Study plan for evaluating bacterial sources in the Ramirez Canyon and Escondido Canyon Creeks watersheds

INTRODUCTION

The beaches adjacent to the mouths of Ramirez Canyon Creek (RCC) and Escondido Canyon Creek (ECC) have exhibited high levels of fecal indicator bacteria. Weekly Heal the Bay (HtB) Beach Report Cards indicate that poor beach conditions occurred at Paradise Cove beach (near the mouth of RCC) about 20% of the time during the dry weather season from 1998-2003 and 65% of the time from 2004 through 2006. Monitoring data near the mouth of ECC are more temporally limited, but indicate poor dry weather beach conditions 98% of the time between the start of sampling in 2005 through the first half of 2006, though poor conditions occurred less than 5% of the time in the second half of 2006 when a berm is believed to have prevented ECC flow from reaching the ocean.

Resolving the problems causing poor beach water quality conditions requires an understanding of bacterial sources, which is unclear in these systems. Bacteria can originate from multiple possible sources in these watersheds, including residential inputs, horse corrals, septic systems, wildlife inputs from open spaces at the upper portions of the watersheds, and shorebirds on the beaches. Selecting the most immediate remedial actions to improve conditions at the beach is difficult until the sources are better understood.

This difficulty of source identification is not unique to these systems. AB 538 required the State to prescribe protocols for source identification studies, but the science of source identification was not well developed enough at the time of the legislation for this to be effective. Genetic techniques to identify sources were first being developed at research laboratories, but studies to evaluate their effectiveness had not yet been conducted. In addition, there were few case studies to illustrate how these techniques could be incorporated into a comprehensive and cost-effective site evaluation. Since that time, the scientific tools have advanced, making the development of assessment protocols more feasible.

To help develop procedures that will lead to cleaner beaches, the Los Angeles County Board of Supervisors allocated funding for bacterial source assessments to be conducted in ECC and RCC. The goal of this project is to use Ramirez and Escondido Canyons as prototypes to develop bacteria source identification protocols, and while doing so, identify the primary bacterial sources in these two watershed systems. This study plan describes that program.

GENERAL APPROACH

The study approach will be adaptive and conducted in phases, with the design of later phases based on outcomes from previous phases. Stakeholder collaboration will be a key

part of the program. Before a new phase begins, results from the previous phase will be examined and discussed with the stakeholder team to develop consensus about how to move forward. Four potential phases are envisioned:

Phase 1: Watershed Characterization

The first phase will involve watershed-wide sampling to identify those sections of the creek system that contain sufficiently high levels of indicator bacteria to warrant further study. Sample sites will include the beach, the creek system immediately upstream of the beach and at the major confluences where additional potential bacterial sources enter the system. Sampling will also be conducted upstream of most anthropogenic influences to assess background concentrations in the system. Sample analyses will be limited to standard indicator bacteria (commonly identified as FIBs) and "Optical Brighteners" in this phase because the primary intent is to isolate sections of the watersheds with high bacterial influence. The optical brightener technique is still developmental and needs some method evaluation, but if successful, would be a highly cost-effective approach for addressing source identification in other watersheds.

Phase 2: Human/non-human Source Identification

The second phase will involve use of two advanced measures to assess whether human or horse fecal sources are present in the areas identified as hot sections in Phase 1. All samples collected during Phase 1 will be filtered and stored frozen for potential analysis, so that no additional field work will be required in Phase 2. The sample processing will be conducted as a second phase because the cost of these measures is several fold higher than for the standard indicators. Processing only those samples that have sufficiently high counts to warrant processing increases cost-effectiveness of the program.

Phase 3: Spatially Intensive Sampling

The third phase will involve enhanced spatial and temporal sampling in those sections of the watershed identified as sources of greatest concern in the first two phases. Whereas Phase 1 is intended to identify sections of the watershed where bacterial sources of concern are thought to originate, Phase 3 is intended to identify the specific input sources. Sampling will take place immediately upstream and downstream of suspected individual sources in that section of stream, such as septic systems or horse corrals.

Phase 4: Library-based Source Identification

If Phase 3 fails to identify specific sources, or if Phase 2 indicates that the bacterial sources are present system wide, then library-based genetic methods will be used to better understand what types of animals are making the largest contribution to the problems at the beach. These genetic methodologies differ from those that will be

employed in Phase 2 in that library based methods measure the source of individual bacterial isolates and can quantitatively specify percentage of fecal inputs coming from different types of animals. This method is reserved for Phase 4 because it is an order of magnitude more expensive, and less reliable, than the Phase 2 methods. However, it has the potential to provide the additional information necessary should the previous three phases fail to identify specific sources to be resolved.

As the program progresses, a clearer picture of the sources of the bacteria found on the beaches of RCC and ECC will emerge. When completed, a review will be conducted to evaluate appropriate protocols for addressing source identification in other watersheds.

SPECIFIC APPROACHES

Phase 1—Spatial Characterization of Bacteria Conditions

We will collect samples at 18 stations in the Ramirez (R) watershed and 9 stations in the Escondido (E) watershed on 15 dates for standard bacterial indicators and optical brighteners. The fifteen dates include once a week sampling for ten weeks in the spring and for five weeks in the fall. The larger amount of sampling allocated for the spring is because of anticipated greater variability associated with more variable flows and because of the opportunity to refine sampling in the fall based on patterns observed in the spring.

Site selection was based on sectioning the watershed based on major changes in land use (e.g. open space vs. residential), clusters of similar land use types, confluences of sub-watersheds, and special areas (creek mouths, Paradise pier, and beach sampling stations), with input from stakeholders. There were also logistic considerations, as samples must reach the laboratory no later than six hours after collection.

The station locations are shown in Figure 1. The different symbols indicate stations sets that will be sampled by an individual sample team within a four-hour period to meet the requirement that sampling processing begin within six hours of collection. Yellow cylinders identify parking spots and access points for stations that are more difficult to access. Stations R1-R1a and R2-R2c are designated as such to indicate they have similar access in difficult areas. A short description of each station, its intended use, and access directions to it are given in Tables 1 and 2 for RCC and ECC, respectively. GPS locations for the stations shown in Figure 1 will be available by March 9, 2007.

Physical reconnaissance has occurred at all stations except four. All sites are accessible. Owner permission is required at three sites and possibly needed at 10 others. The process to gain these permissions is underway. Los Angeles County Department of Public Works and the City of Malibu have been major contributors in helping our gain access. Final determinations of those sites requiring access permission will occur this week and stations requiring access may be moved or deleted if we cannot gain permission.

Spring sampling will begin in March 2007 and is intended to capture dry weather conditions during the rainy season, when base flows are typically higher. Dry conditions for the purpose of sampling will be defined as no rain for the 24-hour period before sampling, and less than 0.1 in rain for the 72-hour period before sampling. If rain conditions prevent sampling in a particular week sampling might occur more than once per week in later weeks. In any case, sampling will not extended beyond the end of May.

Sampling site selection will remain flexible during the study. Stations that are dry or with exceptionally low flow may be bypassed in individual weeks. Results will also be reviewed by the County, HtB, and SCCWRP after the first five sampling periods to assess whether some stations should be eliminated from the program due to continuously low counts. In particular, an assessment will be made whether continued sampling in ECC is appropriate; beach bacterial counts near this creek system have been low since June 2006 and it is unclear whether that is due to a berm at the mouth of the creek or because ECC waters now have low bacterial loads. The Stakeholder team has already identified the possibility of shifting the ECC effort to Latigo Creek, should ECC samples in the spring contain low bacterial counts. The results of this review will be transmitted to all others by e-mail because of time limitations.

The fall sampling will be initiated in September 2007 and is intended to capture conditions at the end of the dry season. A meeting of the Stakeholder team will be held between the spring and fall sampling to reassess the sampling locations and necessary temporal frequency.

Samples in both seasons will be collected between 0700 and 1100 hours at all stations to minimize ultraviolet degradation of bacteria and maximize detection of any potential sewage inflows to the creeks (septic system inputs tend to be highest in the morning, after everyone awakes). Two samples (field duplicates) will be taken at four selected sites on each creek during each sampling date to understand short-term sampling variability. These stations (R3, R8, R11, R14, E4, E6 E10, and E12) represent the ends of major land use sections and the beach. Flow will be measured at each station on each collection date to obtain a measure of bacteria flux in addition to concentration. Flow will be measured in one of three ways at each station. If the water in the creek is 3 inches or greater in depth, a calibrated flow meter will be used to obtain the sample. In this case the cross-sectional profile of the creek will also be obtained. To do this, the width of the water surface across the creek is measured as well as water depth measurements taken at points 25%, 50%, and 75% across the width of the stream. If the stream is shallower, flow volume must be measured by the time it takes to fill a vessel of known volume. This will be done by either finding a point in the creek where the flow is pinched into a small stream and most all the water passing is captured through some combination of funnel, hose and calibrated receptacle (such as a 1 liter graduated cylinder). If the depth is low and no narrow spots are found, the third method that will be used is to direct the flow to a narrow area and measure it by the time it takes to fill a vessel of known volume. As part of the flow kit, the sampling team will carry three lightweight boards with them so that if the first two methods are not usable, they can form a temporary weir of two

boards directing flow to and measuring the water flowing from a notched section of the third. The intent is to get a gross estimate of flow (±50%).

Initiation of analyses for standard bacterial indicators will occur within 6 hours of collection, as will sample filtration and freezing of bacteria source identification samples for potential Phase 2 analysis. Field and laboratory approaches will follow those described by Noble et al. (2006). Briefly, water samples will be aseptically collected in sterile polyethylene bottles and transported to the laboratory on ice. Samples will be analyzed for total coliform, *E. coli*, and enterococci using Colilert-18® and Enterolert® (IDEXX, Westbrook, ME). Sub-samples of each water sample will be immediately filtered onto membranes to capture bacteria optical brighteners and stored on dry ice for later analysis in Phase Two.

Optical brighteners (OB) are chemicals added to detergents to enhance clothing color. OB absorb UV light and emit blue that are detectable with a spectrofluorometer. They provide an inexpensive means of identifying potential septic system leaks. OB will be measured following the method described by Hartel et al. (in press). This method relies on the differential degradation rates of commercial optical brighteners found in laundry detergents and organic matter when exposed to UV light. Water samples taken from streams contaminated with human septage typically contain both optical brighteners and organic matter. Both fluoresce at similar wavelengths, but the fluorescent signal given off by optical brighteners degrades about three times more quickly than that of organic matter when exposed to high intensity UV light. This differential degradation is the basis for distinguishing optical brightners from other organic matter.

Briefly, water samples will be collected and their fluorescence measured using a Turner Designs AU10 fluorometer fitted with a 436 nm narrow range emission filter, optimized to detect optical brighteners. The sample will then be exposed to a controlled amount of high intensity UV light, which will degrade the optical brighteners, but not the fluorescent organic matter in the sample. The sample will then be re-measured. Any loss of fluorescent signal greater than a pre-determined cut-off value is evidence that the water sample was positive for optical brighteners.

As OB are relatively new measures, we will conduct a method evaluation exercise prior to sampling. The exercise will involve a series of positive and negative controls to assess whether the method correctly identifies when sewage is present. Ultimately the field results will also be compared to the human genetic marker applied in Phase 2. A good correlation, should it occur, will provide further evidence of the value of OB as an inexpensive indicator of septic activity in the future.

Phase 2—Human/Nonhuman Source Identification

A subset of samples exhibiting high indicator bacteria levels in Phase 1 will be tested for the presence of human fecal contamination using a *Bacteroides* human genetic marker using the method of Bernhard and Field (2000). Although this method is not applied in typical compliance monitoring, it is among the few methods that have been shown to be

specific for human contamination in a source identification method evaluation test (Griffith et al. 2003).

In addition to the human *Bacteroides* marker, samples with high bacterial counts will also be tested for a *Bacteroides* marker specific for horses using primers developed by Dick et al. (2005). Unlike the human *Bacteroides* marker, this assay has not yet been subjected to independent testing, but the assay performed well in identifying horse fecal material in Oregon where it was developed. The measure will be included in this study since stakeholders identified horse stables as a potential source of fecal bacteria in the ECC and RCC watersheds, and the incremental cost for measuring the additional marker is low. Because the method has not been previously evaluated independently, we will include control samples from known sources as part of the test protocol.

Samples to be analyzed for Phase 2 will be selected by SCCWRP, in e-mail consultation with the Stakeholder Task Force based on spatial distribution of fecal indicator bacteria counts in Phase 1. If agreement about the samples to be processed cannot be reached via e-mail, then a meeting will be scheduled to develop consensus.

Phases 3 and 4

Adapting from the results of the first and second phases, the third and forth phases will be directed toward watershed sections with consistently higher levels of bacteria or flux of bacteria. Source tracking will be directed toward tracking upstream in specific tributaries, within specific areas, or up discharge pipes that have consistently higher bacterial contributions. Design specifics cannot be identified at this time. The design will be based on stakeholder consensus at a meeting scheduled for review of results from Phases 1 and 2.

SCHEDULE

The initial study plan was circulated for agreement among the key parties during January 2007. The present study plan reflects comments received, as well as further input obtained from two site visits. Assuming there is no overriding concern raised with this version of the study plan, sampling will begin in mid-March. Analytical results of the first ten surveys will be available in June 2007 at which time the first subset of samples for human/non-human source identification will be selected.

A stakeholder meeting will be planned for August 2007 to review results from the spring Phase 1 and 2 efforts. The meeting will be used to assess how the fall sampling should be modified based on what was learned from the spring.

A second Stakeholder meeting will take place in January 2008, after results from the fall sampling are available, to plan the design for Phases 3 and 4. These Phases would be implemented in spring of 2008.

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