SAN JUAN CREEK WATERSHED BACTERIAL STUDY

Final Report

Study funded by California Regional Water Quality Board, San Diego Region
Agreement #9-182-190-0 issued to County of Orange Public Facilities and Resources Department (PFRD)

Prepared by: Douglas Moore, Ph.D., Director, Donna Ferguson, M.S., and Elisabeth J. Gonzalez, Ph.D., Orange County Public Health Laboratory

December 24, 2002

EXECUTIVE SUMMARY

In 1998, the lower reach of San Juan Creek (SJC) was listed by the Regional Water Quality Control Board (RWQCB) as water quality impaired in accordance with Section 303(d) of the Clean Water Act due to high levels of fecal indicator bacteria. Thus, in May 2000, the State Water Resources Control Board (through the San Diego RWQCB) provided funding to the County of Orange Public Facilities and Resources Department (PFRD) to perform a study in collaboration with the Orange County Health Care Agency (OCHCA) of the existing bacterial contamination within the San Juan Creek watershed.

San Juan Creek empties into Doheny Beach, which is also frequently posted as exceeding State recreational water quality standards. Orange County Public Health Laboratory (OCPHL) of the OCHCA was subcontracted by PFRD to carry out a bacterial watershed study of SJC to provide the County of Orange with information on the relative magnitudes of the bacterial loadings and sources to develop and implement an effective correction program using a combination of bacteriological monitoring surveys and bacterial source tracking (BST) analysis. The objectives of the sampling study were as follows:

1. Perform a bacterial survey of the water quality of the San Juan Creek watershed under dry weather conditions and locate any areas frequently exceeding bacteriologic water quality standards. Conduct a detailed monitoring survey of the problem areas identified.

2. Determine the source of the indicator bacteria found in the problem areas using bacterial source tracking techniques.

3. Compare two different techniques of bacterial source tracking, ribotyping and Antibiotic Resistance Analysis (ARA) to determine the accuracy of these techniques.

OCPHL collected water samples at various locations throughout the watershed to determine bacterial densities in creeks, storm drains and ocean water and for source identification testing. *E. coli* and *Enterococcus* were isolated from fecal and water samples and sent to reference laboratories to conduct ARA and ribotyping. The data from the bacterial monitoring and BST testing would be used to determine the sources of fecal pollution in SJC including humans, sewage, dogs, cats, horses and seagulls. However, since BST techniques are still in the developmental stages, OCPHL conducted a quality assurance (QA) study with the reference laboratories to determine the accuracy level of ARA and ribotyping prior to using these methods to test SJC watershed samples. This report describes the results of the SJC watershed bacterial monitoring and

source tracking study that was conducted in three phases to accomplish the following tasks:

Phase I (Task 3): Bacteriological Survey of Watershed and Adjacent Beach Recreational Water.

Thirty-six sampling locations, including 26 creeks, 7 storm drains, and 3 ocean sites, were sampled weekly for 11 weeks to identify areas which frequently exceed bacteriologic water quality standards. Water samples were tested for fecal indicator bacteria including total coliforms, fecal coliforms and *Enterococcus*.

Moderate to high levels of fecal indicator bacteria were detected in storm drains and creeks. The highest concentrations of fecal coliforms and *Enterococcus* were found in the storm drains as compared to the creeks and ocean sampling sites. Samples taken from creek sites distant to human habitat also had low to moderate levels of bacteria, suggestive of fecal contamination by non-human sources. The results of Phase I are described further in Chapter 1, "Phase I: Bacteriological Survey of San Juan Creek Watershed".

Phase II (Task 4): Detailed Bacteriological Survey of Identified Problem Areas.

Five sites were selected under the criteria previously described for continued monitoring and source tracking studies:

Pacific Ocean at the mouth of SJC (station number SJ02);
East side of SJC, at the beach, behind the berm (SJC2);
SJC below Pacific Coast Highway (PCH) (SJ06);
SJC above Trabuco Creek (SJ10); and
Trabuco Creek (SJ25).

Fecal indicator levels were determined for 69 samples collected over a 13-week period. *E. coli* testing was added during Phase II since it is more specific than fecal coliforms as in indicator of fecal contamination and to obtain isolates for bacterial source tracking testing conducted during Phase III.

As in Phase I, the bacterial concentrations for fecal indicators were higher overall in San Juan and Trabuco creeks compared to levels detected in the ocean water samples. The lower SJC area below PCH was consistently polluted with higher concentrations of fecal coliforms, including *E. coli* and *Enterococcus*. Overall, the bacterial concentrations found during Phase II were higher than levels for Phase I (excluding the effects of rain). The task 4 results are presented in detail in Chapter 2, "Phase II: Detailed Bacteriological Survey of San Juan Creek Watershed".

Phase III (Task 5): Source Identification by ARA and Ribotyping: Library Preparation and Technique Accuracy Determination.

Recent studies have reported the use of source tracking methods such as ARA and ribotyping to determine sources of bacteriological contamination as being human or animal derived based on differences in antibiotic resistance patterns or ribotype profiles of fecal indicators.

In this study, *E. coli* and *Enterococcus* bacteria were isolated from known species, including humans and animals that may be major contributors to high fecal indicator levels in the watershed. The bacterial strains were used to construct large ARA and ribotyping databases or libraries representative of *E. coli* and *Enterococcus* strains from humans and animals in the SJC watershed area.

To date, source tracking methods have not been widely tested in the field or subjected to rigorous QA testing. Therefore, OCPHL conducted a QA study to assess the suitability of using ARA and ribotyping for source identification of watershed isolates to be conducted in Phase IV. Accuracy and reproducibility of both methods was evaluated using 100 organisms from known sources provided to the contract laboratories as "blind" or proficiency samples. Based on the proficiency testing of known *E. coli* isolates, only 29% and 27% were accurately classified into the source groups using ARA and ribotyping, respectively. As for *Enterococcus*, 46% of 99 isolates were accurately classified. The results and discussion of the data are described in Chapter 3, "Phase III: Final Source Identification Report".

Phase IV (Task 6): Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates.

The objectives of Phase IV were to conduct ARA and ribotyping analyses to determine the relative contributions of human, sewage, horse, cat, dog and seagull feces to the levels of *E. coli* and *Enterococcus* in the watershed. Since the accuracy testing results obtained during Phase III indicated that ARA and ribotyping currently lack the accuracy and reproducibility level required to determine the sources of bacterial pollution, Phase IV was not undertaken.

Conclusions

Bacterial Survey

- Bacterial pollution measured by standard fecal indicator organisms was ubiquitous in storm drains and creeks sampled in the San Juan Creek watershed. Overall water quality measured against REC-1 standards was poor. The levels of indicators varied by the type of sampling location. The highest levels were found in storm drains, followed by creek sites, with the lowest levels detected at ocean sites. It is not known if lower levels in creeks were due to dilution, predation by other organisms, attachment to surfaces or inactivation.

- Concentrations of indicator organisms at storm drains varied temporally and spatially, with levels at some drains up to one log higher throughout the sampling period.

- While this study did not involve sampling at all storm drains in the watershed, the data indicate that storm drains are the major source of dry weather pollution at sampling stations upstream of PCH and below the furthest sampling site.

- Concentrations of fecal coliforms in storm drains ranged from a geometric mean of 1,401 colony forming units (CFU)/100 ml for station SJ07 (Storm drain L01S09 at La Novia and San Juan Creek) to 15,919 CFU/ml for SJ11 (Storm drain L02P02) at Trabuco Creek. These levels are similar to those found in urban runoff in the Newport Beach and Aliso watersheds (unpublished reports). Mean concentrations of indicators detected during the sampling period are not indicative of large or moderate levels of direct sewage contamination (1 to 2 log higher than typical levels). However, occasional spikes in indicator levels were detected in some creek sites and storm drains. The data does not rule out dilution of sewage from leaking pipes, cross-connected lines or the occurrence of intermittent sewage spills.

- Fecal coliform and *Enterococcus* concentrations were markedly higher at the San Juan Creek sampling sites near PCH as compared to sites further upstream. Possible explanations for this finding include indicator bacteria contribution from the intervening storm drain, direct contamination from waterfowl or other unidentified sources, and differences in stream morphology and ecology that allow organisms to regenerate.

- Low to moderate levels of bacteria were also found in creek sampling stations located distant to dense urbanization but within rural land use areas. This indicates that contamination is not limited to urban areas and that human land use activities as well as wildlife may be contributing sources at these sites.

- The concentrations of *Enterococcus* and fecal coliforms, two more specific indicators of fecal pollution than total coliforms, generally correlated by site. Levels of total coliforms often did not correlate with the other indictors.

- Rainfall events resulted in considerably higher levels of indicator bacteria at all the sites.

Bacterial Source Tracking

- In this study, the ARA and ribotyping methods did not demonstrate sufficient accuracy, discriminatory power, or reproducibility required to differentiate *E. coli* and *Enterococcus* isolates originating from human and non-human sources such as dogs, cats, horses and seagulls.

- Source tracking methods are emerging technologies that have not been rigorously tested. While they remain an area of research interest, they may have little or no use in determining the source of pollution in watersheds subject to multiple sources of contamination. Additional investigation is needed to address critical factors such as the monitoring design, type of indicator bacteria used, size and representativeness of the database, number of fecal indicator sources, number of proficiency test samples, type of data analysis used to interpret source identification results, bacterial variation, and geographic differences.

- Until an accurate source tracking technique is found, determining sources of pollution should rely on detailed watershed and sub-watershed surveys using conventional techniques that have been well established.

- Further studies are needed to validate source tracking methods using quality assurance testing.