PROBLEM AND SCOPE

Urban streams and ocean beaches in the Santa Barbara area have fecal indicator bacteria concentrations that exceed public health standards for recreational water.

The source of the contamination and consequently potential mitigation strategies are not known.

Scope of this study includes collection of hydrologic, geochemical, and microbiological data along Mission Creek and East Beach west of Mission Creek.
WORK TASKS

1. Analysis of existing data
2. Collection of hydrologic data from urban streams
3. Collection of hydrologic data from ocean beaches
4. Characterization of dissolved organic carbon composition
5. Genetic and molecular microbiological analysis
6. Tracers of wastewater origin
ANALYSIS OF EXISTING DATA

Statistical analysis of data from urban streams.
1. Correlation with available streamflow data
2. Estimation of loads and potential magnitude and seasonality of source

Statistical analysis of data from ocean beaches
1. Correlation with tidal cycles
2. Seasonality
3. Correlation with flow events
LOCATION OF SAMPLE COLLECTION SITES

EXPLANATION

- Surface-water sites
- Ocean beach sites
BACTERIAL CONCENTRATIONS AND TIDES

Minimum or "neap" tide

Extreme or "Spring" tide

Neap tide

Extremes tide

Most probable number, per 100 ml

Height in feet above MLLW
INSTRUMENT INSTALLATION AND DATA COLLECTION

Shallow water-table wells installed using a cone-penetrometer (CPT) rig

Seepage-runs along streams to evaluate changes in flow and quality (MaFadden, Polinoski, and Martin, 1991)

Seepage meter and subsurface temperature data collection
RADIUM ISOTOPE SYSTEMATICS

Four isotopes having a wide range of half-lives

Chemistry similar to calcium. Large difference in concentrations in ground water and seawater governed by exchange reactions.

Estimates of ground-water discharge supported by estimates calculated from water-level and seepage-meter data under a range of tidal and hydrologic conditions.
Possible sources of fecal bacteria contamination to urban streams and beaches

- Transient human populations
- Birds and direct leakage from sewer lines
- Urban nuisance flows
Mission Creek streamflow and water-quality data, April 19-20, 2005

- Large concentrations and loads for total coliform and *E. coli* at Old Mission Creek (OMC) inflow and mouth
- Absence of enterococci at OMC and in sampled urban drains
- General increase in *E. coli* with distance downstream

**Fecal indicator bacteria**

- **Total coliform** ± 50%
- **E. Coli** ± 6%
- **Enterococci** ± 80%

**Concentration** x **Volume** = **Load**

\[ \text{MPN/L}^3 \times \text{L}^3/\text{t} = \text{MPN/t} \]
Dissolved organic carbon (DOC) and ultraviolet (UV) absorbance data
April 19-21, 2005

Changes in concentration and composition of dissolved organic carbon from different sources associated with fecal indicator bacteria contamination

Fecal indicator bacteria concentrations in Arroyo Burro were selected for comparison and contrast with data from Mission Creek (MC). Arroyo Burro flows perennially as a result of urban nuisance flows and does not receive ground water inflow.
Excitation/emission fluorescence (EEM) data

**Baseflow**

3:49 AM, January 10, 2001

0 2E+05 4E+05 6E+05 8E+05 10E+05 12E+05 14E+06 16E+06 18E+06

300 400 500 600 700

**Recessional flow**

10:40 AM, January 26, 2001

0 2E+05 4E+05 6E+05 8E+05 10E+05 12E+05 14E+06 16E+06 18E+06

300 400 500 600 700
MOLECULAR MICROBIOLOGY

TWO BROAD TYPES OF DATA AND TWO BROAD TYPES OF INTERPRETATIONS PRODUCING VERY DIFFERENT INFORMATION

TYPES OF DATA—Genetic (T-RFLP) and molecular (PLFA) data. Terminal-Restriction Fragment Length Polymorphism (T-RFLP) data reflect the genetic material from specific organisms. Phospholipid Fatty Acid (PLFA) data reflect different organisms having common metabolic processes.

TYPES OF DATA INTERPRETATIONS—Microbial Source Tracking and Microbial Community Structure Analysis. Microbial Source Tracking provides a genetic “fingerprint” fecal indicator bacteria from different sources to identify source of bacteria. Microbial Community Structure Analysis evaluates changes in the abundance, type, and diversity of organisms within the entire microbial population resulting from environmental conditions. These changes are used with other data to infer the source of the microorganisms.
Polymerase Chain Reaction (PCR) - a molecular copy machine

Bacterial Cell

Ribosomes
Evolutionarily conserved, translate genetic information into proteins

70s Ribosome
50s Subunit
30s Subunit
5s
16s
23s

Sequence of the 16S rRNA gene serves as a stable genetic "fingerprint" for each organism

Modified from Menu Ledy, OCWD
Polymerase Chain Reaction (PCR) and Terminal Restriction Fragment Length Polymorphism (T-RFLP)

- **Genome**
- **Hypervariable region (500bp)**

**PCR:** Sequence between primers copied

- **5’ (Forward) primer sequence with fluorescent label**
- **Copies are further duplicated, yielding up to 10^6 X amplification**
- **3’ (Reverse) primer sequence (unlabeled)**

**Result:**
Large number of 5’ fluorescently labeled amplicons containing the hypervariable gene sequence between the primers.

Modified from Menu Ledy, OCWD
Terminal-Restriction Fragment Length Polymorphism (T-RFLP)

Fluorescent label

Uncut 350 bp Amplicon of Organism 1

BstU1 cut site @56 bp

Organism 1
Peak @ 56 bp

Uncut 350 bp Amplicon of Organism 2

BstU1 cut site @210 bp

Organism 2
Peak @ 210 bp

Modified from Menu Ledy, OCWD
Amplicons without and with endonuclease digestion

Without digestion:
- Number of amplicons = 11
- Simpson's reciprocal index = 3.52
- Shannon-Weiner index = 1.57

With digestion:
- Number of amplicons = 33
- Simpson's reciprocal index = 13.4
- Shannon-Weiner index = 3.00
Phospholipid Fatty Acids (PLFA)

LIPID BI-LAYER IN A MICROBIAL CELL MEMBRANE

Carbon/Palmitic  Oleic
Nitrogen  Oxygen  Phosphorus
Water Oxygens

H Heller, M Schaefer, K Schulten,
RasMol Image by E Martz
Changes in phospholipid fatty acid (PLFA) concentrations and composition during stormflow

- **SAR below Prado Dam**
  - Stage height behind Prado Dam, in feet above sea level

- **SAR at diversion downstream from Imperial Hwy**
  - Ratio of straight-chain to branched PLFA, in picomoles per picomoles
  - Total PLFA, in picomoles per milliliter

- **Streamflow**
  - In cubic feet per second

- **Percentage of streamflow derived from runoff downstream of Prado Dam**

**USGS**

*science for a changing world*
Ultraviolet (UV) absorbance and excitation/emission fluorescence (EEM)

Simplified characterization of dissolved organic carbon (DOC) from different sources. Less expensive than hierarchical approaches that separate DOC on the basis of polarity and molecular weight, or on the basis of functional groups.

Can we use these data to characterize the source of dissolved organic carbon and potentially associate that information with the source of fecal indicator bacteria?
TRACERS OF WASTEWATER ORIGIN

Not naturally occurring, associated with human use and consumption, and measurable in low concentrations. Some of these compounds may or may not have associated environmental risks.

More than 60 compounds analyzed in the part per trillion range. Classified into two broad categories:

- Compounds produced by humans
  - Coprostanol (low solubility, associated with colloidal fraction)
  - Urobilin
  - Estrogen

- Compounds used by humans
  - Caffeine
  - Pharmaceuticals and personal health-care products (PPCP’s)
# Project Timeline

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<td>d. Quarterly sample collection</td>
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<td>III. Collection of hydrologic data along oceanfront beaches</td>
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PROJECT STAFF

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