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# LAGUNA WATERSHED STUDY AND WATER QUALITY IMPROVEMENT FEASIBILITY ANALYSIS

*Proposition 50 Clean Beaches Grant Program*

*Agreement No. 07-585-550-2*

## FINAL PROJECT REPORT

City of Santa Barbara  
Creeks Division

March 13, 2013

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Appendix B: Fecal Indicator Bacteria and Human Fecal Pollution in the Laguna Watershed – Phase I

Appendix C: Fecal Indicator Bacteria and Human Fecal Pollution in the Laguna Watershed – Phase II

Appendix D: Grant Summary Form

# 1 BACKGROUND

## 1.1 BEACH WARNINGS

The main water quality problem that the Laguna Watershed Study and Water Quality Improvement Feasibility Analysis (Project) addresses is frequent beach warnings and potential associated health risks, based on high levels of fecal indicator bacteria, at East Beach at Mission Creek (Table 1). The Project is located in Santa Barbara, CA, a city of approximately 90,000 people located in the County of Santa Barbara (Figure 1). Two creeks discharge to East Beach at Mission Creek: Mission Creek, which is itself impaired for fecal indicator bacteria, and Laguna Channel (Table 2). The two streams almost always comeingle in a coastal estuary prior to discharge into the Pacific Ocean. East Beach at Mission Creek has been listed on Heal the Bay’s list of “Beach Bummers” in past years.

**TABLE 1. BEACH WARNINGS AT EAST BEACH AT MISSION CREEK.**

<b>AB411 Year (April 1- October 31)</b>	<b>Number of Beach Warnings at East Beach at Mission Creek</b>
<b>1999</b>	5
<b>2000</b>	5
<b>2001</b>	6
<b>2002</b>	7
<b>2003</b>	1
<b>2004</b>	6
<b>2005</b>	13
<b>2006</b>	16
<b>2007</b>	5
<b>2008</b>	3
<b>2009</b>	1
<b>2010</b>	7
<b>2011</b>	10
<b>2012</b>	2



**FIGURE 1. PROJECT LOCATION IN SANTA BARBARA, CA.**

**TABLE 2. EAST BEACH AT MISSION CREEK IMPAIRMENTS AND ASSOCIATED WATER BODIES.**

Impaired Beach	Pathogen Related Listings*	Waterbody Discharging to Beach (Impairment)
<b>East Beach at Mission Creek</b>	Enterococcus, Fecal Coliform, Total Coliform	Mission Creek (E. coli, Fecal Coliform) Laguna Channel (not assessed)

\*2010 Clean Water Act Section 303(d) Listings.

The City of Santa Barbara (City) has taken an aggressive approach to improving beach water quality and has used several Clean Beaches Initiative (CBI) grants towards this end (see Figure 2 for an overview map of projects located within the City). The City has implemented capital projects such as low-flow storm drain diversions and an ultraviolet (UV) disinfection project (with funding from the Proposition 13 and Proposition 40 Clean Beaches Grant Program; Figure 2). Concurrent with the capital program, the City continues to search for sources of indicator bacterial contamination.

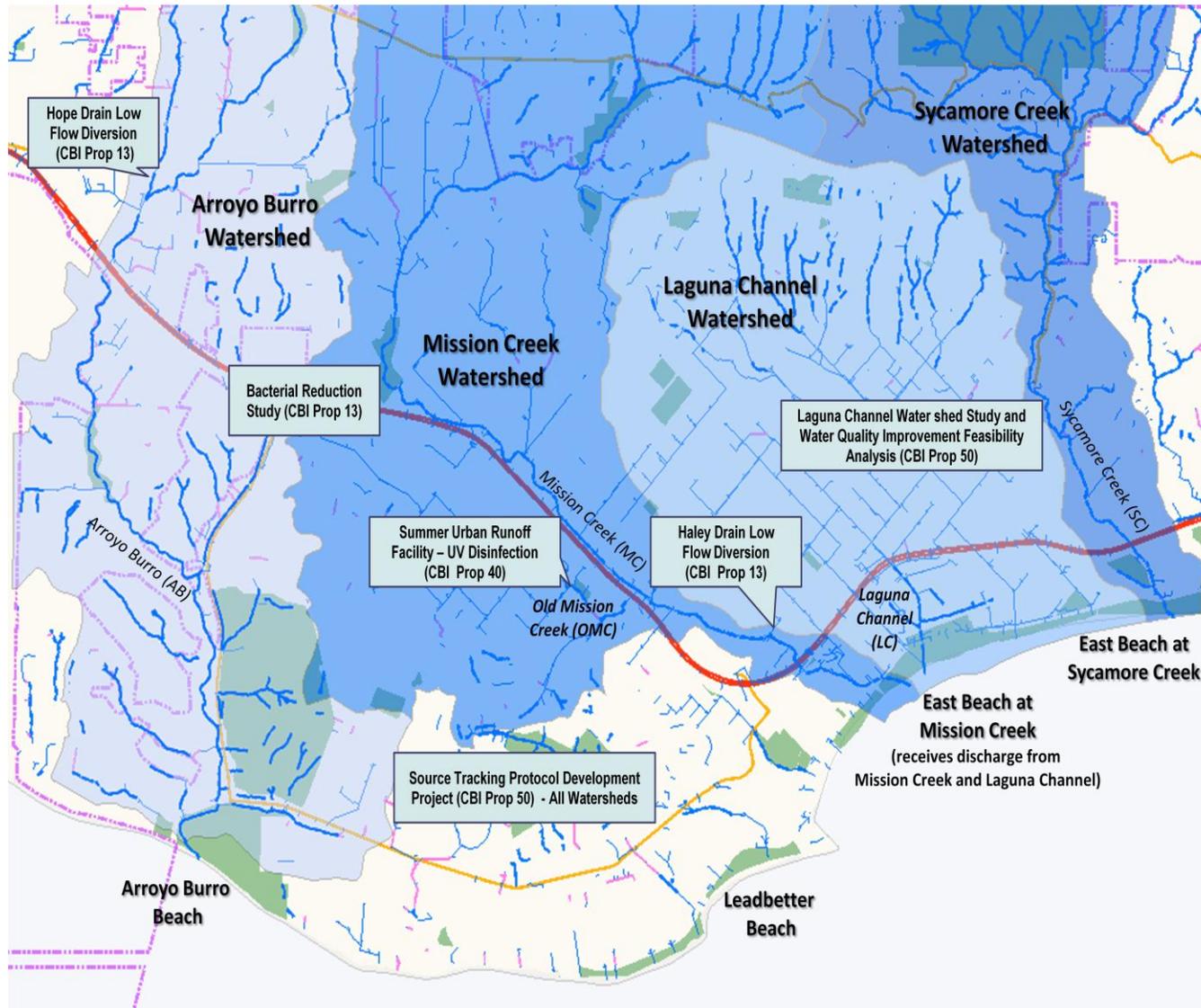


FIGURE 2. OVERVIEW MAP OF AB411 BEACHES, CREEKS (THICK BLUE LINES), STORM DRAIN PIPES (THIN BLUE LINES), WATERSHED BOUNDARIES, AND CBI-FUNDED PROJECTS LOCATED IN THE CITY OF SANTA BARBARA (DASHED PINK LINES).

The relationship between beach warnings and the flow of Mission Creek and Laguna Channel was demonstrated in a statistical analysis of beach warnings previously completed by the City using AB411 monitoring data. The analysis showed that exceedances of AB411 criteria at beaches in the City are far more frequent when the creeks and associated beaches are open and flowing to the ocean, compared to when they are closed. For example, at East Beach at Mission Creek, exceedances of the total coliform standard are 25 times more likely when the estuary is flowing to the ocean, compared to when it is closed by a sand berm, illustrating the importance of water quality in coastal creeks and the storm drains discharging to them.

## **1.2 SOURCES OF INDICATOR BACTERIA**

Fecal indicator bacteria can come from human waste and animal feces, but they can also grow in decaying plant material and even on storm drain surfaces, gutters, kelp, and sand grains. Indicator bacteria growing in the environment are unlikely to pose health risks to humans, and the risk associated with animal fecal sources is unknown. Among types of fecal contamination at coastal, urban beaches, untreated human waste has the greatest potential to sicken beachgoers. Prior to committing to a project designed to eliminate indicator bacteria from Laguna Channel flow, the City sought to understand if the indicator bacteria were truly indicative of human waste.

## **2 PROJECT SUMMARY**

### **2.1 PROJECT PURPOSE**

The project purpose is to complete the Laguna Channel Watershed Study and carry out a feasibility analysis of proposed implementation projects.

### **2.2 SCOPE AND GOALS**

By completion of this Project, the City conducted the first phase of a two-part implementation project to eliminate harmful fecal bacteria from the Laguna Channel prior to discharge at East Beach. The first phase involved a watershed study and feasibility analysis conducted in order to identify the most cost-effective project to eliminate harmful bacteria and pathogens from Laguna Channel prior to discharge to the Pacific Ocean. The Project also included preliminary design, construction cost estimates, and CEQA review for the identified water quality improvement project.

The main goals of the Project were to 1) confirm or disprove the presence of human waste markers discharging from the Laguna Channel, prior to design and construction of an end-of-pipe treatment system, and 2) pending positive results in (1), identify the most cost effective project to improve water quality in dry weather flows discharging from Laguna Channel to East Beach at Mission Creek. The City plans to submit a second CBI Implementation Grant application for construction of the identified project, which consists of relining two miles of sanitary sewer pipes that have been identified as target locations where untreated sewage may be leaking into nearby storm drains and reaching Laguna Channel.

## 2.3 PROJECT DESCRIPTION

The City of Santa Barbara contracted with Geosyntec and the University of California Santa Barbara (UCSB) to conduct a study to evaluate dry weather hydrology, microbiological indicators, bacterial sources and loads, and feasible water quality improvements for the Laguna Channel in Santa Barbara, California. The study was conducted in three parts: 1) dry weather hydrology field reconnaissance and flow monitoring evaluation; 2) identification of sources, routes, and the loading of Fecal Indicator Bacteria (FIB) and DNA-based markers of human waste (human *Bacteroides* marker or HBM) in dry weather creek and storm drain flows; and 3) recommendation of water quality enhancement alternatives, including an assessment of project feasibility, based on findings from 1 and 2.

The results of this effort are described in detail in the report by Geosyntec entitled, “Laguna Watershed Study and Water Quality Improvement Feasibility Analysis” (Appendix A) and two reports by UCSB, “Fecal Indicator Bacteria and Human Fecal Pollution in the Laguna Watershed – Phase I” (Appendix B) and “Fecal Indicator Bacteria and Human Fecal Pollution in the Laguna Watershed – Phase II” (Appendix C). A summary is provided in the following sections.

### 2.3.1 APPROACH AND TECHNIQUES

Existing information, including the City’s Storm Drain Atlas and Geographic Information System (GIS) was used to identify storm drains and subwatershed boundaries within the Laguna Channel Watershed. Field reconnaissance was used to observe qualitative patterns of flow, i.e. using a classification scheme including “dry,” “trickle,” and “flowing,” in the storm drains throughout the Laguna Watershed. Based on visual observations and storm drain maps, a sampling and quantitative flow monitoring study was designed, with sites marked in Figure 3. See Section 3 (Locations of Project Monitoring Locations) for coordinates and additional details about sites.

The following methods were used in support of the Laguna Watershed Study. Methods are described in detail in the Quality Assurance Project Plan (QAPP), including Standard Operating Procedures, and the Monitoring Plan (MP):

- Flow Measurements (Automated and Manual)
- Fecal Indicator Measurements (IDEXX)
- Field Measurements (Dissolved Oxygen, Temperature, Conductivity and pH)
- Microbial Source Tracking Methods (Human *Bacteroides* and 16S PCR-TRFLP)

These methods were applied in Laguna Channel and several storm drain locations feeding the channel.



FIGURE 3. SAMPLING AND FLOW MEASUREMENT LOCATIONS.

Results from flow monitoring and microbial techniques were used to calculate loads coming from different parts of the Laguna Watershed and to confirm whether human waste was present in the watershed.

A microbial source tracking sampling plan was developed and coordinated with automated flow monitoring. A data-logging flow meter was installed at three storm drain locations (intersection of Laguna and Haley, City Annex Yard, and intersection of Gutierrez and Salsipuedes) for a duration greater than two weeks at each location to determine daily or weekly variations in flow rates. A total of 79 samples were collected between July and September of 2008 at locations of flow monitoring and other locations, and analyzed by UCSB for FIB (total coliforms, *E. coli*, and *Enterococcus* spp.) and human waste

markers such as Human-specific *Bacteroides* Marker (HBM). Results of the 2008 microbial testing results are incorporated into the Laguna Watershed Study (Appendix A) and described in detail in Appendix B. Additional water sampling and microbial testing was conducted by UCSB in 2009; results from this effort are described in detail in Appendix C and are not included in the summary of results presented below.

## 2.3.2 RESULTS

### 2.3.2.1 DATA REVIEW

A careful investigation of the City's Storm Drain Atlas and GIS data showed that the Laguna Watershed was smaller than indicated previously on City watershed maps, as seen by comparing the watershed outline in Figure 2 to the watershed outline Figure 4. Previous watershed maps were based on digital elevation data sources. Here, a detailed analysis of the storm drain network found that in some areas, water was shunted, via storm drains, to adjacent watersheds and waterbodies to avoid flooding in the low-lying lower Laguna Watershed. Storm drain maps and GIS layers were also used to delineate subwatershed (or subdrainage) boundaries (Figure 4).

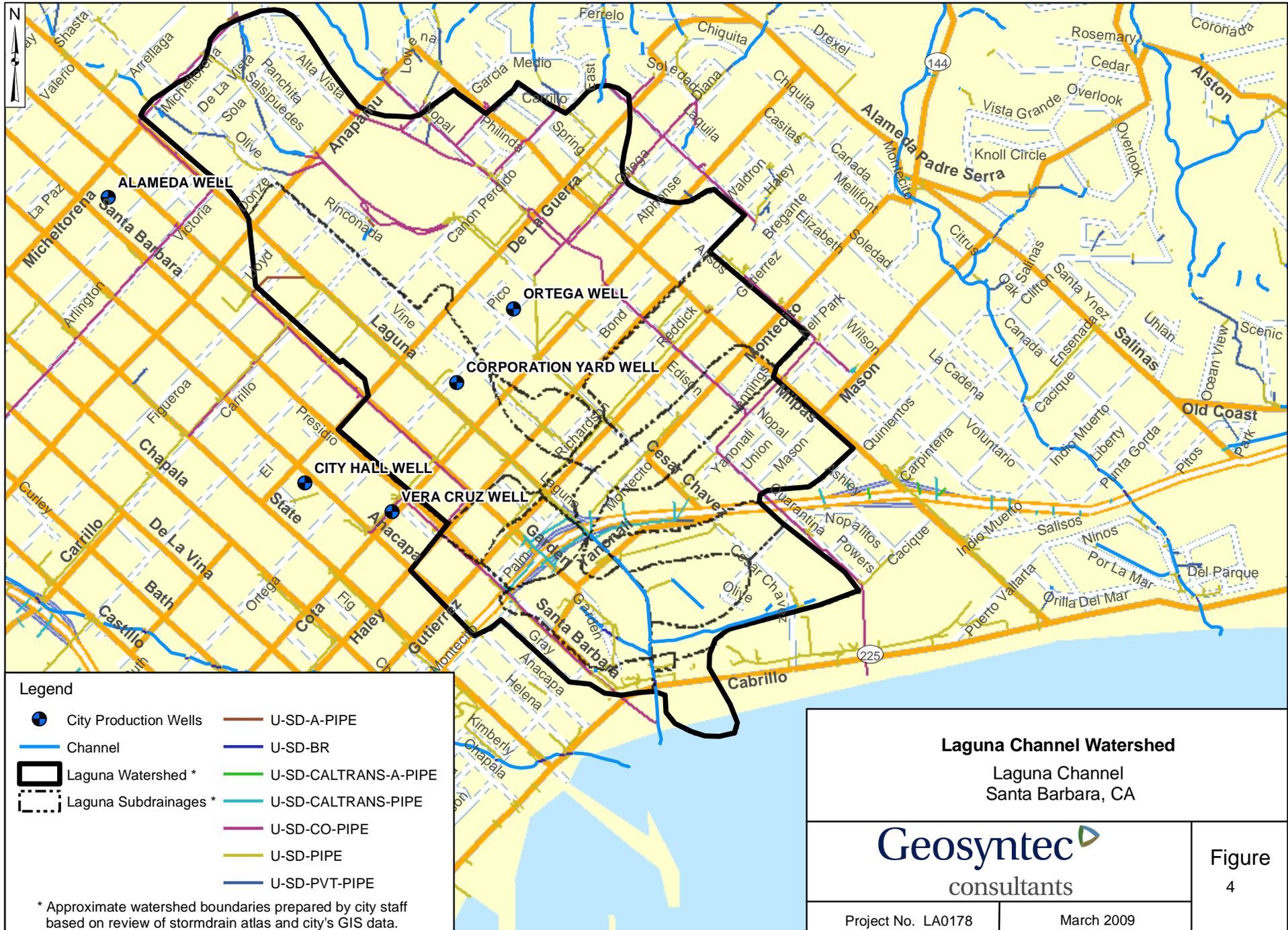
### 2.3.2.2 FIELD RECONNAISSANCE

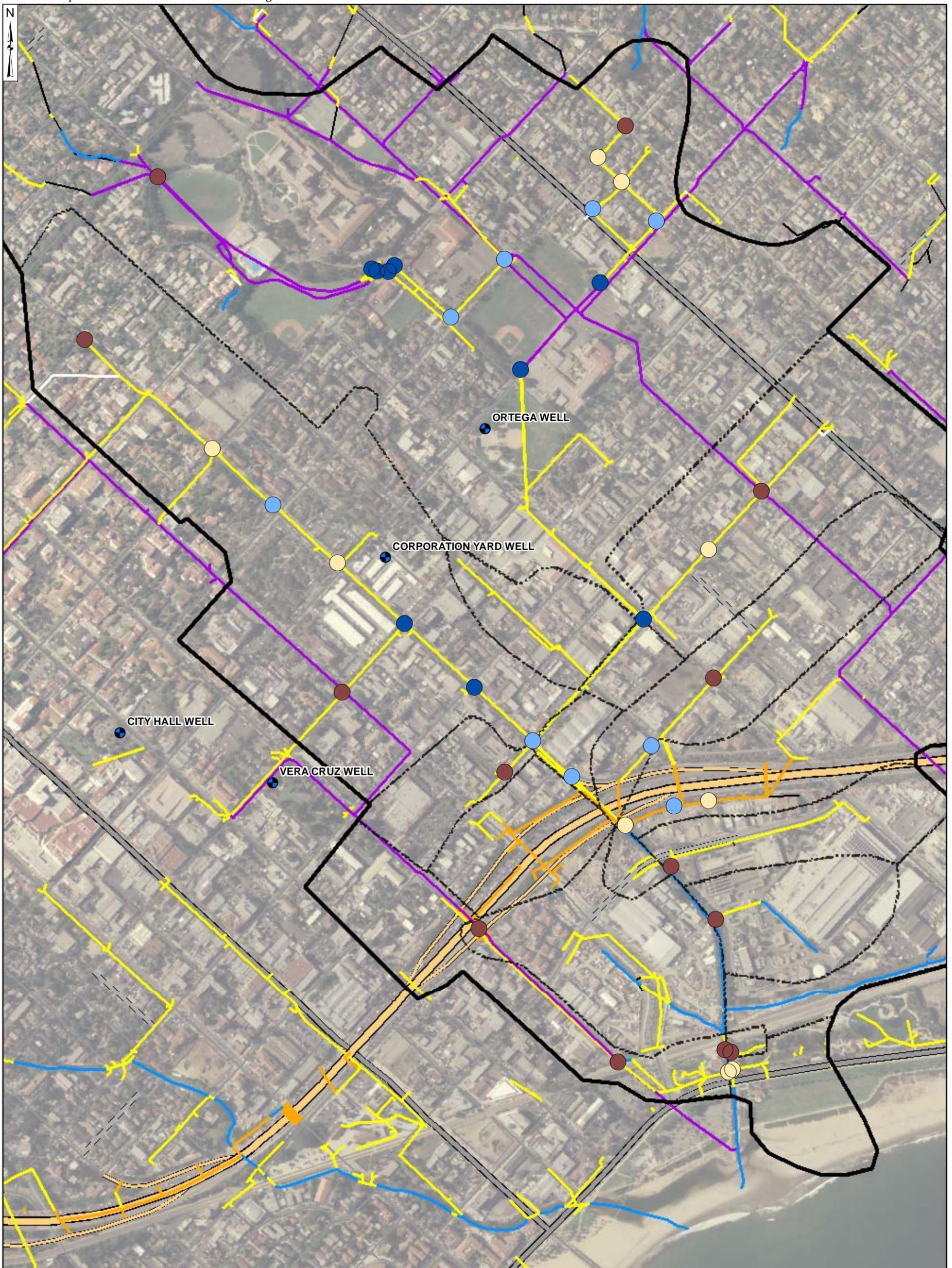
Field reconnaissance showed that the majority of the sources of dry weather flow were identified as coming from the storm drain network north of the Highway 101, rather than from discharges to the open channel south of Highway 101 (orange line in Figure 5). See Appendix A, Section 3.1.1 for more details on field observations.

### 2.3.2.3 HYDROLOGY

An average flow rate of 140 gallons per minute (gpm) or 0.30 cubic feet per second (cfs) was found to be flowing into the Laguna Channel from storm drains, and 65 gpm or 0.15 cfs, was found to be flowing out of Laguna Channel into the Mission Lagoon at East Beach (Figure 6). See Appendix A, Section 3.1.4 for a detailed water budget evaluation.

Dry weather sources include City wells (direct pumping from the Corporate Well to the storm drain and seepage from fittings at the Ortega Well; locations shown in Figure 3) and seepage from groundwater (Figure 8). Substantial variability in flow rates was seen when automated data loggers were used with flow gauges; an example is provided in Figure 7. See Appendix A, Section 3.1.2 for detailed results of automated flow monitoring.





**Legend**

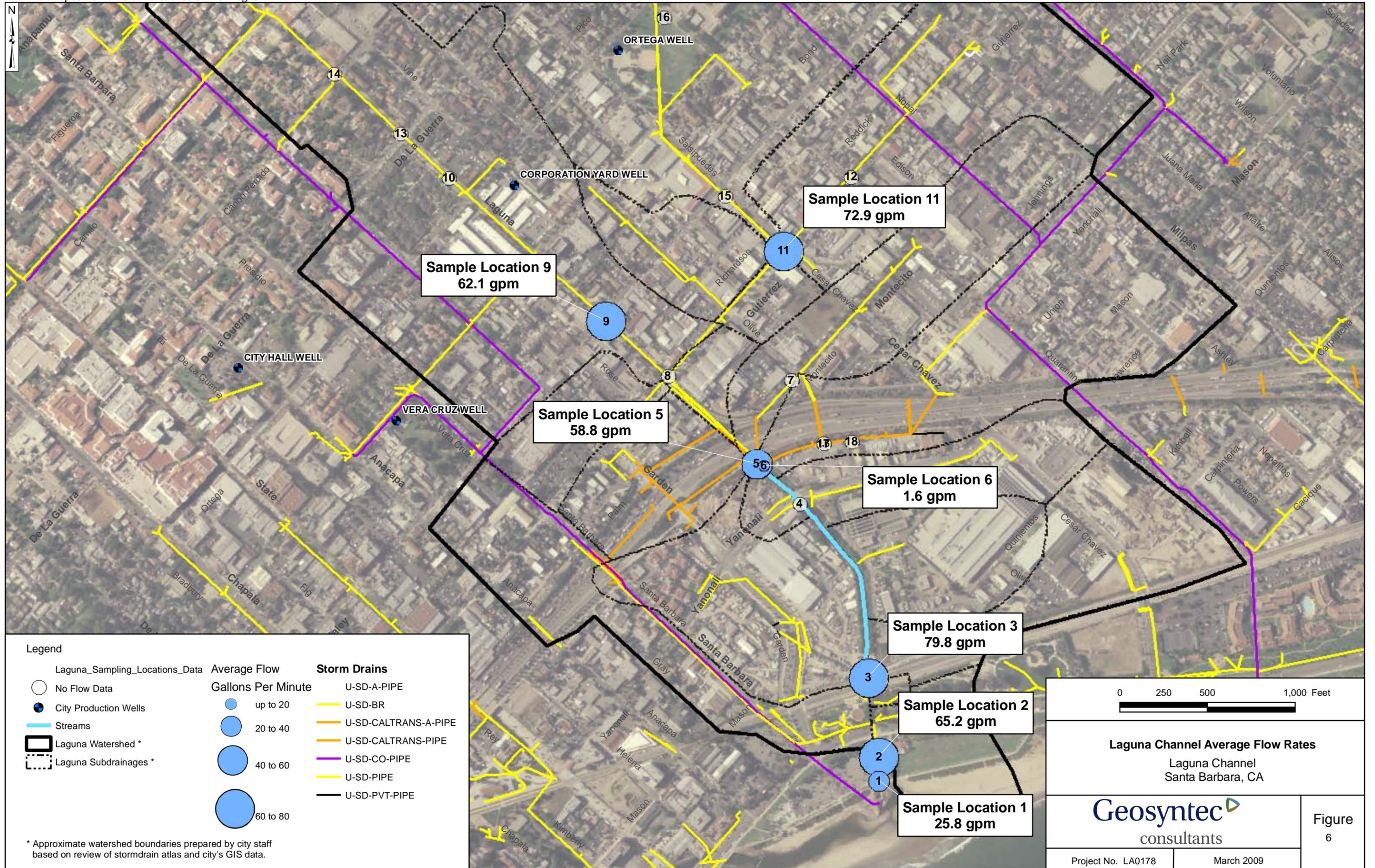
<b>Flow Observations</b>	<b>Storm Drains</b>
● Dry	U-SD-A-PIPE
● Wet but not flowing or trickle	U-SD-BR
● Observable flow	U-SD-CALTRANS-A-PIPE
● Significant flow	U-SD-CALTRANS-PIPE
● City Production Wells	U-SD-CO-PIPE
▭ Laguna Watershed *	U-SD-PIPE
▭ Laguna Subdrainages *	U-SD-PVT-PIPE
— Channel	

\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.

**Quantitative Graphical Summary of Dry-Weather Flow Stormdrain Reconnaissance Findings**  
Laguna Channel  
Santa Barbara, CA

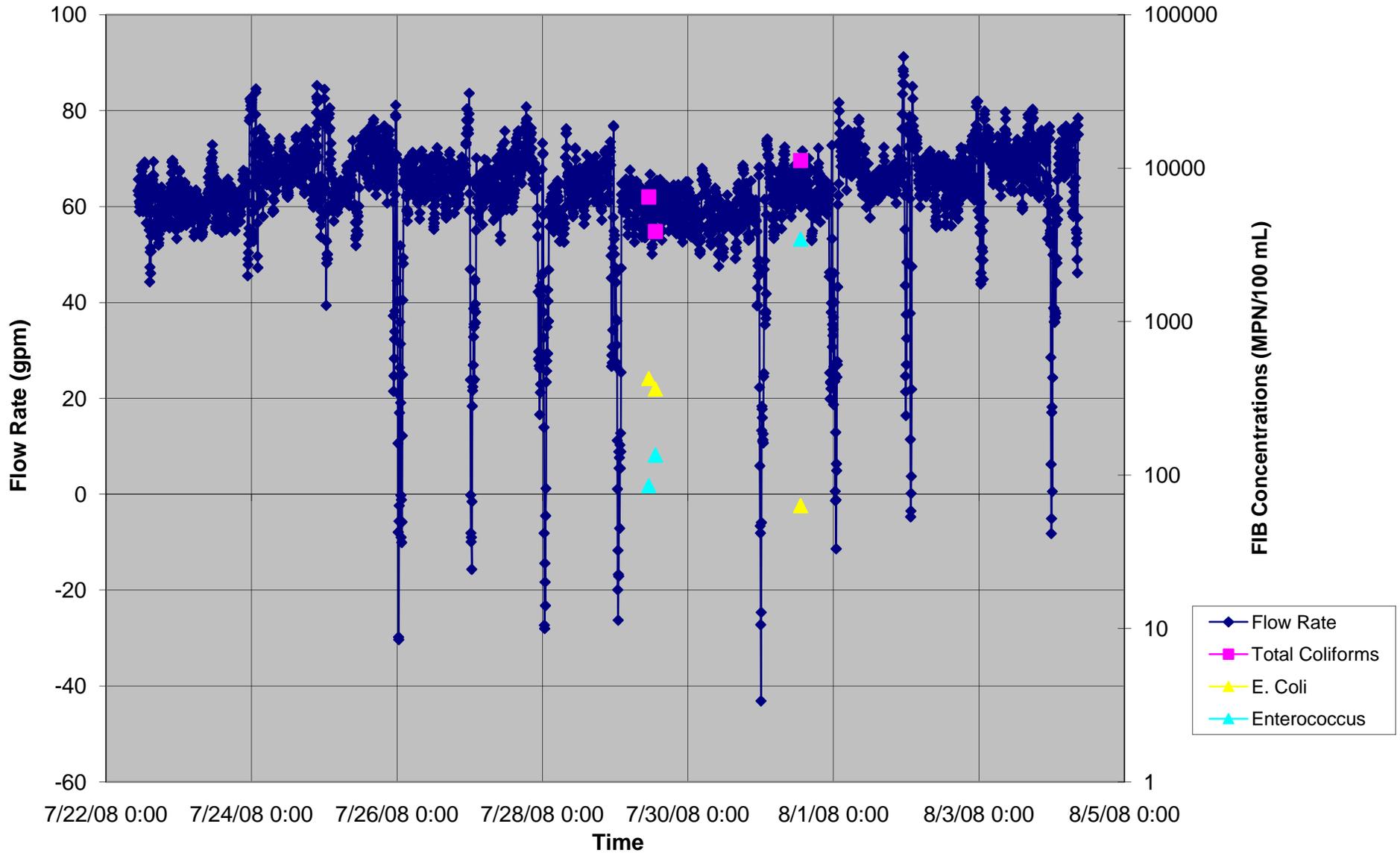
**Geosyntec**  
consultants

Project No. LA0178	March 2009	<b>Figure 5</b>
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\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.

**FIGURE 7**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**  
**Laguna Drain (Sample Location 9)**  
**30-Minute Average Flow Readings and FIB Concentrations, 7/22/08 - 8/4/08**





A) View looking downstream of groundwater seepage in cracks in the Gutierrez Stormdrain Line at monitoring location 11



B) Drop Inlet wall at southern corner of Ortega Park with groundwater trickling into crack in the inlet wall.

**FIGURE 8. GROUNDWATER SEEPING TO STORM DRAIN NETWORK.**

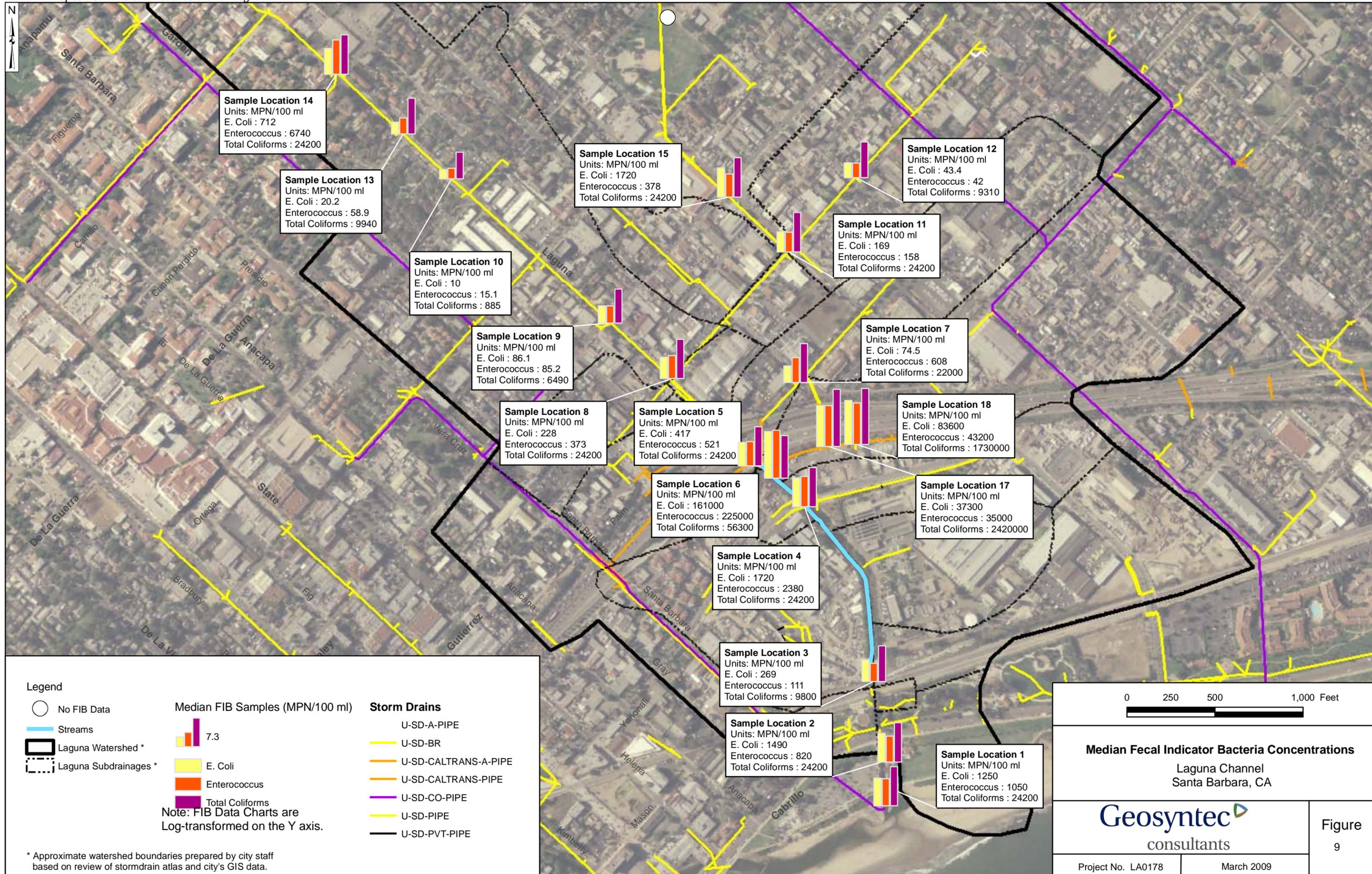
#### 2.3.2.4 MICROBIAL CONCENTRATIONS AND LOADS

Storm drain samples and Laguna Channel samples frequently exceeded AB411 standards. At 13 of 19 sites, the median fecal indicator bacteria (FIB) levels of the samples collected exceeded at least one of the single sample maximums (Figure 9), with total coliform being the most frequent indicator in exceedance. Longitudinal concentration patterns, i.e. from upstream storm drain sites to downstream, open channel sites, were observed in the Laguna Watershed for *E. coli* and enterococcus. Total coliform results were too often above quantification thresholds to observe longitudinal patterns. In the Gutierrez drain, FIB mostly originated from the Salsipuedes drain (Location 15), and rather than the upstream reaches of the Gutierrez drain (Location 12). In the Laguna drain, the FIB concentrations usually decreased from the Laguna drain at Canon Perdido Street (Location 14) to the Laguna drain at De La

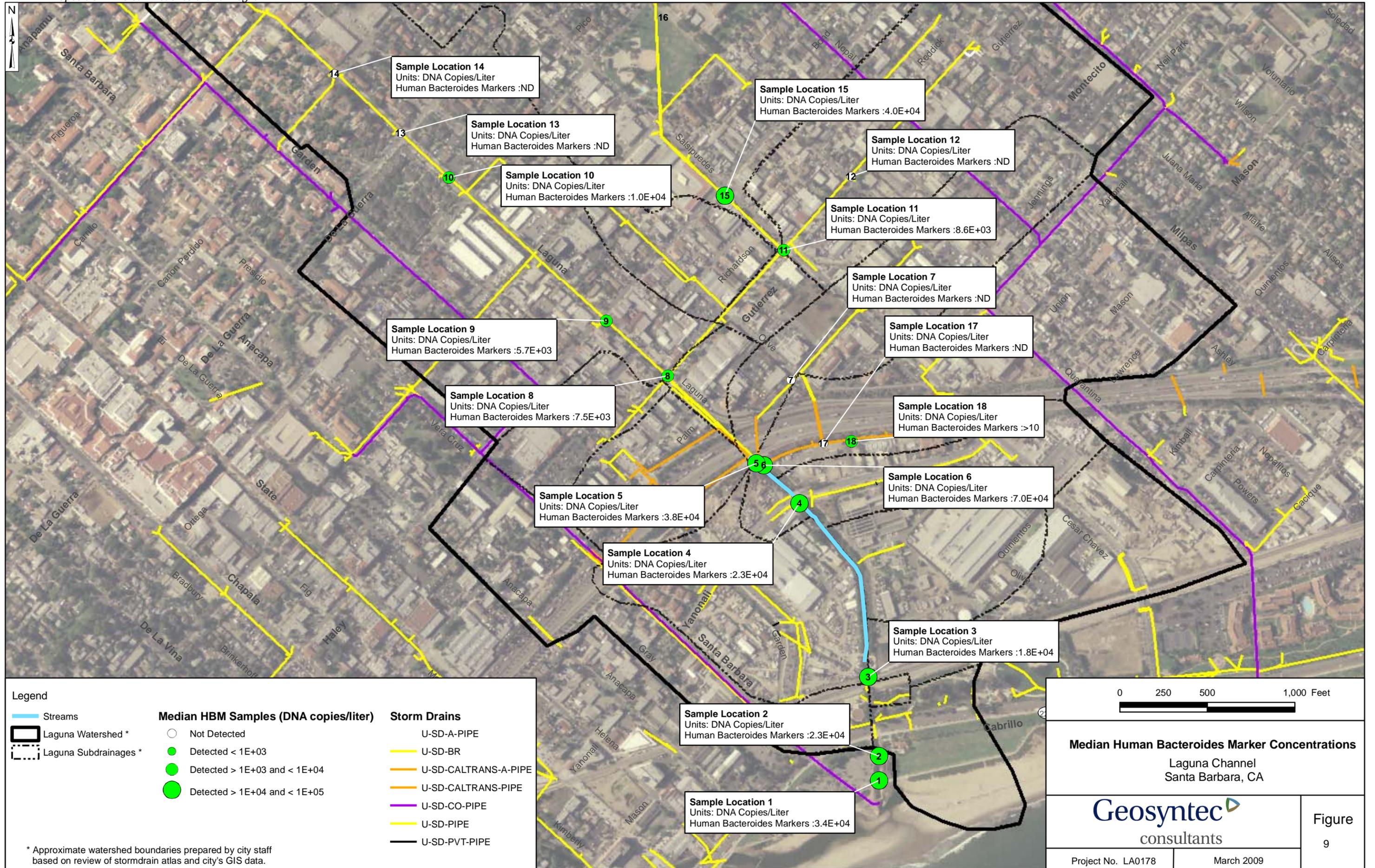
Guerra Street (Location 13), but remained fairly constant downstream of this location (see Appendix A, section 3.2 for data). In the open Laguna Channel, FIB concentrations generally increased from the Laguna drain under the Highway 101 on-ramp (Location 5) to downstream of Yanonali Bridge (Location 4), decrease from Location 4 to upstream of the railroad tracks (Location 3), but increase again from Location 3 to the location in the channel before entering the pump station (Location 2), suggesting FIB sources are located between Locations 5 and 4, and between Locations 3 and 2. Very high FIB concentrations consistently originate from the City Annex Yard (Locations 6, 17, 18), which was a likely significant source of FIB to the channel between Locations 5 and 4 (this source of fecal indicator bacteria has since been addressed by the City). See Appendix A, Section 3.2 for more detailed results

The human-specific *Bacteroides* marker (HBM) was consistently detected at Locations 2, 4, 5, 8, 11, and 15 (Figure 10). HBM were sporadically detected in Laguna Channel Locations 1 and 3 and drains 6, 9, 10 and 18. In some cases, HBM targets were classified as “detectable but non-quantifiable.” For example, at the sampling location upstream of the railroad tracks (Location 3), HBM were detected on three occasions but were only once classified as quantifiable. See Appendix A, section 3.2 for data and additional details. No HBM was detected in storm drain Locations 7, 12, 13, 14 and 17. When detected, HBM usually was present in the order of  $10^4$  copies/L. The highest concentrations, exceeding  $10^4$  copies/L, were observed at the Laguna drain under the Highway 101 on-ramp (Location 5). Concentrations measured in the Laguna Drain at Haley Street (Location 9) were consistently measured to be less than  $10^4$  copies/L. The median result in the Laguna Channel prior to entering the storm drain is  $2.3 \times 10^4$  copies/L. The data indicate that HBM originate from 2 drain sections. The highest concentrations of HBM originate from the Salsipuedes Street drain (Location 15), which flows to the Gutierrez Street drain (Location 11) and then to the Laguna-Gutierrez union (Location 8). Some HBM also appear to originate from a Laguna drain lateral (Location 10), which flows to the Laguna drain (Location 9) and then to the Laguna-Gutierrez union (Location 8). Downstream of the Laguna-Gutierrez union, HBM sources were identified between Locations 8 and 5, and between Locations 3 and 2. These results would suggest that the potential human sources of FIB to the watershed are likely originating in storm drain flows along the Gutierrez and Laguna lines.

Based on FIB loading calculations, which are subject to a significant degree of uncertainty, there is a larger mass flux of FIB per day leaving the open Laguna Channel than entering (Table 1). Although FIB data is not always meaningful for drawing conclusions due to its significant variability, the FIB calculations associated with this project may indicate that the FIB accumulate or have some equilibrium range within the channel and do not come primarily from an up-gradient source. By contrast, based on the analysis of human-specific *Bacteroides* DNA, it appears that there is significant input of human fecal waste into some Laguna storm drains and into Laguna Channel. Obvious spatial correlation between measured FIB and HBM concentrations could not be identified; similar trends between indicator species and HBM concentrations were also not observed. See Appendix A (Section 3.3 and Table 4) for additional details.



\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.



**Sample Location 14**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :ND

**Sample Location 13**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :ND

**Sample Location 10**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :1.0E+04

**Sample Location 15**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :4.0E+04

**Sample Location 12**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :ND

**Sample Location 11**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :8.6E+03

**Sample Location 7**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :ND

**Sample Location 17**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :ND

**Sample Location 18**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :>10

**Sample Location 6**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :7.0E+04

**Sample Location 9**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :5.7E+03

**Sample Location 8**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :7.5E+03

**Sample Location 5**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :3.8E+04

**Sample Location 4**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :2.3E+04

**Sample Location 3**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :1.8E+04

**Sample Location 2**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :2.3E+04

**Sample Location 1**  
Units: DNA Copies/Liter  
Human Bacteroides Markers :3.4E+04

\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.

### 2.3.3 WATER QUALITY IMPROVEMENT PROJECT FEASIBILITY ANALYSIS

Based on the flow observations, measured bacteria concentrations, load calculations, current industry practices, and publically-available studies and reports on FIB treatment and water quality improvement projects, Geosyntec evaluated various methods to reduce the bacteria concentrations that exceed public health standards for recreational waters. A multi-tiered approach was recommended for the treatment of dry weather flows in order to meet the project goal of ultimately reducing the risk of human illness at East Beach by reducing the loading of human fecal contamination into the Mission Lagoon, to which the Laguna Channel discharges.

First, prior to the construction of any treatment facility, source control measures should be implemented where appropriate and feasible.

Recommended source controls included:

- Continued public outreach;
- Investigation into and repairs of potential sewer leaks;
- Restoration of the low flow diversion from the Annex Yard to the El Estero Wastewater Treatment Plant;
- Investigation into and possible diversion of cross connection to the Laguna storm drain line upstream of the Laguna Street and Ortega Street intersection;
- Supplemental sampling within the lower Laguna Channel and the pond at Chase Palm Park.

If elevated bacteria levels and/or human marker signal persist despite source control implementation, structural treatment should then be implemented as necessary. Structural treatment design flow rates should be based on the state of the watershed post-implementation of source controls (for instance, the implementation of source controls could result in reduced upstream bacteria levels, and therefore result in the requirement of a lesser degree of treatment than originally anticipated). These include a combination of filtration, ultraviolet (UV) treatment, ozone treatment, subsurface flow wetlands, and other source control and structural treatment Best Management Practices (BMPs) such as routine storm drain and channel maintenance to remove vegetation and other debris, public education, and the diversion of some flows in the upper watershed to the sewer system. Complete diversion at the outlet of the Laguna Channel was determined to be infeasible due to flow rates beyond the capacity of the existing sanitary sewer. Treatment alternatives were considered with the following criteria, as appropriate:

- Predicted reduction in contaminant load, including treatment capacity.
- Predicted reduction in indicator bacteria levels and beach warnings at East Beach.
- Costs, including design, construction, and lifetime maintenance.
- Resource consumption, including power and potable water.
- Operations and maintenance requirement.
- Loss of clean groundwater, in analysis of diversions.
- Real property ownership.

- Proximity of storm drains to existing utilities, e.g., to sewer lines for diversions.
- Consideration of environmental review.
- Consider the role of mixing with Mission Lagoon, the potential for mixing and regrowth, and the residence time in the lagoon in dry weather.

In order to select the most appropriate and effective treatment alternative, a decision matrix was used to rank capital cost, operations and maintenance cost, public perception, land requirements, and project safety for each treatment option (Table 3). See Appendix A, Section 4 for details. Ozone and UV disinfection were determined to be the most in line with the project design goals and site constraints. These conclusions are consistent with the 2003 Laguna Channel study performed by Enartec with contributions from Geosyntec Consultants. It is recommended that the treatment of dry weather flows be implemented using a multi-tiered approach. It should also be noted that the disinfected runoff from Laguna Channel is not expected to entirely retain its quality upon comingling with the untreated waters of Mission Creek due to mixing, bird contributions, and regrowth in the lagoon.

#### 2.3.4 IDENTIFIED WATER QUALITY IMPROVEMENT PROJECT: RELINING TARGET SANITARY SEWER PIPES

The results in the Watershed Study (Appendix A) showed that there were consistent, positive results for human waste markers in several storm drain locations in the Laguna Channel Watershed, justifying end-of-pipe treatment for Laguna Channel. The cost-benefit analysis of treatment alternatives recommended that, in addition to suggested source control efforts, a UV disinfection facility appeared to be a viable, and necessary, step for protecting the health of swimmers at East Beach. One of the suggested source control measures was sewer leak investigation and repair. At the time of the Watershed Study's completion, the City (and other communities) did not possess tools to identify leaks from sanitary sewer lines into storm drains. Extensive sewer pipe repair and rehab without confirmed, identified leaks was not a viable option at this point in the Project.

The City tried to pursue rapid permitting, design, and construction of a UV disinfection facility at the Laguna Pump Station. However, permitting complexities prevented the City from continuing down the rapid construction route. Several projects in the area were already in motion, and the City's Environmental Analyst concluded that issuing a Notice of Exemption for the UV project would be an example of "piecemealing" a project that, in sum, may have had significant environmental impacts. The projects, including the potential UV disinfection project, were combined and expanded into the Mission Lagoon Restoration and Water Quality Improvement Project. This project is currently in the early stages of design and permitting.

Simultaneous to the effort to install a UV disinfection project, the City completed the Proposition 50 CBI-funded Source Tracking Protocol Development Project, which provided methods to search for leaking sewer lines and to identify high-risk areas for sanitary sewer leaking, in order to target them for repair and replacement. During a thorough investigation, one leak in the Laguna Watershed was identified and repaired, but additional leaks are thought to exist based on patterns of human-waste marker results described above.

**Table 3. Treatment Alternative Decision Matrix**

Treatment Alternatives	Criteria (weight)						Total	Discussion
	Safety (1)	Public Perception (2)	Land Required (1)	Ecological Impacts (1)	Capital Cost (1)	O&M (2)		
<b>Chlorine</b>	0	1	4	1	4	4	<b>19</b>	Chlorine is a toxic compound that leaves residuals that can threaten aquatic life downstream. Treated water would appear cleaner; however, due to residuals and the risks of on-site chemical storage, negative public perception is expected. Compared to the other alternatives, the land requirements, capital cost, and O&M are low. Capital cost includes the treatment tank and initial chlorine supply. O&M consists of regular cleaning of the system and chlorine re-supply.
<b>UV</b>	5	4	4	5	5	4	<b>35</b>	UV is a safer option with no known downstream ecological affects. Public perception would be positive because no chemicals are involved. Because UV does not remove odors or make water visibly clearer, water may appear untreated to the public. The system may be placed in the existing pump house and does not require additional land. The capital cost is low compared to other alternatives. O&M includes regular inspection, cleaning, bulb replacement, and energy supply.
<b>Ozone</b>	4	4	4	4	3	3	<b>28</b>	Ozone involves on-site production of an unstable chemical. Depending on the influent's chemical composition, ozone treatment could produce brominated disinfection by-products. Ozone does not produce disinfection residuals and dissipates when exposed to oxygen. Public perception would be good because treated water would be visibly cleaner; however, because ozone is a chemical, the public may wrongfully assume it is not good for the environment. This facility may be placed in the existing pump house. The capital cost is greater than UV and O&M includes inspection, cleaning, and energy supply.
<b>Peracetic Acid</b>	2	0	4	4	4	4	<b>22</b>	This treatment type is less safe than UV or ozone because of the compound's explosive nature. For this reason and the lack of implementation examples, the public may have a negative response. The footprint, capital cost, and O&M would be similar to that of a chlorine facility due to its comparable configuration.
<b>Subsurface (SSF) Wetlands</b>	5	3	0	5	1	5	<b>27</b>	SSF wetlands are very safe and would likely not impact downstream ecology. Public perception would be good due to its aesthetic character and use of natural processes. However, because a large wetland is necessary and installation includes construction on the beach, the public may dislike disturbances. Capital cost would be large due to land requirement and additional permits. The O&M is less frequent, but potentially more labor intensive.
<b>Infiltration Trench</b>	5	2	0	5	1	5	<b>25</b>	An infiltration trench is safe because it involves natural treatment and is completely underground. Negative public perception may arise because of misconception that the water is secretly discharged instead of treated through natural filtration. The facility would have no odor problems and would not affect the beach's aesthetics or ecology. The footprint and capital cost of this system is undesirable because it requires a large amount of costly land. Despite large initial cost, O&M would require no energy and would need maintenance less frequently than the other mechanical treatment options.

Using the GIS-based techniques described in the Final Report for the Source Tracking Protocol Development Project, the City now seeks to repair two miles of high-risk sanitary pipes in the Laguna Watershed. The City will complete this construction and conduct additional human-waste marker testing prior to installing end-of-pipe UV treatment as part of the Mission Lagoon Restoration and Water Quality Improvement Project. If sewer repairs eliminate the signal of human waste markers discharging from Laguna Channel, the UV disinfection facility will not be included in the Mission Lagoon Restoration and Water Quality Improvement Project. Therefore, the City has identified the sanitary sewer pipe relining project as the preferred water quality improvement project for the Laguna Channel Watershed. Because source control efforts were not included in the decision matrix, a brief analysis of the sewer pipe relining project is provided here:

1. *Predicted reduction in contaminant load, including treatment capacity:* The entire load of consistent sources of human waste are expected to be eliminated. This does not include sporadic events, such as RV dumping.
2. *Predicted reduction in indicator bacteria levels and beach warnings at East Beach:* Based on the analysis conducted in the Laguna Watershed Study for treatment alternatives, fecal indicator bacteria levels in ocean samples, and associated beach warnings are not expected to be reduced. Pathogen levels will certainly be reduced, protecting swimmers at East Beach; however, fecal indicator bacteria regrowth in storm drains will obscure any reductions from sewer pipe relining.
3. *Costs, including design, construction, and lifetime maintenance:* Design and construction is expected to cost approximately \$488,000.
4. *Resource consumption, including power and potable water:* None.
5. *Operations and maintenance requirement:* Will fit within Wastewater Division normal sanitary sewer maintenance program.
6. *Loss of clean groundwater, in analysis of diversions:* N/A
7. *Real property ownership:* N/A
8. *Proximity of storm drains to existing utilities, e.g., to sewer lines for diversions:* N/A
9. *Consideration of environmental review:* Notice of Exemption.
10. *Consider the role of mixing with Mission Lagoon, the potential for mixing and regrowth, and the residence time in the lagoon in dry weather:* Not considered separate from treatment alternatives reviewed in Laguna Watershed Study (Appendix A).

Given the enormous benefit of true source control, i.e., the permanent elimination of pathogens from the storm drain system and beaches downstream, repair of potentially leaking sewer lines is the strongest contender for water quality improvement in the Laguna Watershed.

The segments of pipe expected to be relined are shown in Figure 11 (this map is tentative pending further survey and GIS work). The project is estimated to cost approximately \$500,000 and has received a CEQA Notice of Exemption.



## 2.4 SCOPE OF WORK/ACTIVITIES COMPLETED

The project was completed according to the scope described in the grant agreement, with activities completed as follows. Discussion of each item, if necessary, is provided in *italic text*. Note that the heading numbers in this section match those in the grant agreement.

### 1.1 Dry Weather Hydrology

1.1.1 Drainage sub-areas and associated storm drain systems were mapped based on City topographic maps and Storm Drain Atlas (both are in the Geographic Information System [GIS]). *This task was complicated by the fact that two storm drain systems lie within the watershed, often running in parallel, yet they discharge to different beach areas.*

1.1.2 The accuracy of the City's storm drain atlas was confirmed with field reconnaissance.

1.1.3 Additional field reconnaissance was used to identify sumps, groundwater pumps, and other sources that provide dry weather flow to storm drains.

1.1.4 Multiple flow measurements were taken at key storm drain outlets and nodes in order to estimate magnitude and variability of flow rates during dry weather.

1.1.5 A storm drain outlet located below the tide gate was investigated as a possible source of microbial contamination.

1.1.6 The role of Laguna and Mission lagoons (at times the lagoons are joined) was addressed. The proportion of flow to East Beach that comes from Laguna Channel versus Mission Creek was estimated, as was the residence time in Laguna lagoon. This task does not refer to a detailed model but to a qualitative treatment that will inform the feasibility analysis (see 1.3 below). *See Appendix A, Laguna Watershed Study for comparison of flow rates and conceptual model.*

### 1.2 Contamination Loading (Sources, Routes, and Loads)

1.2.1 Existing City data on indicator bacteria and human waste Deoxyribonucleic acid (DNA) markers in the Laguna watershed were reviewed. *See Appendix A, Laguna Watershed Study.*

1.2.2 Data gaps were identified in water quality and microbial source tracking data. *See Appendix A, Laguna Watershed Study.*

1.2.3 A sampling plan was developed and implemented for identifying pollution sources, using indicator bacteria tests and DNA methods (see 2 below). The sampling plan took into account variability in flow rates.

1.2.4 Based on the sampling results obtained pursuant to 1.2.3, the flux of microbial contamination entering Laguna Channel and lagoon was quantified. Estimates were made of the contaminant loads from key storm drain outlets and nodes. See Appendix A, *Laguna Watershed Study*.

### 1.3 Feasibility Analysis

1.3.1 A list of water quality improvement projects that will succeed in reducing microbial contamination and beach warnings at East Beach was developed. The list includes consideration of a disinfection facility that would be installed at the outlet of Laguna Channel near existing pump station. Also, diversions to sanitary sewer where appropriate were considered.

1.3.2 The list of improvement projects was analyzed according to the following factors, as appropriate:

- Predicted reduction in contaminant load, including treatment capacity.
- Predicted reduction in indicator bacteria levels and beach warnings at East Beach.
- Costs, including design, construction, and lifetime maintenance.
- Resource consumption, including power and potable water.
- Operations and maintenance requirement.
- Loss of clean groundwater, in analysis of diversions.
- Real property ownership.
- Proximity of storm drains to existing utilities, e.g., to sewer lines for diversions.
- Consideration of environmental review.
- Consideration of the role of mixing with Mission Lagoon, the potential for mixing and regrowth, and the residence time in the lagoon in dry weather.

1.3.3 Based on the analysis, an implementation and concept plan was developed for reducing microbial contamination at Laguna Channel and East Beach.

2. DNA Microbial Source Sampling and Analysis. Microbial source tracking was used to provide information about the presence of human waste in the channel and answered questions about locations of “hot spots” and potential sources. Sampling took place in conjunction with the watershed study.

2.1 Samples were collected from the lower Laguna Channel at several locations to confirm the presence of human fecal DNA markers.

2.2 Samples were processed for fecal indicator bacteria (using IDEXX methods) and *Bacteroides* (using Quantitative Polymerase Chain Reaction [qPCR] methods) according to the QAPP.

2.3 Based on results from the lower channel, additional samples were collected and processed in the storm drain system to assess where the contamination originates. Information gathered during the watershed study guided where sampling took place upstream in the storm drain system.

### 3. Preliminary Design Plans

3.1 Construction design drawings were completed to 35 percent detail for the identified water quality improvement project. *The identified water quality improvement project is the relining of two miles of suspected leaking sanitary sewer pipes in Laguna Watershed.*

3.2 An assessment of operational requirements for the identified water quality improvement project was completed. *The operational requirements of the sewer pipes, once the project is constructed, will not change and will fit within the annual operational and maintenance plan of the Wastewater Division of the City of Santa Barbara.*

3.3 A 35 percent construction cost estimate was developed.

3.4 A detailed Project description for environmental review was developed.

3.5 Design drawings (35 percent) and construction cost estimate (35 percent) were submitted to the Grant Manager.

4. CEQA and Permitting For Identified Project.

4.1 CEQA and permitting information was submitted to the Grant Manager. *A Notice of Exemption was provided.*

## 2.5 PARTNERS INVOLVED

The following partners were involved with the Project:

1. Geosyntec Consulting (Brandon Steets, P.E.): Geosyntec assisted in field reconnaissance, completed all of the dry-weather hydrology, and the Laguna Watershed Study and Water Quality Improvement Feasibility Analysis.
2. University of California, Santa Barbara (Dr. Patricia Holden): Dr. Holden and her laboratory group assisted in field reconnaissance, completed all water sampling and laboratory analysis, and conducted data analysis for microbial parameters.

## 3 LOCATIONS OF PROJECT MONITORING ACTIVITIES

All field activities in the research project described here are considered “Monitoring Activities.” There were no Management Practices included in the grant agreement. Therefore, this section describes locations of all Project Monitoring Activities completed. Project sites are numbered and shown in Figure 3. Coordinates of each location are provided in Table 5. Activities associated with each site are coded in Figure 3 and listed in Table 5. Details about locations can be found in Appendix A, Section 2.

**TABLE 4. PROJECT LOCATIONS AND SITE DESCRIPTIONS**

Site No.	Longitude	Latitude	City of SB Station ID	Description
1	34.413717	-119.685524	LC Pumplag	Laguna Channel, Mission Lagoon overflow
2	34.414182	-119.685595	LC Pump	Laguna Channel, just upstream of pump station
3	34.415455	-119.685829	LC Railroa	Laguna Channel, upstream of railroad tracks
4	34.418230	-119.687125	LC Yanonal	Laguna Channel, Yanonali bridge
5	34.419017	-119.688181	LC Hwy101	Laguna Drain, under US101 onramp
6	34.418821	-119.687824	HW-H09-03	Drain from annex yard, outfall
7	34.420126	-119.687331	MH-H09-06	Drain Montecito @ Olive
8	34.420183	-119.689674	CB-H09-45	Drain Laguna @ Gutierrez
9	34.421038	-119.690838	DI-H09-42	Drain Laguna @ Haley
10	34.423280	-119.693931	MH-G08-06	Laguna drain lateral
11	34.422200	-119.687529	CB-H08-03	Drain Gutierrez @ Salsipuedes
12	34.423360	-119.686271	CB-H08-29	Drain Gutierrez @ Quarantina
13	34.423944	-119.694817	MH-G08-10	Drain Laguna @ De La Guerra
14	34.424871	-119.696081	MH-G08-04	Drain Laguna @ Canon Perdido
15	34.423129	-119.688778	MH-H08-11	Drain Salsipuedes @ Haley
17	34.419176	-119.686732	DI-H09-08	Drain upstream Ortega Park
18	34.419243	-119.686189	DI-H09-10	Drain from annex yard, middle
19	34.424195	-119.689965	CB-H08-13	Drain E. Cota St. & Salsipuedes St. (sidewalk)
20	34.428051	-119.690388	CB-H07-30	Drain De La Guerra St. & Nopal St. (sidewalk)
21	34.428267	-119.687887	MH-H07-03	Drain Milpas St. & Ortega St.
22	34.415195	-119.678357	AV-J10-01	Beach drain discharge (across Fess Parker's resort)
23	34.417827	-119.678877	MH-J09-06	Drain Quarantina St. & Cacique St.
24	34.422347	-119.682677	MH-J08-08	Drain Yanonali St. & Nopal St.
25	34.424519	-119.680352	MH-J08-06	Drain Yanonali St. & Alisos St.
26	34.426056	-119.687611	MH-H08-16	Drain Nopal St. & E. Cota St.

**TABLE 5. PROJECT MONITORING LOCATIONS AND ACTIVITIES**

Site No.	FIB (IDEXX)	Microbial Source Tracking	Field (PH, DO, T, Conductivity)	Flow
1	X	X	X	X
2	X	X	X	X
3	X	X	X	X
4	X	X	X	
5	X	X	X	X
6	X	X	X	
7	X	X	X	
8	X	X	X	
9	X	X	X	
10	X	X	X	
11	X	X	X	X
12	X	X	X	
13	X	X	X	
14	X	X	X	
15	X	X	X	
17	X	X	X	X
18	X	X	X	
19	X	X	X	
20	X	X	X	
21	X	X	X	
22	X	X	X	
23	X	X	X	
24	X	X	X	
25	X	X	X	
26	X	X	X	

## 4 PROJECT PERFORMANCE

### 4.1 PROJECT ASSESSMENT AND EVALUATION PLAN

A Project Assessment and Evaluation Plan (PAEP) was developed at the onset of the Project. The PAEP is summarized in Table 6.

**TABLE 6. PROJECT ASSESSMENT AND EVALUATION PLAN TABLE.**

<b>Project Goals</b>	<b>Baseline Measurements and Information</b>	<b>Output Indicators</b>	<b>Outcome Indicators</b>	<b>Measurement Tools and Methods</b>	<b>Targets</b>
1. Understand the sources of water and loads of pollution (primarily microbial contamination) entering the Laguna Channel.	<p>1. Indicator bacteria data has been collected by the County of Santa Barbara at East Beach since 1998.</p> <p>2. The City has monitored two locations on Laguna Channel since 2001 for indicator bacteria.</p> <p>3. DNA source tracking has confirmed the presence of human waste in creek samples, storm drain samples, and the surf zone.</p> <p>4. The City has a detailed Storm Drain Atlas last updated in 2000.</p>	<p>1. Completeness and coverage of maps of storm drain network and hydrology.</p> <p>2. Completeness and coverage of flow rate (magnitude and variability) and direction of dry weather inputs to Laguna Channel Drainage System.</p> <p>3. Completeness and coverage of the loads of pollution (primarily microbial contamination) entering the Laguna Channel.</p>	<p>1. Identification of pollution hotspots and allocation to contamination to certain storm drain segments.</p> <p>2. Water quality and flow data that will aid in the choosing of the appropriate treatment options.</p>	<p>1. City of Santa Barbara. 2000. Storm Drain Atlas</p> <p>2. DNA based microbial source tracking methods.</p> <p>3. Flow gauges installed to record data around the clock.</p>	Report showing the major sources and direction of flow through the storm drain network. Will include fecal indicator bacteria and DNA marker loads from major sources of flow.
2. Understand the most appropriate project(s) for reducing the flux of harmful microorganisms entering the Laguna Lagoon and East Beach in dry weather.	<p>1. Bacterial Reduction Study (2002)</p> <p>2. Laguna Channel Disinfection System Preliminary Design Report (2003) County of S.B.</p>	<p>1. A list of potential treatment projects.</p> <p>2. Cost Benefit Analysis of each treatment option.</p>	<p>1. Report outlining the different treatment options for the watershed.</p> <p>2. Identification of the most appropriate project(s) for reducing the flux of harmful microorganisms entering the Laguna Lagoon and East Beach in dry weather.</p>	1. Current literature on water treatment technology.	Report showing the different treatment options and preferred options for the Laguna Watershed. Will include a cost/benefit analysis for each option.

## 4.2 RESULTS OF PAEP

### 4.2.1 PROJECT GOAL 1: WATER SOURCES AND POLLUTANT LOADS

#### 4.2.1.1 UNDERSTAND THE SOURCES OF WATER AND LOADS OF POLLUTION (PRIMARILY MICROBIAL CONTAMINATION) ENTERING THE LAGUNA CHANNEL.

This goal was fully met by reaching the desired outcome, i.e., a report (Laguna Watershed Study) showing the major sources and direction of flow through the storm drain network, along with associated load of microbial contaminants (fecal indicator bacteria and DNA-based human waste markers). The outcome indicators were both met. First, the City and its partners were able to identify pollution hotspots and allocate contamination to certain storm drain segments. Second, water quality and flow data aided in the choosing of the appropriate water quality improvement project. Specifically, the diffuse and consistent pattern of contamination with human waste markers led to the conclusion that leaking sanitary sewer pipes likely contribute to the contamination problem. Therefore, pipes will be repaired prior to the installation of a resource-intensive capital project relying on treatment of Laguna Channel flow.

### 4.2.2 PROJECT GOAL 2: IDENTIFY APPROPRIATE PROJECT(S)

#### 4.2.2.1 UNDERSTAND THE MOST APPROPRIATE PROJECT(S) FOR REDUCING THE FLUX OF HARMFUL MICROORGANISMS ENTERING THE LAGUNA LAGOON AND EAST BEACH IN DRY WEATHER.

This goal was fully met by reaching the desired outcomes. First, a report showing the different treatment options and preferred options for the Laguna Watershed, which includes a cost/benefit analysis for each treatment option, and second, the identification of relining two miles of target sanitary sewer pipes to reduce the flux of harmful microorganisms entering the Laguna Lagoon and East Beach in dry weather. The output indicators were fully reached. A list of potential treatment projects was outlined in the Geosyntec Report and a cost-benefit analysis of each treatment option was provided.

## 5 PUBLIC OUTREACH

Public outreach for the project included updates about the Project in the City's Annual Water Quality Monitoring and Research Report and presentations to the City of Santa Barbara's Citizens Advisory Committee to the Creeks Division.

## 6 PROJECT FUNDING

This Project was supported by the Proposition 50 Clean Beaches Initiative Grant Program and local Measure B matching funds. The project was budgeted accurately with the exception of Professional Services funds required for Project Design and Environmental Review. The Project was under budget in these areas and the \$51,850 was not requested for reimbursement, as shown in Table 7. All grant funds were derived from the Proposition 50 Clean Beaches Initiative Grant Program. The City's match represents 17% of the Project total cost.

**TABLE 7. PROJECT COSTS**

Item	Budgeted Grant Funds	Budgeted Match	Budgeted Project Total	Actual Grant Funds Spent	Actual Match	Actual Project Total
<b>Personnel</b>		\$48,400	\$48,400		\$34,200	\$34,200
<b>Professional Services</b>	\$220,000		\$220,000	\$168,150		\$168,150
<b>TOTAL</b>	<b>\$220,000</b>	<b>\$48,400</b>	<b>\$268,400</b>	<b>\$168,150</b>	<b>\$34,200</b>	<b>\$202,350</b>

## 7 ITEMS SUBMITTED FOR REVIEW

**TABLE 8. ITEMS SUBMITTED FOR REVIEW**

Item	DESCRIPTION
A.	PLANS AND COMPLIANCE REQUIREMENTS
1.	GPS information for Project site and monitoring locations
2.	Project Assessment and Evaluation Plan (PAEP)
	Non Point Source Pollution Reduction Project Follow-up Survey Form
3.	Monitoring Plan (MP)
4.	Quality Assurance Project Plan (QAPP)
5.	Copy of final CEQA/NEPA Documentation
B.	WORK TO BE PERFORMED BY GRANTEE
1.1.1	Drainage sub-area map(s)
1.1.4	Flow measurement data and summary of findings
1.2.4	Estimated contaminant loads and summary of findings
1.3.3	Proposed implementation project(s) and concept plan
2.1.4	DNA Microbial Source Tracking Results
3.5	Project Design (35 percent) and Construction Estimate (35 percent)
4.1	CEQA Documentation for Implementation
F.	REPORTS
1.	Grant Summary Form
2.	Progress Reports by the twentieth (20 <sup>th</sup> ) of the month following the end of the calendar quarter (March, June, September, and December)
3.	Annual Progress Summary
4.	Natural Resource Projects Inventory (NRPI) Project Survey Form
5.	Draft Project Report
6.	Final Project Report

## 8 LESSONS LEARNED

Several lessons were learned during the course of the Project:

- Storm drain maps should be scrutinized very carefully at the project onset in order to determine accurate subdrainage boundaries. If surface flow lines and directions are ambiguous, field work should be conducted in order to determine connections among pipes.
- Dry-weather flow rates can be highly variable in storm drains, varying by 100% over the course of a single day, potentially affecting estimates of pollutant loads.
- Low flow rate measurements may have high precision with a given method and test location, but accuracy is more challenging to achieve.
- Consistent markers for human waste likely signal a sewage leak or illicit connection rather than a sporadic source such as RV dumping. Finding leaks can be extremely challenging and time consuming, but in the long run may save money and energy when compared to capital project installation and maintenance. An overview of tools for finding human waste in storm drains can be found in a report from a different CBI-funded project completed by the City, "Source Tracking Protocol Development." The report, "Tools for Tracking Human Fecal Pollution in Urban Storm Drains, Creeks, and Beaches" can be found on the City's website at:  
*[http://www.santabarbaraca.gov/Resident/Community/Creeks/Reports\\_and\\_Studies.htm](http://www.santabarbaraca.gov/Resident/Community/Creeks/Reports_and_Studies.htm)*

## 9 FOLLOW UP ACTIVITIES

The next steps for the City are to rehabilitate and replace sanitary sewer pipe segments based on data generated by this project and techniques developed in the Source Tracking Protocol Development Project. Two miles of pipes have been identified for relining in calendar year 2013. At the conclusion of the relining project (and following sufficient rains to flush storm drains), the City will conduct additional tests for human waste markers in key storm drains. If results remain positive for human waste markers, the City will consider the role of end-of-pipe treatment for the Laguna Channel.

The City is also conducting more extensive surveillance of potential RV dumping into storm drains than it has conducted previously. An estimate of RVs parking overnight on City streets and in Safe Parking Lots will be compared to the number of RVs that dump waste tanks (at no cost to the RV owner) at the Marborg Waste Facility in Santa Barbara. Previous results using canine scent tracking and a log of enforcement calls do not suggest a large problem with dumping, but the City is taking extra steps to confirm this result.

## **APPENDIX A**

Laguna Watershed Study and Water Quality Improvement Feasibility Analysis

*Prepared for:*

**City of Santa Barbara, Creeks Division**  
630 Garden Street  
PO Box 1009  
Santa Barbara, California

# **Laguna Watershed Study and Water Quality Improvement Feasibility Analysis**

## **Laguna Channel Santa Barbara, California**



*Prepared by:*

**Geosyntec**   
consultants

engineers | scientists | innovators

924 Anacapa Street, Suite 4A  
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*with assistance from:*

*UCSB Bren School of Environmental Science &  
Management; laboratory of Dr. Patricia Holden*

Project Number LA0178

March 2009

## **Laguna Watershed Study and Water Quality Improvement Feasibility Analysis**

This report was prepared by the staff of Geosyntec Consultants under the supervision of those whose signatures appear hereon. The findings or professional opinions were prepared in accordance with generally accepted industry practice. No attempt to verify the accuracy of the data provided by third parties was made. No warranty is expressed or implied.

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## EXECUTIVE SUMMARY

Geosyntec and the University of California Santa Barbara (UCSB) conducted a study for the City of Santa Barbara, Creeks Restoration and Water Quality Improvement Division (City) to evaluate dry weather hydrology, microbiological indicators, bacterial sources and loads, and feasible water quality improvements for the Laguna Channel in Santa Barbara, California. The study was conducted in three parts: 1) dry weather hydrology field reconnaissance and flow monitoring evaluation; 2) identification of sources, routes, and the loading of Fecal Indicator Bacteria (FIB) and DNA-based markers of human waste (human *Bacteroides* marker or HBM) in dry weather creek and storm drain flows; and 3) recommendation of water quality enhancement alternatives, including an assessment of project feasibility, based on findings from 1 and 2. This study was funded by a state Clean Beaches Initiative (CBI) grant with a goal of reducing the risk of human illness at East Beach by reducing the loading of human fecal contamination into the Mission Lagoon, to which the Laguna Channel discharges. The Pacific Ocean at East Beach is a 303(d) listed waterbody for fecal and total coliform.

Based on visual observations, the majority of the sources of dry weather flow were identified as coming from the upper storm drain network. In order to quantify flow, and to calculate a water budget for the Laguna Channel, key locations near the trunk of the Laguna storm drain network were selected for continuous automated flow monitoring throughout the dry season. Additionally, discrete manual flow measurements were collected where the automated flow gage could not feasibly be installed. A FIB sampling plan was developed and coordinated with automated flow monitoring. A data-logging flow meter was installed at three storm drain locations (intersection of Laguna and Haley, City Annex Yard, and intersection of Gutierrez and Salsipuedes) for a duration of no less than two weeks at each location to determine daily or weekly variations in flow rates. A total of 79 samples were collected between July and September of 2008 at locations of flow monitoring and other locations, and analyzed by UCSB for FIB (total coliforms, *E. coli*, and *Enterococcus* spp.) and HBM.

An average flow rate of 140 gallons per minute (gpm) or 0.30 cubic feet per second (cfs) was found to be flowing into the Laguna Channel from storm drains, and 65 gpm or 0.15 cfs, was found to be flowing out of Laguna Channel into the Mission Lagoon at East Beach. Based on FIB loading calculations, which are subject to a significant degree of uncertainty, there is a larger mass flux of FIB per day leaving the Laguna Channel than entering. Although FIB data is not always meaningful for drawing conclusions due to its significant variability, the FIB calculations associated with this project may indicate that the FIB accumulate or have some equilibrium range within the channel and do not come primarily from an up-gradient source. By contrast, based on the analysis of human-specific *Bacteroides* DNA, it appears that there is significant input of human fecal waste into some Laguna storm drains and into Laguna Channel. An

obvious spatial correlation between measured FIB and HBM concentrations could not be identified; similar trends between indicator species and HBM concentrations were also not observed.

Based on the flow observations, measured bacteria concentrations, load calculations, current industry practices, and publically-available studies and reports on FIB treatment and water quality improvement projects, Geosyntec evaluated various methods to reduce the bacteria concentrations that exceed public health standards for recreational waters. These include a combination of filtration, ultraviolet (UV) treatment, ozone treatment, subsurface flow wetlands, and other source control and structural treatment Best Management Practices (BMPs) such as routine storm drain and channel maintenance to remove vegetation and other debris, public education, and the diversion of some flows in the upper watershed to the sewer system. Complete diversion at the outlet of the Laguna Channel (Location 2) was determined to be infeasible due to flow rates beyond the capacity of the existing sanitary sewer. In order to select the most appropriate and effective treatment alternative, a decision matrix was used to rank capital cost, operations and maintenance cost, public perception, land requirements, and project safety for each option. UV and ozone were determined to be the most in line with the project design goals and site constraints. These conclusions are consistent with the 2003 Laguna Channel study performed by Enartec with contributions from Geosyntec Consultants.

It is recommended that the treatment of dry weather flows be implemented using a multi-tiered approach. First, prior to the construction of any treatment facility, source control measures should be implemented where appropriate and feasible. Then, if elevated bacteria levels persist despite source control implementation, structural treatment should then be implemented as necessary. Structural treatment design flow rates should be based on the state of the watershed post-implementation of source controls (for instance, the implementation of source controls could result in reduced upstream bacteria levels, and therefore result in the requirement of a lesser degree of treatment than originally anticipated). It should also be noted that the disinfected runoff from Laguna Channel is not expected to entirely retain its quality upon comingling with the untreated waters of Mission Creek due to mixing, bird contributions, and re-growth in the lagoon. In fact, a mass balance analysis to assess the impact of a 99% (2-log) reduction in the FIB concentrations in the Laguna Channel discharges resulted in a 19-31% reduction in FIB concentrations in the Lagoon. However, such a reduction in FIB applied through the AB411 dry season (April – October) was only predicted to reduce the number of beach postings from 22 to 21 (annually) and from 7 to 6 (dry season only). Therefore, the benefits of the project, much like the City's Mission Creek (SURF) project, would be to address human fecal contamination coming from the Laguna Channel and discharging to the lagoon and the Pacific Ocean, with significant uncertainty associated with the effects of Mission Lagoon.

Although the benefits of the project include reducing the risk of human illness in the Mission Lagoon and at East Beach, there are potential downsides that need to be acknowledged prior to the implementation of these recommendations. One disadvantage is the significant capital cost

of project implementation, combined the long-term cost of energy, operation, and maintenance. Other potential problems include the potentially negative public perception of channel flow disinfection and the unknown ecological impacts to the lagoon after discharging disinfected water.

## 1.0 INTRODUCTION

### 1.1 Study Purpose

The City of Santa Barbara Creeks Restoration and Water Quality Improvement Division (City) retained Geosyntec Consultants (Geosyntec) to monitor dry weather flows in the Laguna Channel, a major flood control channel that drains the eastern portion of downtown Santa Barbara. This project, funded by a state Clean Beaches Initiative (CBI) grant, is in support of the City's efforts to address concerns related to water quality and bacterial loading from dry weather flows in the Laguna Creek watershed during the dry season. The term 'dry weather' is defined as having no measurable rain events in the prior 72 hours. The City previously identified ozone disinfection at the outlet of the Laguna Channel as an appropriate option for reducing pathogen concentrations in dry weather runoff that currently discharges to the Pacific Ocean at East Beach (Enartec, 2003). Prior to moving forward with such a project, the City required an investigation to better characterize the dry weather flows, estimate bacteria and human fecal sources and loading rates, and identify potential source control implementations that could reduce demand on a proposed treatment system. Additionally, the City is considering alternative structural treatment processes to ozone, and has requested a feasibility study to evaluate different treatment options.

The ultimate goal of the study is to reduce the risk of human illness at East Beach (the Pacific Ocean at East Beach is a 303(d) listed impaired waterbody) by reducing the loading of human fecal contamination into the Mission Lagoon, to which the Laguna Channel. This study will focus on improving the quality of water being discharged to East Beach and the Pacific Ocean to levels that are below State water quality standards for recreational use and bathing<sup>1</sup>. It is important to understand the sources of dry weather runoff, the sources of fecal contamination in the runoff, and the runoff flow rates and flow rate variability to effectively achieve this goal. In addition, an analysis of Fecal Indicator Bacteria (FIB) present in the channel and analysis of human waste genetic markers (although not well correlated) was conducted by the University of California, Santa Barbara (UCSB) to provide additional insight as to human fecal sources and loading processes. Dry weather hydrology monitoring and evaluation was conducted, and assisted in the identification of appropriate dry weather sampling locations at which to directly enumerate human fecal indicator levels in the channel.

This project includes three fundamental components: (1) characterization of dry weather hydrology through visual observation and automated gauging of storm drain flows; (2) identification of sources, routes, and the loading of FIB contamination in dry weather runoff;

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<sup>1</sup> State of California Department of Health Services for ocean beach body-contact standard as established in Assembly Bill 411 for fecal indicator bacteria.

and, (3) recommendation of water quality enhancement alternatives, including an assessment of project feasibility, based on findings from (1) and (2).

## **1.2 Study Location and Description**

The Laguna Channel watershed encompasses approximately 630 acres, including much of eastern downtown Santa Barbara (**Figure 1**). Runoff from the area is conveyed via storm drain into the Laguna Channel at a location east of the Garden Street on-ramp to the southbound Highway 101 and runs towards the discharge point onto East Beach near the intersection of Garden Street and Cabrillo Boulevard. Because the channel ultimately discharges to an elevation that is below mean sea level, water must be pumped from the channel at the base of the watershed to a higher elevation onto the beach. At this discharge point, a lagoon forms which, at times, contains FIB in concentrations that exceed AB411 standards for marine recreational use and contribute to FIB exceedance (and hence swimmer warning postings) in the Pacific Ocean at East Beach near the Mission Lagoon. Between 2001 and 2007, an average of 22 beach warnings were posted per year (7 during the dry seasons). In the past, East Beach has generally received dry weather grades of D's and F's according to the Heal the Bay's Beach Report Card program. Similar to the recent University of California, Santa Barbara (USB) human bacterial study of the Laguna Channel, other prior studies have been conducted on the Mission Creek and Arroyo Burro drainages and storm drains to identify bacterial sources and prioritize locations for diversion or disinfection (UCSB, 2008).

The land use distribution within the Laguna Creek watershed is mixed in that the upper portion of the watershed is mainly residential and the imperviousness of the ground surface is moderate (Questa Engineering Corporation, August 2005). The lower portion of the watershed is mixed residential and industrial with the farther downstream portions of the watershed being mostly industrial land use. The imperviousness of the ground surface in these lower areas is high due to paving and reduced open spaces. The general limits of the storm drain network that drain water to the Laguna Channel and the general limits of the Laguna Creek watershed are shown on **Figure 2**. In general the watershed is encompassed within the boundary of Anacapa Street to the west, Micheltorena Street to the north, and Voluntario Street to the east. The topographical profile of the study area is variable, with the surface gradient being fairly shallow in areas near the beach at the lower Laguna Channel portion, and becoming steeper in the northern reaches of the watershed.

Upstream of Highway 101, the majority of the Laguna storm drain system consists of a network of circular-shaped reinforced concrete pipes, ranging in diameter up to approximately 54-inches, and several long sections of single or double rectangular concrete drains. Between the Highway 101 Southbound onramp at Garden Street and downstream to the outlet at East Beach, the Laguna Channel is open and unlined except for a short section at the pump station and tide gate where it is lined with concrete. In the unlined portion of the open channel, there is dense

vegetation along the banks characteristic of a riparian ecosystem. Small fish and crawfish can be found in the water of the open channel, as well as numerous waterfowl and other birds.

## 2.0 STUDY APPROACH

The following sections describe the methodology and techniques used to perform this study. The approach was concisely described in Geosyntec's Scope of Services dated January 11, 2008 (see **Appendix A**), and was adjusted in the field as necessary to achieve the goals of the project. Initially, a review of available literature relevant to the goals of this study was conducted to gain insight regarding similar dry weather studies and feasibility of treatment options and outcomes. Subsequently, the following sections describe the methodology for evaluating dry weather hydrology, selection and use of an automated flow monitoring device, FIB sampling and analytical techniques, and recommendations of feasible water quality enhancement alternatives.

### 2.1 Literature Review

Geosyntec reviewed numerous publications and papers that were relevant to the goals of this study to gain insight regarding similar dry weather studies and feasibility of treatment options and outcomes. The reference papers that were evaluated varied greatly in their scope and magnitude, and therefore there have, in some cases, contradictory results and recommendations regarding treatment of FIB for the specific conditions presented. Below is a summary of key information retrieved during the literature review process. **Appendix B** includes a detailed description of the items reviewed during this study.

The majority of the disinfection treatment projects reviewed are located within Southern California. Many were a part of the state's California Beaches Initiative and, despite their variation in size and cost, chose to implement a UV disinfection system to reduce the amount of bacterial loading and ultimately reduce the number of beach postings. The reason for implementing a UV system was based on the option's nonhazardous nature, the absence of chemicals during treatment, the lack of disinfection byproducts, and its comparable effectiveness to other chemical treatments options such as chlorine and ozone disinfection (Weldon and Hartman, 2006).

Costs differed between projects depending on design flow, the amount of bacterial loads, and ultimately the size of the system itself. Many of these UV treatment projects were designed to treat about 150 (Moonlight Beach and Westside SURF projects) to 170 (Aliso Beach) gallons per minute of flow and ranged from \$1.3 million (Westside SURF project) to \$2 million (Poche Beach CBI project) in total project cost. Annual operation and maintenance costs ranged from \$18,000 (Westside SURF project) to \$250,000 (Poche Beach CBI project).

UV treatment consistently performed well and significantly reduced the bacteria concentrations in the treated effluent. The treatment system installed at Moonlight Beach (Weldon and Hartman, 2006) reduced 99% of total coliform, fecal coliform, and Enterococcus at the facility and their Heal the Bay beach water quality grading went from D's and F's to A's and B's.

Other projects implemented alternative methods of treatment including storm drain to sewer diversion (Haley and Hope Diversion Project) and an infiltration trench (Kure Beach). Upstream diversion systems at storm drains were chosen for locations with high pollutant loading, feasible installation, and consistent flow (City of Santa Barbara, 2007). These projects installed units of various capacities, diverting water from 30 gallons per minute to 300 gallons per minute. An infiltration trench was also installed at Kure Beach in North Carolina (Burchell et. al., 2007). This system has been successful since installation and it has been recorded to reduce the volume of water being discharged into the ocean and decreased the bacterial concentrations by over 97%. This system also has not significantly increased the groundwater table elevation. Such stormwater diversion projects serve as useful examples of potential source control alternatives for the upper Laguna Watershed.

A constant lesson learned in almost all of the treatment projects reviewed is that although treatment is successful at the facility itself, bacteria loads increase downstream of the facility due to regrowth, regeneration, animal inputs into the open channel, and bird droppings along the wrackline. These studies stated that the opportunity for bacteria regrowth and regeneration can be reduced by installing the treatment system further downstream and closer to the discharge site.

The Preliminary Design Report for the Laguna Channel, submitted by Enartec, with contribution from Geosyntec Consultants (2003), was also reviewed in accordance to this study's scope of work (**Appendix A**). An ozone treatment facility was chosen as the most appropriate treatment option for the Laguna Channel. This system was designed to treat a flow of 150 gallons per minute and the system was estimated to cost a total of \$140,000. Ozone was recommended due to its ability to treat other organic contaminants including grease, oil, and pesticides. An ozone treatment system also requires less pretreatment which equals less filter/strainer backwash.

Aside from implemented projects other studies were reviewed to gain valuable information regarding FIB loads, correlations, and monitoring. One study (Noble et. al., 2006) described a successful, multi-tiered approach to FIB monitoring in the Santa Monica Bay. This approach included traditional measurements of FIB, molecular assays developed and conducted for BMP and Enterovirus, and the sequencing the enterovirus to determine the likely types amplified in the assay. This method of monitoring helped not only to indicate loads but also to indicate the source locations. The multi-tiered method is consistent with this current study of the Laguna Channel, where both FIB and genetic human-specific markers are used to prioritize dry and wet weather treatment planning.

Information regarding FIB loads and correlations was also reviewed. One study showed that dry weather concentrations are consistently higher than wet weather concentrations and that dry weather concentrations increase where urban residential commercial land uses contribute to the watershed (URS, 2002). Studies also showed that FIB loads had a correlation to high temperatures (Tiefenthaler et. al., 2008) and that dry weather loads may derive during dry

weather from storm drain piping and channels and the erosion of sediments in urban landscapes (Reeves et. al., 2004).

## **2.2 Dry Weather Hydrology Evaluation**

To evaluate dry weather hydrology of the Laguna Channel watershed, Geosyntec first performed a visual inspection of the lower Laguna Channel followed by a visual inspection of the upper storm drain network. The inspection procedure consisted of walking from downstream to upstream along the channel/storm drain and observing/recording sources of dry weather flow.

The main section of open channel, between the southbound Highway 101 Garden Street onramp and the pump station, was investigated first. If discharges to the channel were observed, the following information was recorded:

- Size and shape of the contributing pipe or conduit;
- Color of the discharge;
- Approximate flow rate (estimated based on visual observations only);
- Maximum depth and velocity of the inflow;
- Presence/degree of odor;
- Presence/degree of turbidity;
- Presence/degree of groundwater seepage (or weeping banks); and
- Presence/degree of algae or organic debris.

Manual flow measurements within the open channel itself were collected using a depth velocity probe where feasible. Observations were recorded on a field data sheet, and photographs and GPS coordinates were taken at significant locations. Completed observation sheets are included in **Appendix C**.

To better identify potential sources of dry weather flow to the Laguna Channel, Geosyntec used the City's Storm Water Atlas (Storm Water Atlas). This map of the existing storm drain network was used to identify key nodes of the network and inspect inlets to identify the drainage areas of each significant outlet to the channel.

The storm drain network upstream of the open channel section of the creek consists of various size pipes that ultimately drain to the main Laguna storm drain along Laguna Street. This 48-inch diameter buried underground reinforced concrete pipe discharges to the open channel directly beneath the southbound Highway 101 Garden Street onramp at Garden Street. To

evaluate the sources of dry weather flow in the storm drain network, Geosyntec inspected the storm drains at each main intersection or branch of the network, where possible, starting at the furthest downstream point (i.e., trunk line along Laguna Street). If there was flow or standing water observed at an intersection, the following visual data (similar to that collected for the downstream channel) was collected:

- Size and shape of storm drain;
- Approximate flow rate (using visual estimation);
- Approximate depth at the center of flow;
- Presence/degree of odor;
- Presence/degree of algae or other organic debris;
- Presence of possible irrigation or groundwater infiltration occurring nearby; and
- Presence/degree of groundwater seepage from cracks in the drain.

A storm drain section or branch with observed flow was followed upstream to the next accessible manhole or inlet and again inspected for water flow. Flow was traced upstream until no flow was observed or until a significant contributing source was observed.

### **2.2.1 Automated Flow Gage Selection and Installation**

Geosyntec evaluated numerous commercially-available models of data-logging flow meters to select the most appropriate device for this study. Based on low flows observed in the storm drains, Geosyntec identified flow monitoring devices that would accurately collect measurements under low flow, shallow depth conditions. A Hach Company American Sigma Series 920 flow meter (920) connected to an area-velocity and level probe (AV probe) was selected as the most appropriate meter for this project. The 920 is capable of logging data retrieved from the AV probe at a user programmed interval, and records the AV probe's instantaneous reading of both flow velocity and depth. The 920 can be programmed according to the shape and size of the pipe to calculate flow using standard hydraulic flow equations. The calculations take into effect the relationship of depth of liquid, velocity, and the shape of the pipe to calculate a flow rate.

The AV probe is installed in the storm drain using stainless steel bands that connect to an expanding or contracting adjustable band. The AV probe is secured to the bands using pre-drilled holes that screw into the bottom of the AV probe. The AV probe uses an ultrasonic signal that points upstream into the flow and reads the Doppler shift of small particles or sediment in the water column to measure velocity. The sensor reads the Doppler shift in a negative or

positive direction, depending on the orientation of the probe. The probe was installed such that flows toward the pump station were logged as a positive velocity value. A highly sensitive oil-filled pressure transducer located on the bottom of the probe measures the depth of water above the transducer. The AV probe also collects an atmospheric pressure reading for reference due to potential changes in atmospheric pressure.

Error in the readings and calculated flow rates may be attributable to irregularities in the shape of the storm drain. The 920 uses pre-programmed flow channel geometry that the user can select and change for size or diameter, but it assumes that the channel is symmetrical and in the case of pipes, it assumes that the cross-section is circular. Because all of the surveyed drains were not perfectly symmetrical, the calculated flow rates were likely slightly over or underestimated based on irregularities. Additionally, excessive turbulence or high suspended sediment in the flow can also introduce uncertainty into the velocity readings. Turbulence can cause the AV probe to read a negative or lower than average velocity, while high suspended sediment load can cause velocity readings to spike or show a non-reading<sup>2</sup>. As recommended in the user's manual for more accurate readings, routine maintenance and cleaning of the AV probe's reading surface and pressure transducer were conducted on a weekly basis for probes installed in the field. Other potential sources of uncertainty could include water depths less than the minimum detectable depth of 0.8 inches or low flow velocities less than the minimum detectable velocity of 0.05 feet per second. Readings below the 920's minimum detectable range are potentially subject to error. Efforts to validate the recorded flow rates of the 920 included flow estimation (using leaf and stopwatch method to measure velocity) and geometric measurements (wetted width and cross sectional depth) within each storm drain pipe fitted with an AV probe. Upon probe installation, manual flow depths were measured to be within the detectable range; 1.5 and 2.1 inches were measured at Laguna/Haley and Salsipuedes/Gutierrez (Locations 9 and 11) respectively. Instantaneous manual flow velocities were also measured within the detectable range; 1.6 ft/s and 2.1 ft/s were measured at Laguna/Haley and Salsipuedes/Gutierrez (Locations 9 and 11) respectively. No depth or velocity was measured at the Annex Yard drain (Location 17) due to significant accumulation of mud on the channel bottom. Field observation sheets and flow validation data are included in **Appendix C**. The user instruction manual and specifications cut sheet for the 920 and AV probe are included as **Appendix D**.

### **2.2.2 Manual Flow Measurement Equipment and Procedure**

Flow was measured manually by UCSB staff at selected sampling locations when feasible, with the exception of sample locations with the automated flow monitoring equipment installed. Manual flow measurements in Laguna Channel were performed using a Marsh-McBirney Flo-Mate Model 2000, electromagnetic velocity probe, using the velocity-area method. At least six

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<sup>2</sup> Based on personal communications with the product supplier.

depth-velocity measurements were collected at each location, depending on the width and geometry of the channel. The feasibility of manual flow measurements using a Flo-Mate Model 2000 velocity probe was determined from preliminary field measurements. For some locations sand bags were used to narrow the channel width so that sufficient velocity ( $> 0.01$  m/s or 0.03 ft/s) and water depth ( $> 0.02$  m or 0.07 ft) were obtained. Product specifications and user manual for the Marsh-McBirney Flo-Mate Model 2000 are included in **Appendix E**.

### **2.2.3 Water Budget Evaluation**

Using the results from automated flow monitoring equipment and manual flow measurements, a water budget was constructed for the Laguna Channel to evaluate contributory flows from observed or potential sources including groundwater well discharge, urban runoff in storm drains, cross connections to storm drains, basement sump pumps, or infiltration from irrigation or groundwater seepage. The average flow rate from the Laguna storm drain network and other drains to the channel was estimated from automated flow measurements, and compared to manual flow measurements collected in the open channel portion and at the pump station at the base of the watershed. Discrepancies in the different flow measurement methodologies and the potential error associated with each method were also evaluated.

## **2.3 Sampling and Analytical Methodology**

In order to quantify dry weather FIB concentrations and fluxes at various locations in the storm drain network, UCSB developed and implemented a dry weather flow sampling and analysis plan. Geosyntec coordinated with UCSB on the development of the plan based on insight gained from field observations of the storm drain network and channel.

The Laguna watershed sampling plan was designed to quantify the temporal variability of the dry weather loads of fecal indicator bacteria and human-specific *Bacteroides* markers transported by the drains and channel to the pumping station on the beach. More specifically, it was designed to quantify 1) the loads estimated at discrete points in the watershed, 2) the variability of bacterial load during transport in the Channel to the pumping station, and 3) the total load received at the pumping station.

### **2.3.1 Field Sampling**

Grab samples (approximately 2 L) for FIB and HBM analysis were collected manually at each sampling location using a sterile beaker. The sample was passed through 25 micrometer ( $\mu\text{m}$ ) pore size Miracloth (Calbiochem, San Diego, CA) and stored on ice until processing, which was completed within 6 hours. In addition to FIB and HBM, general water quality data including dissolved oxygen, temperature, and salinity were measured with a YSI Model 85 meter; pH was measured in the lab with a Corning pH meter 430. For a detailed description of laboratory methodology, please refer to the UCSB report located in **Appendix F**.

### 2.3.2 FIB Sampling and Analysis Methodology

Microbiological sampling was performed by UCSB. The sampling locations were selected based on field observations, visual flow assessments, and preliminary FIB concentration data. Additional sampling location selection criteria included: flow being observable during sampling, FIB being present in significant amounts (i.e., *E. coli* and *Enterococcus* exceeding 10 Most Probable Number per one hundred milliliters [MPN/100ml]), and/or nearby upstream fecal sources being expected. **Table 1** presents a description of the Laguna watershed sampling locations and **Figure 3** illustrates each location within the Laguna watershed. A total of 79 samples were analyzed between July and September 2008 from the base of the Laguna Channel at the pump station (see **Figure 4**) to further reaches of the upstream storm drain network. **Table 2** summarizes the sampling dates and times.

**Table 1: Monitoring Locations**

Sample Location	Sample Location	Distance to Tidal Gate (ft) <sup>*3</sup>
1	Overflow from Mission Lagoon, collected in Channel before entering pump station	-86
2	Laguna Channel before entering pump station	40
3	Laguna Channel upstream of railroad tracks	491
4	Laguna Channel downstream of Yanonali bridge	1,604
5	Laguna drain under Highway 101 onramp, middle channel	1,935
6	Drain outlet from City Annex Yard	1,905
7	Drain Olive @ Montecito Street	2,480
8	Drain Laguna Street @ Gutierrez Street	2,652
9*	Drain Laguna Street @ Haley Street	3,122
10	Drain Laguna @ 702 Laguna Street	4,338
11*	Drain Gutierrez @ Salsipuedes Street	3,639
12	Drain Gutierrez @ Quarantina Street	4,214
13	Drain Laguna @ De La Guerra Street	4,711
14	Drain Laguna @ Canon Perdido Street	5,225
15	Drain Salsipuedes @ Haley Street	4,093
16	Drain upstream Ortega Park (no flow observed)	5,314
17*	Drain Annex Yard 1 (location flow equipment)	2,289
18	Drain Annex Yard 2 (upstream of flow equipment)	2,458

\* Sampling location was monitored for flow with the automated flow meter.

<sup>3</sup> Distance measured upstream from tidal gate along drainage network (including storm drains and channels)

## **2.4 Bacterial Load Calculations and Microbial Budget Evaluation**

FIB and HBM load estimates were calculated using the mean flows and concentrations. The flow rate data obtained from the automated flow meter and manual flow measurements were used to calculate an average flow rate at various locations within the Laguna Channel and the Laguna storm drain network. The FIB laboratory analytical results from each sample location were averaged over the sampling period for each FIB constituent. Using the average flow rate and the average FIB concentration, an average daily load was calculated<sup>4</sup> for FIB at selected sampling locations. Mathematically, arithmetic mean values for flows and concentration values are most appropriate for estimating cumulative average loads regardless of whether the dataset is parametric or non-parametric.

Geosyntec assessed previous and current water quality (including pH, dissolved oxygen, temperature, salinity, and conductivity), FIB, and HBM data and differentiated (when possible) sources of indicator bacteria using water quality characteristics and FIB trends by constructing a microbial budget, or mass balance, for the Laguna Channel and its tributaries. Geosyntec first reviewed existing City data on indicator bacteria and human waste DNA markers in the Laguna watershed in preparation for the microbial budget. Other water quality data were reviewed to evaluate their usefulness in tracking sources of dry weather flow and bacteria influx. Data gaps were identified and evaluated for constructing a complete microbial budget.

## **2.5 Water Quality Enhancement Alternatives**

As presented in Section 2.1, a literature review of reports and studies on projects with similar objectives was conducted in an effort to evaluate effectiveness, cost, and feasibility of available urban runoff bacteria load reduction strategies. In addition, the initial results of this dry weather flow monitoring study, and FIB loading calculations were evaluated to provide planning and design-related information on potential sources and treatment flow rates. Based on the literature and data review, design criteria for the bacteria load reduction strategies were developed.

Bacteria load reduction strategies were evaluated in an effort to identify the most appropriate system to reduce fecal contamination and the frequency of posted warnings at East Beach. The analysis included a review of the originally-proposed ozone disinfection facility at the outlet of Laguna Channel (Enartec, 2003), source control opportunities, and alternative disinfection approaches including chlorination, biocides, UV, ozone, treatment through subsurface wetlands, and subsurface infiltration.

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<sup>4</sup> Arithmetic mean values are most appropriate for estimating loads even for non-normal data sets.

### 3.0 DATA EVALUATION AND RESULTS

The following is a summary of visual observations conducted in the field, data evaluation and flow calculations, water budget evaluation, results of bacterial sampling, and load calculations.

#### 3.1 Dry Weather Hydrology and Flow Monitoring

##### 3.1.1 Field Observations

Initially, to understand the dry weather hydrology of the Laguna Channel watershed, a field reconnaissance of the lower Laguna Channel was conducted by Geosyntec and City staff on February 27, 2008 and March 26, 2008. A follow-up visit was conducted on March 12, 2009 in order to verify the initial reconnaissance flows. Local observations were also made prior to the installation of each automated flow meter (July through August 2008). **Figure 5** displays a graphical summary of dry-weather field reconnaissance observations. During the field inspection, there were no significant sources of dry weather flow observed to be contributing to the open channel, other than storm drain outlets. Minimal dry weather flow was seen trickling from covered storm drain outlets that drain to the channel, although none of these were found to be a significant source of flow to the channel. Evidence of trash, debris, and human waste such as fecal matter, was observed along the length of the channel as well as abundant bird populations and dense vegetation. Some slight groundwater seepage was observed along the banks of the lower Laguna channel, although no water was observed to be flowing from the banks.

Field inspections to evaluate dry weather flows in the upper Laguna storm drain network were also conducted with the City on March 26, 2008. During the field inspections, flow was observed to be entering the open channel from the storm drain network at the Highway 101 overpass location. The water flowing into the channel at this location was observed to be a greenish color, slightly turbid and exuded a sulphurous odor. The odor was observed when the sediment and vegetation was disturbed at the water's edge, also resulting in the observation of dark colored highly organic sediment. Some foam was observed on the surface of the water flowing into the channel. In addition, adjacent to the Highway 101 overpass a second, 66-inch diameter storm drain pipe outlet was observed to contain discolored standing water but no observable flow into the channel. This pipe typically conveys runoff from the City Annex Yard, and a small portion of storm water runoff from Montecito and Yannonali Streets on the north side of Highway 101. Due to the flow of water entering the channel at the Highway 101 overpass location, the storm drain networks were subsequently inspected.

The base of the storm drain network that drains to the open Laguna Channel consists primarily of two main lines: the Laguna drain and the Gutierrez drain. During inspection of the storm drain network, flow was observed in the Laguna drain at the Haley Street intersection, but not upstream of Ortega Street.

A follow up inspection conducted on March 12, 2009 (following a period of at least one month of no rain events) indicated that significant flow is coming from a lateral located at the western corner of de la Guerra Street and Laguna Street. This flow was visually estimated at a consistent rate of approximately 5 gallons per minute.

Low flow was observed in the Gutierrez drain at the intersection with Salsipuedes Street, although no flow was observed in the Gutierrez drain upstream, on the opposite side of the street of this intersection. Flow was not observed in the line beneath Salsipuedes at the next available catch basin in the north end of Ortega Park. A drop inlet on the south side of Ortega Park that ultimately drains to the Gutierrez drain was observed with a crack in the wall of the inlet that had a trickle of water flowing into the catch basin (see **Figure 6**). While this was not considered a significant source of water in the storm drain network, it did indicate that shallow groundwater could contribute to the flow observed downstream.

A follow up inspection of potential flow contributing to the Salsipuedes/Gutierrez drains, conducted on March 12, 2009, indicated that contributing flow is coming from two main storm drain lines that connect to the Salsipuedes/Gutierrez drains near the north end of Ortega Park and the Santa Barbara Junior High School. Flowing water was observed in the 48-inch diameter storm drain pipe beneath East Ortega Street near Milpas Street. This storm drain network has inlets or catch basins in the area bounded by the streets of North Milpas to the south, East Ortega Street to the east, Alisos Street to the north and East Cannon Perdido to the west. In catch basins near Ortega and Milpas Streets leaf or detritus catchers showed significant amounts of human waste (trash and food) as well as decaying vegetation.

Additionally, the follow up inspection indicated another source of contributing flow comes from a 36 inch diameter storm drain line whose trunk is located at the southwest corner of Nopal Street and East de la Guerra Street. This drain network has inlets and catch basins along Nopal Street to the north, East de la Guerra Street, Quarantina Street, East Anapamu Street between Alta Vista Road and Olive Street, and the Santa Barbara High School to the south and west. While this follow up inspection was completed in the wet season, it was conducted following a dry period of at least one month, and is consistent with observations that the City had made on November 18, 2008, which were also conducted following a dry period of approximately three weeks. The flows observed during the follow up inspections and by the City are considered to be indicative of dry-weather flows.

### **3.1.2 Automated Flow Monitoring**

Automated flow monitoring equipment was installed near the base of the Laguna and Gutierrez storm drain lines at the following two intersections: Laguna and Haley Streets (Location 9), and Gutierrez and Salsipuedes Streets (Location 11). Numbers were assigned to each sampling location. The list of these locations and their corresponding numbers can be seen in **Table 1**. In addition to these installation locations, based on observations of intermittent flow conducted

during FIB sampling at the City Annex Yard storm drain outlet (Location 6), automated flow monitoring equipment was also installed near the end of this storm drain (the downstream City Annex Yard drain, Location 17) in order to evaluate flow patterns. The locations of automated flow monitoring equipment deployments are shown on **Figure 3**. As noted in Section 2.2.1, a manual flow measurement was collected at each automated flow gage location and the validation results indicated that the gage flow readings were within 2 - 11 percent of the manual flow measurements. Field notes and data validation calculations are included in **Appendix C**.

#### Laguna and Haley (Location 11)

The first automated flow gage was installed at the Laguna drain near the intersection of Laguna and Haley Streets on July 22, 2008, and removed on August 4, 2008. The flow meter measured flow rates ranging from 48 gallons per minute (gpm) to 91 gpm, and the overall 14-day average flow rate was calculated to be 62 gpm or 0.14 cubic feet per second (cfs). A graph showing flow data from the meter in this location is presented as **Figure 7**. The graph shows the rolling 30-minute average of the 5-minute interval instantaneous automated flow measurements versus time and FIB sample results at the times they were collected on a secondary y-axis on a logarithmic scale. A rolling 30-minute average was used for flow to dampen measurement anomalies and to more clearly demonstrate temporal flow patterns.

A reversal in flow direction occurred at this storm drain at a somewhat regular interval of 24 hours at or near midnight during the monitoring period. Based on discussions with the manufacturer of the flow monitoring equipment it was found that high turbidity or suspended sediment load in the flow, or high turbulence or current eddies can sometimes account for a flow reversal in the recorded data. Additionally the manufacturer indicated that these readings will typically be seen as infrequent or intermittent data points that only occur over a short time interval. Since the recorded flow data showed a reversal typically for a period of more than 30 minutes, an inspection of the drain was conducted by City staff at the anticipated next flow reversal period on the night of August 12, 2008. Neither a regular flow reversal or excessive turbulence event was not observed in the storm drain during the inspection period but crayfish and raccoons were observed in the storm drain. The manufacturer suggested turbulence as the reason for the flow reversal. One potential reason for the interference could be a basement sump pump draining to the storm drain network, causing turbulent or turbid flow that affected the recorded readings. Interference with the standard flow pattern could also potentially be due to regular nighttime actions of raccoons or other small animals causing turbulence near or disturbance of the flow meter.

#### Gutierrez and Salsipuedes (Location 11)

The second automated flow gage was installed on the Gutierrez drain near the intersection of Gutierrez and Salsipuedes Streets (Location 11) on August 4, 2008, and removed on August 22, 2008. The flow meter measured flow rates ranging from 0 gpm to 210 gpm, and the 19-day

average flow rate was calculated to be 72 gpm or 0.16 cfs. A graph showing flow data from the meter in this location is shown on **Figure 8**. The graph shows a rolling 30-minute average flow and FIB sample results at the times they were collected on a secondary y-axis on a logarithmic scale. The graph shows that in approximately the first six days of monitoring, a sharp spike or increase in flow is seen approximately every 9.5 to 10 hours. This flow pattern could be indicative of a sump or basement pump or irrigation timers on a regular pumping cycle. The flow logging data also indicated that flow rate dropped to 0 gpm between the afternoon of August 11 and August 12, 2008. The water level data for this time period were observed to increase significantly. It was later found through City staff that the pump at the tide gate was shut down on the morning of August 11 and that water had backed up into the storm drain network. Once the pump was turned back on in the morning of August 12, the flow data and the water level data returned to the range of flow observed prior to the stop. After the pump at the tide gate was turned back on, the pattern of flow increase every 9.5 – 10 hours was not observed and an overall slight decrease in average flow for the period from August 12 to August 22 was observed. The reason for this change in flow pattern is unknown.

A double rectangular concrete pipe enters the catch basin at Guierrez and Salsipuedes Streets (Location 11). The catch basin and pipes were noted to contain a large volume of sediment and organic material. The catch basin effectively acts as a depositional sump in the drain network, being 4-6 inches lower than the invert of the pipes that enter and exit. One pipe exiting this catch basin was effectively blocked off from flow due to a sediment mound. The sediment in the catch basin was silty sand and when disturbed produced black-colored suspended material and a sulfurous odor, and there was a filamentous white substance near the water and sediment mound interface. This white substance is believed to be sulfur-reducing bacteria; this substance and odor was also observed at the Ortega well which discharged approximately 0.3 miles upstream in the Salsipuedes drain. Evidence of animal interaction in these storm drains was also observed based on crayfish carcasses and raccoon footprints in the sediment piles.

#### Annex Yard (Location 17)

The automated flow gage was installed in the Annex Yard Line (Location 17) on September 3, 2008, and removed on September 16, 2008. The flow meter measured flow rates ranging from 0 gpm to 45 gpm, and the 13-day average flow rate as calculated to be 1.6 gpm, or 0.0036 cfs. A graph showing flow data from the meter in this location is shown on **Figure 9**. (The graph shows a rolling 30-minute average flow, FIB sample results at the times they were collected on a secondary y-axis on a logarithmic scale.) The graph shows that the flow at this location was irregular and that there was no discernable pattern, probably due to the nature of the source of water. This storm drain receives truck wash-down water from the City Annex Yard. The trucks haul landscaping debris, which consists of dead plants and soil, and dump the material for offsite disposal. Then the truck beds are washed out and a side drain catches the runoff and conveys it to the storm drain system. The manhole access to the main Annex Yard storm drain is a grate with a trash capture bag suspended. When installing the flow meter, it was noted that the bag

was completely full. Upon entering the storm drain, it was observed that there was a slight sulfurous or sewer-like odor, and the invert of the storm drain contained mud and organic debris approximately 3-4 inches thick. There was standing water in the drain at the time of installation and flow channels were observed in the mud. At this location there exists a malfunctioning diversion structure that should send wash water to the sanitary sewer. During this study period, unsuccessful attempts were made to retrofit the device. The City is currently working on a permanent solution.

Field observations suggest that the Laguna storm drain network contains a variety of potentially significant and continuous FIB sources (e.g., sediment, decomposing organic material, raccoons, etc.), but no clear indications of human fecal contamination inputs.

### 3.1.3 Water Sources

The sources of water flowing into the storm drain network are variable and difficult to precisely locate due to their diffusive nature and sporadic timing; however, the major sources are believed to be from a variety of locations. One source of water is the City's production wells. Inspections of City production wells that currently discharge to the Laguna storm drain network were conducted on March 26 and August 12, 2008. Inspections were conducted at the Corporation Yard well, Ortega Park well, City Hall well<sup>5</sup>, and Vera Cruz well<sup>5</sup> (**Figure 3**). The Corporation Yard production well is located in the City's maintenance yard located at the intersection of Laguna and Ortega Streets. Based on conversations with City staff, this well is believed to be screened in a groundwater aquifer that is under artesian conditions and so water from this well is discharged directly to the Laguna storm drain line via a maintenance yard lateral. The water flowing from this well was manually measured using a 5-gallon bucket and stopwatch. The flow rate was measured at least three times on two separate field inspections in June 2008 and ranged from 22 to 34 gpm.

The Ortega Park well is also screened in an artesian aquifer and discharges at the surface from the well head to the Salsipuedes Street drain. Based on discussions with City staff, the City is conducting a feasibility study for a water treatment plant where the well head is connected to the sewer system via above ground piping that connects. Observations of this well head indicated that a small amount of flow drains to the storm drain network and eventually to the Gutierrez Line. The flow from the Ortega Park well was measured three times on August 12, 2008, and averaged approximately 0.55 gpm. During the inspection of the Ortega well, the presence of a strong sulphurous smell was observed which may be indicative of sulfur reducing bacteria growing in the well vault.

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<sup>5</sup> Based on the City's recent delineation, this is now believed to be outside of the study watershed.

Another potential source of water is groundwater infiltrating into the storm drain network. Groundwater in the lower elevation areas of downtown Santa Barbara can be as low as 1 foot below ground surface (ft bgs) in some wet seasons. Based on groundwater quality monitoring data obtained from the Geotracker© Database maintained by the State Water Resources Control Board, groundwater in lower downtown Santa Barbara can range from 1.23 to 3.45 feet bgs or between 5.00 to 7.50 feet above mean sea level (amsl). The elevation of the inverts of manholes along the Gutierrez and Laguna drains are in the range of 0.26 to 6.28 feet amsl indicating that groundwater infiltration through cracks and connections into the storm drain pipes through cracks and connections is another potential source of water found in the channel. **Figure 6** shows a section of the Gutierrez storm drain pipe near the Gutierrez drain at Salsipuedes Street (Location 11). It can be seen that groundwater is seeping into the storm drain along small cracks and connections in the pipe. While the flow from this source may be very low at each crack, thousands of feet of storm drain pipes with cracks could lead to a significant amount of inflow. Irrigation of landscaping and parks can also lead to high groundwater in this area which contributes to the groundwater seepage potential. **Figure 6** also shows a catch basin inlet on the south end of Ortega Park near the Salsipuedes Street storm drain that has a crack with groundwater trickling into the drop inlet. While this observed flow is not locally quantifiable, it confirms that, in general, groundwater inflow sources can be a significant contribution to the network. Shallow groundwater elevation data from the Geotracker Database is not available in the area around the open channel portion of the study area and therefore it is not known if groundwater is a significant source or sink for this section.

Based on follow up inspections of the storm drain network and watershed upstream of Ortega Park, it is apparent that a number of potential sources of flow inputs are contributing to the flow observed in the Gutierrez line. Flow from the storm drain network located at East Ortega and North Milpas Streets contributes a significant portion to the Gutierrez line. Although the source of flow in this storm drain network was not identified, flow could be from surface drainage of irrigation from both residential and commercial buildings, and from potential cross connections from commercial and residential sources. Flows were also observed in the line located near Nopal and East de la Guerra Streets. Again, the specific sources of flow in this line were not identified, although one source appears to be coming from a section of storm drain line located beneath or near the Santa Barbara High School campus. Additional input could also come from residential cross connections in this neighborhood.

Although no flow was observed to be flowing from the County storm drain line that discharges to the lagoon directly adjacent to the tide gate, the outlet gate was partially submerged; therefore, because this line could not be inspected at the outlet for discharge, it cannot be ruled out as a potential water source to the lagoon.

Further evaluation of magnitude and sources of flow throughout the study area are presented in the following section.

### 3.1.4 Water Budget Evaluation

To evaluate the water budget in the channel, a comparison of the measured flows at key locations was conducted. **Table 3** shows a summary of the average manual and automated flow measurements. **Table 3B** displays the individual manual flow measurements. **Figure 10** graphically displays the average flow rates at the automated and manual flow monitoring locations. Manual measurements were taken in the open channel whereas automated flow measurement devices were used within the storm drain. The daily discharge volume was obtained using the average automated flow monitoring results from the Laguna drain at Gutierrez (Location 9), the Gutierrez drain at Salsipuedes (Location 11), and from the downstream drain at the Annex Yard (Location 17). The combined average input flow rate from each of these locations total 140 gpm or 0.30 cfs<sup>6</sup>. This estimate assumes that there are no other input sources to the open channel beyond those measured or observed in the field. Overflow from Mission Lagoon, collected in the channel before the pump station (Location 1) was not included as an input source since it is overflow from the Mission/Laguna Lagoon and slight leakage through the tide gate, both of which intermittently contribute a minor volume into the Laguna Channel pump station at the base of the watershed. While the water that drains into the channel at this location does not directly come from the Laguna Channel watershed it is a significant volume, and should be considered in the estimation of the treatment system design flow.

The manual flow measurements collected in the channel before entering the pump station (Location 2) were assumed to be representative of the total outflow of the Laguna Channel. Flow was manually measured at the pump station as described in Section 2.2.2. Manual flow monitoring was conducted eight times at Location 2, between the dates of July 11 and August 20, 2008. An average flow rate of 65 gpm or 0.14 cfs was measured at Location 2.

In comparing the upstream average flow rates to the downstream estimates at Location 2, there is a difference of 71 gpm or 0.16 cfs entering the channel from the storm drains greater than what is pumped around the tide gate. One explanation for this difference could be direct water surface evapotranspiration, although this process alone is unlikely to account for the significant surplus upstream flow.

Another potential explanation for the difference could be measurement error inherent to both the manual and automated flow monitoring methods. There are limitations to both methods. The manual method is generally considered to be subject to uncertainty of plus or minus 40 to 50 percent, due to cross sectional and velocity measurement inaccuracies at these low and shallow flows, while the automated measurement device has an uncertainty of plus or minus 2 to 11

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<sup>6</sup> These locations were monitored at successive two-week intervals and not monitored concurrently. The cumulative flow estimate should be considered an appropriate value for rough assessment and planning purposes.

percent based on the flow validation measurements collected in the field during installation of the gage.

When the combined average flow from the storm drain network based on automated flow measurements is compared to the manual flow measurements collected at the first daylight of the storm drain network at the Highway 101 on-ramp (Location 5), it is apparent that there is a discrepancy between the results from these two flow monitoring techniques. Average flow at Location 5 was found to be 59 gpm (measurements ranging from 36 to 119 gpm), while the average combined flow of the upstream storm drains was 136 gpm. Additionally, at the base of the watershed at the pump station (Location 2), the average manual flow measurements were similar to those at the Highway 101 on-ramp (Location 5), indicating a consistency in the manual flow measurements, assuming that no significant flow input or output is occurring in the open channel.

The discrepancy of the flow measurements could be potentially explained by the limitations of the manual flow measurements. Depth measurements collected at the Highway 101 on-ramp (Location 5), where the storm drain network daylights to the open Laguna Channel, range from 1.3 to 2.3 inches. Additionally, the velocity measurements collected at the same location ranged from 0.03 to 0.10 feet per second (fps). The velocity probe user's manual indicates that the minimum detectable velocity is 0.05 fps and therefore some of the readings fall below the detectable velocity. Field depth measurements were also around the minimum accurate depth range of 1 to 1.5 inches. In addition, measurements collected over time at Location 5 show a steady decrease in both velocity and flow. Per the user's manual, factors which could contribute to this observation include poor maintenance of the electrodes on the velocity probe and inadequate or infrequent zeroing of the probe before use. In discussions with the UCSB field representative conducting the manual measurements in the open channel, it was found that little or no maintenance to the probes electrodes was conducted, and that the probe was never zeroed during the span of the project as recommended by the manufacturer's specifications.

Additionally, at the Highway 101 on-ramp (Location 5) there are three rectangular concrete outlet culverts. One of the culverts connects to a small dry storm drain line from East Montecito, but the other two are assumed to be the 54-inch double concrete pipes of the main Laguna storm drain line. During the collection of manual flow measurements, the UCSB researcher indicated flow was only observed to be visible from one of these two pipes, although water was in and around the outlet of both culverts. This second culvert was never measured for possible smaller flow contributions. Therefore, minor flows from this line may have been overlooked in the manual measurements. Additionally, there are Caltrans laterals from the below-sea-level inlets around the Garden underpass that could not be inspected for flow, therefore flow gains/losses could be resulting from these as well. Given that the channel is unlined for the majority of the distance from the Highway 101 on-ramp to Cabrillo Boulevard, infiltration from the open channel into the groundwater is another potential explanation for the difference. The groundwater elevations were found in the Geotracker Database from a site located directly

adjacent to the channel at Yannonali and Garden Streets. The groundwater table elevation in this location was found to be at an elevation of 0.57 to 4.29 feet above mean sea level during the time of this study. Based on these relatively high groundwater elevations it is unlikely that groundwater infiltration could explain much of the flow losses between the upper watershed locations and the measurements from the lower channel.

In an attempt to cross-check the manual flow measurements in the channel before entering the pump station (Locations 1 and 2), flow gauging data for the pump station was requested from the City, however no monitoring of dry weather flow was conducted and neither were pump electric usage rates and operation times available. In order to reduce uncertainty in the overall Laguna water budget and of the daily range of flow rates in the lower channel, additional flow monitoring is recommended for this area (using a zero'ed or calibrated probe) if the City goes to final design for the pipes and treatment system.

### **3.2 Laboratory Analytical Results**

The laboratory analytical results were completed by UCSB in the laboratory of Dr. Patricia Holden of the Bren School of Environmental Science and Management. UCSB generated a report of their findings for this study and the following two sections are a summary of the analytical findings and conclusions from their report. A copy of the report including a more detailed discussion of the results is included as **Appendix F**.

#### **3.2.1 FIB Analytical Results**

The laboratory analytical results of FIB samples collected for this study are presented in **Table 4**. These results are subject to an uncertainty of plus or minus 20 percent. **Figure 11** shows the median FIB concentrations at each sampling location represented by a bar graph for each FIB constituent. The single sample maximum for marine recreational waters<sup>7</sup> for Enterococcus and total coliform are 104 MPN/100ml and 10,000 MPN/100 ml, respectively. There is currently no listed standard for E. coli, although the standard for fecal coliform is 400 MPN/100ml. In the case that the ratio of fecal coliform to total coliform exceeds 0.1, then a total maximum of 1,000 MPN/100ml must be not be exceeded. The aforementioned standards apply to the Pacific Ocean at East Beach, to which the Laguna Channel, and ultimately the Mission Lagoon discharge.

The majority of the samples collected at all locations exceeded at least one of the single sample maximums. The highest median E. coli (EC) and Enterococcus (ENT) concentrations were found at the drain outlet from the City Annex Yard (Location 6). The median concentrations of EC and ENT at Location 6 were 161,110 and 224, 680 MPN/100 ml, respectively. The highest

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<sup>7</sup> Water quality standards by the State of California Department of Health Services for marine recreational waters as established in Assembly Bill 411.

median total coliform (TC) concentrations were found at the downstream Annex Yard drain (Location 17) at a value of 2,419,570 MPN/100 ml. The Laguna drain at 702 Laguna Street (Location 10) and the Gutierrez drain at Quarantina Street (Location 12) generally had the lowest median ENT and EC concentrations, although one spike in ENT concentration was observed on August 20, 2008 at the Gutierrez drain at Quarantina (Location 12). Also at the Olive drain at Montecito Street (Location 7), a spike in both EC and ENT was observed in the morning of July 31, 2008. The lowest median TC concentrations were measured in samples collected from the Laguna Channel upstream of the railroad tracks (Location 3), the Laguna Drain at Haley Street (Location 9), the Laguna Drain at 702 Laguna Street (Location 10), the Gutierrez drain at Quarantina (Location 12), and the Laguna Drain at De La Guerra Street (Location 13). When evaluating data sets that are non-parametric or have a non-normal distribution, such as the results for FIB were reported in this study, the median of the data set is considered more representative of the overall trend or characteristics of the data collected. Additionally, for graphical purposes and for comparison purposes, the median of the FIB results at each sample location were used in **Figure 11**.

Similar longitudinal concentration profiles were observed in the Laguna Watershed for EC and ENT. In the Gutierrez drain, FIB mostly originated from the Salsipuedes drain (Location 15), and not from the upstream reaches of the Gutierrez drain (Location 12). In the Laguna drain, the FIB concentrations usually decrease from the Laguna drain at Canon Perdido Street (Location 14) to the Laguna drain at De La Guerra Street (Location 13), but remain fairly constant downstream of this location. Comparison between the Laguna drain at De La Guerra Street (Locations 13) and the Laguna drain at Haley Street (Location 9) is difficult, because concurrent samples were not collected. The FIB concentrations in Laguna drain (Location 9) and the Gutierrez drain (Location 11) are similar, so each drain segment may similarly contribute to FIB concentrations downstream in Laguna drain (Location 8).

In the open Laguna Channel, FIB concentrations generally increase from the Laguna drain under the Highway 101 on-ramp (Location 5) to downstream of Yanonali Bridge (Location 4), decrease from Location 4 to upstream of the railroad tracks (Location 3), but increase again from Location 3 to the location in the channel before entering the pump station (Location 2), suggesting FIB sources are located between Locations 5 and 4, and between Locations 3 and 2. Very high FIB concentrations consistently originate from the City Annex Yard (Locations 6, 17, 18), which is a likely significant source of FIB to the channel between Locations 5 and 4.

### 3.2.2 HBM Analytical Results

The HBM analytical results completed by UCSB are summarized in **Table 6** and shown in **Figure 12** by the magnitude of the median at each sampling location. HBM were consistently detected at Locations 2, 4, 5, 8, 11, and 15. HBM were sporadically detected in Laguna Channel Locations 1 and 3 and drains 6, 9, 10 and 18. In some cases, HBM targets were classified as “detectable but non-quantifiable.” For example, at the sampling location upstream of the

railroad tracks (Location 3), HBM were detected on three occasions but were only once classified as quantifiable. No HBM were detected in storm drain Locations 7, 12, 13, 14 and 17. When detected, HBM usually were present in the order of  $10^4$  copies/L. The highest concentrations, exceeding  $10^5$  copies/L, were observed at the Laguna drain under the Highway 101 on-ramp (Location 5). Concentrations measured in the Laguna Drain at Haley Street (Location 9) were consistently measured to be less than  $10^4$  copies/L. The median result in the Laguna Channel prior to entering the storm drain is  $2.3 \times 10^4$  copies/L. Based on measured HBM levels in sewage samples ( $7.8 \times 10^9$  copies/L) (Sercu, 2009), the result measured at Location 2 equate to approximately 0.0003% sewage.

The data indicate that HBM originate from 2 drain sections. The highest concentrations of HBM originate from the Salsipuedes Street drain (Location 15), which flows to the Gutierrez Street drain (Location 11) and then to the Laguna-Gutierrez union (Location 8). Some HBM also appear to originate from a Laguna drain lateral (Location 10), which flows to the Laguna drain (Location 9) and then to the Laguna-Gutierrez union (Location 8). Downstream of the Laguna-Gutierrez union, HBM sources were identified between Locations 8 and 5, and between Locations 3 and 2. The HBM concentration changes between Locations 5 and 4 were inconsistent, with order of magnitude decreases on some days and increases on other days. These results would suggest that the potential human sources of FIB to the watershed are likely originating in storm drain flows along the Gutierrez and Laguna lines. Furthermore, data are limited and results are highly variable, therefore limited conclusions can be drawn at this time.

HBM inputs detected in the upper reaches of the Laguna watershed storm drain network do appear to correlate with higher FIB loads downstream. A similar correlation between the two indicators can be seen in both data sets with respect to the increase in HBM and FIB concentrations between Locations 3 and 2 in the open Laguna Channel. However, upon further examination of the individual FIB indicators at each location, it can be seen that E. coli concentrations, which are generally considered to be roughly equivalent to fecal coliform concentrations, actually show a decrease between Locations 3 and 2 while ENT and TC show higher concentrations downstream. Another key observation is the fact that FIB was detected at Locations 13 and 14, upstream of the Laguna Drain at 702 Laguna Street (Location 10). However, no HBM was detected in the Laguna Drain at De La Guerra Street (Location 13) or Canon Perdido Street (Location 14). HBM and FIB trends suggest that there are other FIB sources of non-human origin contributing to the total load and that HBM does not correlate to FIB in all cases.

### 3.2.3 Water Quality Results Evaluation

During sampling, UCSB collected water quality data in the field for conductivity, salinity, temperature, and dissolved oxygen (DO), and analyzed pH in the laboratory. The water quality parameters results are presented on **Table 5**.

Based on these results, conductivity and salinity at the overflow from Mission Lagoon (Location 1) are consistently and significantly greater than all other sampling locations. This data helps to validate the visual observation that water flowing into the channel at this location is from the Mission/Laguna Lagoon, via leakage of the tide gate. The salinity and conductivity at all other sampling locations were lower and consistent with one another.

Additionally, when comparing the average water quality values between storm drain network and open channel locations, a number of patterns are noticeable. Temperature in the storm drains is generally higher than in the open channel, possibly due to illicit cross-connections or groundwater (hot springs) inflow. Average storm drain (Locations 7 through 18) temperatures ranged from 21.3°C (Location 14) to 26.5°C (Location 10), while the average open channel (Locations 1 through 6) temperatures ranged from 19.7°C (Location 7) to 23.5°C (Location 1).

The DO concentration results in the open channel were fairly consistent, whereas the storm drain results demonstrated more variability. The average DO concentrations in the open channel were generally higher than in the storm drain network. In the storm drain network the DO was lowest at Locations 9, 10, 14, 17 and 18. These locations are in the Laguna Street main trunk section of the storm drain network, and in the City Annex Yard drain. Drains along the Salsipuedes and Gutierrez drains (Locations 11, 12, and 15) exhibited consistently higher DO concentrations than the other sampled drains. At the confluence of the Gutierrez and Laguna drains (Location 8), a mixing of the two flows occurs, which is assumed to stabilize the DO concentrations downstream.

pH levels were fairly constant throughout the study area and averages ranged from 7.5 (Location 10) to 8.3 (Location 18).

Field parameter results indicate that there are significant differences in the storm drain water quality as compared to water quality measured in the open channel. Further discussion of the significance of these results is presented in Section 3.4.

### **3.3 Bacterial Load Calculation Results**

The FIB measurements, along with the average flow rates at Locations 1, 2, 3, 5, 9, 11, and 17 were used to estimate the total daily load in units of MPN per day for each of the FIB types. The results of the load calculations are presented on **Table 3**. Calculations of average annual load use the average flow rates and average FIB concentrations. Arithmetic mean values for flows and concentration values are most appropriate for estimating cumulative average loads regardless of whether the data set is parametric or non-parametric. For comparison purposes, the channel inlet Locations of 1, 9, 11, and 17 were compared to the outlet (Location 2). The daily FIB loads were highly variable by sampling locations, spanning a range of three orders of magnitude. The highest daily loads of all FIB types were found at Location 17 (City Annex Yard storm drain). In general, the total daily loads for storm drain locations were in the range of  $10^8$  to  $10^9$ .

MPN/day for EC and ENT, and in the range of  $10^9$  to  $10^{10}$  MPN/day for TC. Comparison of the Gutierrez and Laguna storm drain loads at Locations 11 and 9, respectively, indicates that FIB loading is slightly higher in the Gutierrez storm drain although the calculated loads for all constituents were found to be in the same order of magnitude.

At the outlet (Location 2), average daily loads for EC, ENT, and TC were estimated at  $5.30 \times 10^9$ ,  $3.52 \times 10^9$ , and  $8.61 \times 10^{10}$  MPN/day, respectively. When all the inlet loads are summed and compared with these, there appears to be a net reduction or loss of FIB loads in the channel. Additionally, when the daily loads for the open channel locations (Locations 1, 2, 3, and 5) are compared, it appears that the loads are similar in magnitude, and demonstrate only slight variation. This finding supports the fact that flow within the open channel is relatively constant (relative to FIB concentration) and FIB loading variability within the open channel is due primarily to FIB concentration variation.

A comparison of changes between in-channel sampling locations indicates that the FIB loads within the storm drain network slightly decrease from upstream to downstream (comparing Locations 9 and 11 to Location 5). This is consistent with the above finding that summed inlet loads exceed loading at the outlet. The combination of high FIB concentrations and low flows result in a higher estimated daily FIB load at the City Annex Yard (Location 17) than at most other sites. This would indicate that the majority of FIB loading to the Channel is from the storm drain network upstream, in comparison to re-growth in the natural portion of the channel downstream.

### **3.3.1 Comparison of Laguna Channel Load Results**

Total estimated input loads of FIB to the Laguna Channel (calculated as the sum of the FIB load measured at Locations 9, 11, and 17) were reviewed in comparison to other local Clean Beach Initiative projects where treatment or diversion systems were recommended and installed. These projects include the Arroyo Burro and Mission Creek Dry Weather Diversion and Bacterial Reduction Study, (City of Santa Barbara, 2007), and the Mission Creek Bacterial Reduction Project (City of Santa Barbara, 2008).

The estimated loads were based on FIB sampling conducted in 2006 for inlets to Mission Creek. Based on the results of the 2006 sampling, the reported loads were  $1.24 \times 10^{12}$  MPN/day for TC,  $1.82 \times 10^{11}$  MPN/day for EC, and  $1.26 \times 10^{11}$  MPN/day for ENT. Similarly, based on samples collected in 2007 associated with the Westside SURF project for inlets to Old Mission Creek, the calculated loads were  $1.1 \times 10^{11}$  MPN/day for TC,  $1.6 \times 10^{10}$  MPN/day for EC, and  $1.7 \times 10^{10}$  for ENT. When comparing these values to those calculated for this current study, the Laguna Channel loads are significantly lower. The loads calculated from the 2006 Mission Creek sampling are one order of magnitude lower for TC and EC and two orders of magnitude lower for ENT. The 2007 Old Mission Creek sampling resulted in ENT load calculations of one order

of magnitude lower than the calculations from this study; however, the TC and EC loadings were the same order of magnitude as those calculated for the Laguna Channel.

### **3.4 Summary of Dry Weather Hydrology and FIB Loading**

The total inflow to the Laguna Channel pump station, based on manual flow measurements, was estimated at 136 gpm (total daily volume of 190,000 gallons). This takes into account inflows from the upstream storm drain network. By comparing the inflows and outflows before entering the pump station (Location 2), it is apparent that there is a higher flow entering the channel than exiting it. Assuming minimal losses within the open channel due to groundwater infiltration or evapotranspiration, as discussed in Section 3.1.4, this discrepancy may be explained by the error attributable to the various flow measurement techniques (i.e., manual measurements in the open channel versus automated flow gage measurements in the storm drain). Further open channel measurements (using a zero'ed or calibrated velocity probe) are recommended to better characterize the variability in flow.

Daily FIB loads were calculated at select locations based on flow measurements and average FIB concentrations. Results were variable between storm drain sample locations, but on the whole showed consistently higher FIB loads within the storm drain network as compared to the open channel. The higher FIB loads in the storm drain network may be attributable to the higher water temperatures, lack of UV light, lower DO concentrations, or presence of accumulated sediment, all of which are conducive to FIB production, regrowth, and viability. For instance, two separate studies by Tiefenthaler et. al. (2008) and Reeves R. et. al. (2004) found higher temperatures to correlate with higher FIB concentrations in both developed and undeveloped watersheds.

Field measurements recorded significantly higher velocities in the storm drains than in the open channel. Higher velocities will typically lead to higher overall sediment resuspension and mixing within the flow profile. Significant amounts of sediment were observed in the storm drain at several monitoring locations, specifically at the Gutierrez drain at Salsipuedes Street (Location 11) and the downstream Annex Yard drain (Location 17). The sediment likely comes from surface water runoff during storm events, which accumulates over time within the storm drain network. Reeves et. al. (2004) found that sediment shed in storm drains can be a significant source of FIB loading. In this study, accumulated sediment in catch basins and storm drain gutters was tested and found to contain very high levels of FIB. This is likely due to the fact that microbes adhere to soil particles and when disturbed by water flows or other external sources (e.g., animal perturbation), have access to nutrients and organic matter for growth, and are protected from UV light.. Additionally, Anderson et. al. (2005) at the University of South Florida found that persistence, or the lowest rate of decay, of FIB in subtropical freshwater and saline water was found in sediment inoculated with a population of FIB. This indicates that sediment will tend to trap and maintain a population of FIB longer than would be maintained within the water column downstream. This suggests that a constant source of FIB originating

from accumulated sediment is potentially being transported by the dry weather flows and contributing to the overall FIB loading in the water column.

FIB loads within the open channel were found to be generally lower than those calculated within the storm drain system and were rather consistent between individual sampling locations due to relatively consistent flow along the channel. Though loading rates were lower in the open channel than the storm drains, animal contact and plant matter can still contribute to the total FIB load (Tiefenthaler et. al., 2008). Additionally, HBM results and field observations at the Laguna drain under the Highway 101 onramp (Location 5) suggest that the open channel is also likely a source of human-influenced FIB.

Boehm et. al. (2004) completed a study of the Talbert Watershed outlet and the interaction of shallow unconfined groundwater with surf zone FIB concentrations and water chemistry. This may be applicable to the Laguna Channel as there was some groundwater seepage observed into the earthen channel. Groundwater exchange or flux into the surf zone via natural gradient driven transport and tidal fluctuations at the land/ocean interface was found to be capable of providing nutrient loads that possibly promote enhanced growth or increased persistence of FIB. Additionally, the study evaluated the mechanism for delivery of FIB to the surf zone through groundwater transport and found there is evidence to suggest groundwater polluted with FIB can add to concentrations in the surf zone. This same pathway could occur anywhere along the open channel where a broken or leaking sewer line was contributing FIB of HBM to groundwater that was seeping into the channel.

The open channel water velocities are significantly lower than those measured in the storm drain system. Lower velocities lessen the potential for the disturbance of FIB-laden sediments. This in effect reduces the potential for sediment (and sediment associated FIB) resuspension in the open channel. As discussed previously, lower water temperatures in the open channel can also inhibit FIB production and viability (Tiefenthaler et. al., 2008 and Reeves et. al., 2004). Furthermore, the open channel is exposed to direct sunlight, further reducing FIB concentrations in comparison to the fate and transport processes in the storm drain network upstream.

In summary, it appears that the majority of the FIB loading to the Laguna Channel originates in the storm drain network upstream of Highway 101. The open channel receives a constant input of FIB from the consistent inflows of FIB-laden water and sediments from the upper watershed. The open channel shows more consistent, but lower, flow rates and FIB loads and is likely regulated by exposure to UV light, lower water temperatures, and lower flow velocities leading to minimal sediment-bound FIB resuspension. However, HBM analyses indicate that there are potential human fecal contaminant loading sources in both the storm drain networks and the in the open channel.

## **4.0 WATER QUALITY ENHANCEMENT ALTERNATIVES AND FEASIBILITY ANALYSIS**

A multi-tiered implementation approach is recommended in order to reduce FIB and HBM concentrations at the tide gate outfall of the Laguna Channel as well as the number of posted warnings at East Beach. Source control options are recommended as the first tier solutions to possibly reduce upstream FIB loads. Because it is difficult to quantify the efficiency of source control, it is necessary to monitor receiving water quality downstream the post-implementation. If bacteria levels are not reduced to acceptable levels through the implementation of source controls alone, structural treatment alternatives (essentially end-of-pipe treatment systems) can be implemented near the channel outfall (Location 2). Existing and recommended source controls are discussed below, and structural treatment alternatives are evaluated based on various design criteria (i.e., aesthetics, maintenance, capital cost, etc.) in order to determine the most appropriate and feasible treatment option for the Project.

### **4.1 Source Control Recommendations and Water Quality Enhancement Projects**

This section provides a summary of source control measures currently implemented by the City as well as recommendations for further water quality enhancement and source control measures. Recommendations are based on field observations, FIB and HBM field measurements, as well as current proven municipal and industrial source control practices.

#### **4.1.1 Locally Implemented Source Control Measures**

The City currently employs several dry weather water quality enhancement projects with the continued goal of protecting creeks and coastal oceans, reducing the number of beach closures, protecting public health and safety, and improving recreational access to the popular Santa Barbara beaches. One such improvement project is the Mission Creek Summer Urban Runoff Facility (SURF) project which diverts dry weather urban runoff to an underground UV treatment facility, prior to discharging treated flows to the ocean at East Beach. The facility began operating in March of 2007, and results indicate that the system significantly reduces FIB loads at the outlet.

The City also been studying the Laguna Creek watershed and FIB treatment options; the City conducted a feasibility study for various FIB treatment systems in 2003 (Enartec, 2003). The study reviewed various treatment options for the reduction of FIB in the dry weather flows in the Laguna Channel, evaluated anticipated FIB reduction factors, and provided the cost and feasibility for each of the proposed systems.

Another project that the City has undertaken to reduce bacterial loading at East Beach and in local creeks has been the Dry Weather Diversion and Bacterial Reduction Project (City of Santa Barbara, 2007). The evaluation of sampling and FIB source tracking resulted in the City's decision to install diversion structures at Hope Avenue and Haley Avenue on the City's west

side. The dry weather flows in these storm drain locations were found to be significant contributions to the overall FIB load in Arroyo Burro Creek and Mission Creek, and these flows were also found to test positive for HBMs in a study conducted by UCSB. The structures divert dry weather flows to the sanitary sewer for treatment via pump and gravity-feed systems. The implementation of this system has successfully reduced the volume of dry weather flow to each watershed.

In addition to maintaining a rigorous monitoring and sampling program to evaluate the bacterial conditions in creeks and at the beaches, the City has recently taken steps to improve the water quality in the Laguna Channel. This includes the installation of fencing around bridges (specifically at the Yannonali Street Bridge and the southbound Highway 101 onramp at Garden) in an attempt to reduce illicit dumping and access to the creek.

The City also conducts outreach programs to educate and inform the public of the measures taken to reduce FIB in creeks and beaches. These programs include:

- Informational pamphlets to pet owners and RV owners on illicit dumping;
- Volunteer creek cleanup days;
- Illicit discharge detection program;
- Installation of portable toilets for the homeless population (such as at Yanonali Street where the labor line forms, and at Chase Palm Park where homeless are found along the railroad track); and
- Regular pickup of waste and feces from creek banks.

#### **4.1.2 Source Control Recommendations**

Based on field observations, analytical results for FIB and HBM, previous studies (Sercu et. al., 2009), and standard industry practices regarding treatment or source control methods for FIB in urban watersheds, a combination of methods in the open channel and storm drain network are recommended to further reduce the FIB loading in the watershed. These methods include the diversion of flow sources in the upstream storm drain network to the sewer system, routine cleanout and maintenance of sediment and dead vegetation in catch basins, regular trash and debris removal in the open channel, and continued public outreach and education.

#### **Continued Public Outreach**

Continuation of public outreach programs by the City to supplement the on-going program is recommended. This will continue to educate the public of the harmful effects of illicit dumping on the water quality in Laguna Creek and at East Beach. Outreach to the general public and

specific potential polluters such as recreational vehicle (RV) owners, pet owners, and homeless populations serves to educate the public and provide a constant reminder of the importance of water quality. This can be achieved through public meetings, distribution of information pamphlets, and postings of no dumping signs in critical areas. One such critical area is at the Yannonali Street Bridge where day-laborers typically congregate. The City currently supplies and maintains a portable toilet at this location to deter the improper use of the creek. Additional signs posted in this area would serve as a reminder to use the portable toilets.

A sizable population of RV or motor home residents exists intermittently at Ortega Park and near East Beach. The City has investigated the possibility that RV users within the Laguna Channel watershed have been illegally dumping sewage waste into the storm drain. The City has made considerable efforts investigating potential dumping by RV owners in these areas including surveillance and on-site interviews. Although it was not observed during investigations, it is recommended that these efforts are maintained to ensure that illegal dumping does not occur.

#### Sanitary Sewer Leak Investigation

In addition to public outreach, it is recommended that the City continues to work with the El Estero Waste Water Treatment Plant (El Estero) to investigate and replace damaged sewer lines. El Estero replaces approximately 2 percent of damaged lines in the city annually. Based on HBM data evaluated in this study it appears there are consistent trends of human fecal contamination in the Gutierrez storm drain line. Specifically in sample Locations 8, 11, and 15, the HBM results indicate positive findings for human DNA markers in nearly all the samples collected. During field monitoring and sampling there were no obvious sewer cross connections and no observable discharges from RV residences that would indicate a potential source of these consistent HBM results. A possible explanation might be a leaking or broken sewer line located somewhere upstream of sample Location 15 (upstream from Ortega Park). The damaged pipe may be discharging contaminated water into the surrounding soil, which could then infiltrate through cracks in the storm drain pipes. It is therefore recommended that the City work with El Estero to prioritize the investigation into, and potential replacement of, any leaky sewer lines in the areas of the watershed where groundwater may infiltrate into the storm drain or open channel.

#### Diversion to Sewer System

Diverting inflows from the Laguna Channel to the sanitary sewer could serve as an effective source control measure by eliminating that contributing during dry weather. The diversion flows that are proposed are likely minimal as compared to unused the capacity of the City's wastewater treatment plant. In addition, storm drain lines are typically located near sewer lines, so it is likely that a short connection from the storm drain to the sewer line could be easily completed, such as at the Hope and Haley Street diversions. If the hydraulic head is not available for a

simple gravity diversion, a sump could be constructed within a storm drain catch basin and flows could be pumped to the adjacent sewer line.

There are three locations or areas where diversions in the Laguna Channel watershed are recommended. The Annex Yard drain pipe supplies a relatively low volume of runoff to the Laguna Channel system but supplies a relatively large FIB load to the creek due to elevated FIB concentrations. The water that discharges to the storm drain system during dry weather periods is from the washout of work trucks. This wash water drains to the storm drain line and then discharges into the open channel. There is currently a system in place that is designed to clarify and divert this runoff to the nearby El Estero Wastewater Treatment Plant. However, this diversion is not currently in operation; it is recommended that this connection be restored in order to reduce this consistent FIB loading to the creek. Completion of this diversion could reduce dry weather design flows of a downstream treatment system by approximately 1.6 gpm.

During field monitoring and sampling, a cross connection to the main Laguna storm drain line was identified just upstream of the Laguna Street and Ortega Street intersection (Location 10). On one sampling occasion water was observed to be actively flowing from this connection; on other occasions, ponded water was observed. It has not been determined if this connection is from a basement sump pump or a sewer connection as it is not currently shown on as-built drawings or the Stormwater Atlas supplied by the City. However, despite the original source, this location is known to supply intermittent flows that serve as significant sources of FIB and HBM loading to the system. To reduce the downstream FIB and HBM loading, it is recommended that these flows be diverted to the sewer system.

Based on discussions with the City of Santa Barbara's Public Works Department, it is understood that there is potential for the diversion of water from other groundwater production wells in the downtown area (some of which are under artesian aquifer conditions) to the Ortega Treatment Plant. These wells include the Ortega Park well and the Corporation Yard well. In combination, these wells contribute approximately 36 gpm or 51,000 gallons per day to the Laguna watershed (35 gpm from Corporation Yard Well and 0.6 gpm from the Ortega Well). The diversion of flow from the Corporation Yard well alone to the sewer system would eliminate a significant volume of water to the storm drain that currently provides a constant load of FIB to the system. It is recommended that the diversion of these wells to the Ortega Treatment Plant should be completed to eliminate this upstream source of flow, which may scour storm drain sediment that is high in FIB.

#### Routine Maintenance and Cleanout of Channel and Catch Basins

During field monitoring and sampling events, sediment buildup was observed in several catch basin sumps. An example of this is at sample Location 11 beneath the intersection of Gutierrez and Salsipuedes Streets. The catch basin sump at this location was nearly full of sediment, dead vegetation, and animal waste; the buildup had begun to block off the dry weather flows into one

or two inlets of the downstream Gutierrez line storm drain pipes. The sediment accumulated in this location was observed to be black in color with a sulfurous smell (typically indicative of high organic content from decomposing vegetation and anoxic conditions). Past studies have shown that FIB thrive under such conditions. The Annex yard storm drain pipe was also observed to contain a large volume of highly organic sediment and rotten vegetation; this is likely due to the fact that flows through the pipe are typically too weak to regularly flush out the sediment and vegetation, and the existing inlet trash capture devices at the Annex yard are full and requiring cleanout.

Sediment and organic debris act as a constant source of FIB loading in the storm drain system when not removed periodically. Therefore, it is recommended that a program is established to ensure periodic inspections and cleanout of sediment and vegetation in catch basin sumps and sections of the storm drains where accumulation is found. This can be done at key locations in the storm drain network to reduce these sources of FIB loading. The Annex Yard line, the Gutierrez lines at sample Location 11, and the Laguna line at Locations 8 and 9 are key areas for inspection and maintenance. Additionally, it is recommended that the routine trash collection and feces removal programs along the open channel portion be continued. Additional removal of dead vegetation along the banks and within the channel may also be useful in controlling the production of FIB.

#### **4.1.3 Additional Source Control Evaluation through Supplemental Sampling**

Waterfowl have been observed to congregate at both the small pond at Chase Palm Park (adjacent to the Laguna Channel) and the Mission Lagoon (located at East Beach). The presence of large bird populations and resultant bird feces could potentially be a significant source of FIB to the Laguna Channel and Mission Lagoon. These stagnant bodies of water provide favorable environmental conditions for FIB production and sustainability. In a study conducted in 2006 (Li-Ming He, 2006), FIB in sediment underlying stagnant water bodies was shown to be much higher than beneath flowing bodies of water. It is recommended that the water and sediments in the lower portion of the channel and in the pond at Chase Palm Park be sampled to evaluate any potential contribution that these potential sources may have to the FIB loading in the Laguna Channel and at East Beach. Additionally, as shown through the study conducted by Boehm et. al. (Boehm, 2004), near coastal groundwater can be a source of nutrients for FIB production in the beach zone where groundwater mixes with ocean water. Additional investigation into the possibility of groundwater contamination near the lagoon is recommended.

#### **4.2 Discussion of Treatment Alternatives**

The treatment alternatives reviewed for this project consist of systems involving both mechanized and natural disinfection processes. It is necessary to keep in mind that even after treatment, there is a significant risk of bacterial regeneration and reactivation within the lagoon prior to discharge to the Pacific Ocean. There is also a seasonal impact of the coastal lagoon on

bacterial concentrations in the Ocean. A study performed by Steets and Holden (2002) concluded that although coastal lagoons reduce fecal coliform loading in the ocean by housing fecal coliform sediments during the summer (low flows), the bacteria-loaded sediments are released into the ocean during winter seasons (high flows). Additionally, an increase in bacterial concentrations downstream from the point of treatment can be caused by birds and marine life on the shore or animal activity within the open channel or lagoon. Finally, regardless of re-growth and bird issues, the treated water will ultimately be mixed with discharges from Mission Creek and thereby result in a somewhat cleaner (diluted) but not entirely disinfected lagoon.

The most cost effective and feasible location to install the recommended treatment alternative is at the existing pump station adjacent to the shore. At this location, a power supply is available, dry flows are already captured and pumped out of the channel, the system would be protected from vandalism, and the potential for bacterial re-growth would be limited due to its short distance from the Ocean. This is also the location where Enartec recommended installing the treatment option according to their Laguna Channel Disinfection System Preliminary Design Report for the County of Santa Barbara (2003). Furthermore, installation of the treatment system at this location would not impact flooding issues for the Laguna Channel since operation would occur during dry weather only and there would be no impact to channel flow capacity.

Sizing criteria presented for each treatment option are based on an estimated design flow rate and the water quality standards acceptable for body contact recreation (REC-1). An average flow of 65 gpm (ranging from 49 to 86 gpm) was measured in the channel near the entrance of the pump station. Based on these flow measurement, a conservative value of 100 gpm was selected as a suitable design flow rate, and was used in the evaluation and sizing of each treatment option. AB411 standards for bacterial monitoring in marine recreational waters are provided in **Table 7**. Based on the maximum allowable and measured median concentrations at the inlet to the pump station (Location 2), the highest required percent reduction is for Enterococcus at 87.32%. To achieve this percentage, the sizing criteria should be conservatively based on a 2-log reduction (99%). Based on average measured FIB concentrations, a 99% removal design basis will provide adequate treatment.

**Table 7: AB411 Standards for Bacterial Monitoring in Marine Recreational Waters (State Water Resources Control Board)**

Constituent	Single Sample Maximum (organisms per 100ml)	Location 2 Median (MPN/ 100ml)	Reduction Required
Total Coliform	10,000 1,000 if FC/TC $\geq$ 0.1	> 24,196	> 58.67 %
Enterococcus	104	820	87.32 %
Fecal Coliform	400	1,497 (E. coli)	73.28%

In order to assess the potential impact on FIB loads downstream of the Laguna Channel pump station, a mass balance was completed for the Mission Lagoon based on inflows from Mission

Creek and the Laguna Channel. Dry weather (no rain in the previous 72-hours) water quality data collected between 2001 and 2007 was provided by the City for Mission Creek at Montecito and Laguna Channel at Chase Palm Park. Flow rates of 0.54 cfs (242 gpm) from Mission Creek (per the Creeks Division) and 100 gpm from the Laguna Channel (treatment design flow rate) were used in the mass balance. Mixed FIB concentrations in the Mission Lagoon were computed based on average FIB concentrations and flow rates and assuming a 99% reduction in the concentration of FIB in the Laguna Channel discharge. Over the 2001-2007 study period, such a level of treatment would result in an average reduction of 31% for total coliform, 19% for E. coli, and 28% for Enterococcus in the Lagoon, as compared to the estimated concentrations in the Lagoon with no treatment. It should be noted that the measured Mission Creek FIB concentrations already account for the presence of the SURF facility since it is located upstream of the Mission Creek monitoring site at Montecito Street, therefore no data adjustments were necessary.

As a rough estimate of the impact of the project on the number of posted beach warnings, the AB411 FIB data collected by the County at East Beach were adjusted by the reduction percentages presented above based on the Lagoon mass balance analysis. Reductions were applied to the measured FIB concentrations for April 1 through October 31 (AB411 dry weather) of each year. The number of annual posted beach warnings are presented in **Table 8** below. The values shown in the parentheses indicate the number of *dry season* postings, counted as April 1 through October 31 only (rest of year is excluded). Results suggest that if the Project were to be implemented, the average number of beach postings may be reduced from 22 to 21 per year and from 7 to 6 per dry season. These results make sense considering the relative magnitude of the Mission Creek inflow as compared to that of the Laguna Channel in combination with the fact that FIB concentrations vary on a log scale and as such exceedance frequencies are not impacted to a significant degree with 19-31% concentration reductions.

**Table 8. AB411 Annual Predicted Warnings Posted at East Beach (dry season results shown in parentheses)**

Year	Warnings	Predicted Warnings
2001	29 (6)	27 (6)
2002	20 (6)	17 (6)
2003	12 (1)	12 (1)
2004	20 (5)	19 (4)
2005	30 (12)	28 (10)
2006	31 (15)	30 (14)
2007	12 (5)	11 (4)
<b>Average</b>	<b>22 (7)</b>	<b>21 (6)</b>

A 99% reduction in HBM would result in a treated concentration of 233 copies/L, which is equivalent to approximately 0.00000003% sewage (reduced from 0.000003% sewage untreated), or raw sewage that is diluted 33 million times, based on a HBM sewage sample concentration provided in a paper by Bram et. al. (2009). No HBM data is available for Mission Creek so a mass balance analysis cannot be completed for the Lagoon.

#### 4.2.1 Mechanized Disinfection Alternatives

Disinfection is the process that destroys or inactivates disease-causing pathogens, such as bacteria, viruses, and protozoa. For the purpose of this study, the bacterial disinfection feasibility and efficiency will be discussed below for the following disinfection alternatives: chlorination, UV disinfection, ozone, and peracetic acid disinfection.

##### Chlorination

Chlorine is the most widely used chemical disinfectant for wastewater in the United States. It can inactivate more than 99% of pathogenic bacteria (Geosyntec, 2008). However, due to the risks of storing this chemical on site and the harmful impacts the residual can have on the downstream habitat and environment, this disinfection treatment will be dismissed for further discussion and is not recommended for use at this site.

##### Ozone

Ozonation is another alternative used for bacterial disinfection. The preliminary design report for the Laguna Channel Disinfection System prepared by Enartec (2003) states that ozone disinfection is the most beneficial bacterial treatment option for this specific project. This conclusion was based upon a variety of factors which include the system's simplicity, reliability, flexibility, controllability, implementation ability, and odor control ability.

Advantages to ozone disinfection include the following:

- Ozone's bacterial disinfection effectiveness is superior to most chlorine compounds;
- It does not produce disinfection residuals;
- Ozone rapidly dissipates and decomposes when exposed to oxygen; and
- Ozone requires a short contact time.

Disadvantages of ozone disinfection include the following:

- It is more costly than chlorinated disinfection treatment options;
- Ozone gas is chemically unstable and it is dangerous to transport; therefore, it must be produced on-site (EPA, 1999); and
- Brominated disinfection byproducts are potential for concern in waters containing bromide. Other byproducts include aldehydes, ketones, and carboxylic acids (Natural Drinking Water Clearinghouse, 1999).

Three necessary parts of an ozone disinfection facility include an ozone production chamber, a contactor tank, and an ozone destruction device. Due to ozone's molecular instability and the dangers associated with having the gas stored on location, an on-site ozone production facility is necessary to produce the chemical throughout treatment. Ozone can be produced from any gas containing oxygen molecules; therefore pure oxygen or atmospheric air can be used in the

production chamber. Pure oxygen is the more costly option; however, it would produce ozone more efficiently than atmospheric air and would need much less treatment before the production phase. Atmospheric air is obviously more attainable and less costly than pure oxygen; however, because ozone generators require clean, dry gas for efficient production, the oxygen would need to be compressed, cleansed, and dehumidified (EPA, 1999).

The contactor tank is the location where the water comes into contact with the gas. A commonly used contactor device in North America is a bubble diffusion system that ranges from 15 to 24 feet deep (Ferguson et. al., 1991). During contact with ozone, the entire cell is inactivated due to the damage ozone causes to the cell membrane, nucleic acids, and some enzymes (Paraskeva and Graham, 2002). Throughout treatment, the ozone off-gas from the contactor device must be destroyed on location. This is done most commonly by a thermal-catalytic system attached to the contactor (Tate, 1991).

Ozone's disinfection efficiency is relatively good. Its effectiveness against *E. coli* is very high due to the bacteria's sensitivity to this treatment. A 4 log reduction (99.99 percent removal) can be achieved in less than 1 minute of contact time, with an ozone residual of 9 µg/L and at a temperature of 12°C (Geosyntec 2008). Ozone has also shown to remove greater than 50 percent of total coliform from influent concentrations (Metcalf & Eddy, 2003). Ozone also has the ability to remove many naturally occurring odors (Droste, 1997), which will likely increase the public's confidence in the treatment process.

The sizing criteria of an ozone disinfection system heavily rely on the design flow rate and the quality of the water being treated. The design flow rate is used to calculate the amount of ozone needed to be produced on site. With a design flow rate of 100 gpm and a minimum efficiency of 99% bacterial removal, the approximate footprint of the ozone treatment plant would be 8 feet by 8 feet. It is important to keep in mind that further water quality testing is necessary to determine which oxygen demanding compounds are present in the water and at what concentrations. These compounds will increase the amount of ozone needed to properly disinfect the water. The description of the ozone process train is provided in **Table 9**.

To increase ozone's disinfection efficiency, the disinfection system is usually designed in conjunction with a pretreatment system that removes larger suspended solids. Although ozone can treat turbid water, the disinfection system would be more effective (i.e., a smaller ozone dose would be required to treat less turbid water) if paired with a mechanical separator or a coarse strainer (Smith-McCollum, 2007). One strainer option is the Eaton Model 2596 automatic, self-cleaning strainer with an automatic backwash system.

**Table 9: Ozone Treatment Train Assembly (Moldzio, G. B., 2008)**

Treatment Component	Description
Ozone Generator	<ul style="list-style-type: none"> <li>• Air or water-cooled</li> <li>• Fully automated</li> <li>• Oxygen or compressed air as feed gas</li> <li>• 220 volts, 60 hertz</li> </ul>
Air Compressor	<ul style="list-style-type: none"> <li>• Oil-free</li> <li>• Feeds compressed air to the oxygen concentrator</li> </ul>
Oxygen Concentrator	<ul style="list-style-type: none"> <li>• Feed oxygen at a purity greater than 95% into the ozone generator</li> </ul>
Venturi Injection Station	<ul style="list-style-type: none"> <li>• Mixes ozone gas with water</li> <li>• Stainless steel injector with multi-stage turbine pump</li> </ul>
Retention Tank	<ul style="list-style-type: none"> <li>• Two-minute retention time for disinfection</li> </ul>
Catalytic Ozone Destruction Unit	<ul style="list-style-type: none"> <li>• Removes ozone off-gas from the reactor tank</li> </ul>
Electronic Control Cabinet	<ul style="list-style-type: none"> <li>• Interfaces the ozone generator with injector pump and main pump</li> </ul>
Metal Skid	<ul style="list-style-type: none"> <li>• Base for injection system</li> </ul>

The public's perception of the treatment facility's implementation would likely be positive if it is well communicated that ozone is not similar to chlorine in the way it breaks down (i.e., chlorine residual remains in the treated water, whereas ozone completely disassociates and does not leave any residual in the effluent). However, because ozone is a chemical, the misconception that all chemicals are bad for the environment may surface during public discussion. Even so, this can be easily clarified and would not likely cause a large roadblock during implementation.

Careful operations and maintenance of an ozone disinfection facility is important to ensure efficient water treatment. In order for the water to be contacted uniformly, a near plug flow must be attained in the contactor tank. An energy supply of 96 kWh is also necessary for the function of the facility described above.

At a minimum, maintenance for an ozone facility is likely to include:

- Regular leak inspections;
- Periodic replenishment of the oxygen supply, if applicable;
- Consistent cleaning of all parts of the system; and
- Monitoring the ozone production chamber's temperature (EPA, 1999).

## UV Disinfection

UV disinfection is another treatment alternative for dry weather flows within the Laguna Channel. A UV bulb emits a wavelength of up to 254 nm, which penetrates the cell wall of a microorganism and is absorbed by cellular materials such as nucleic acids. This absorption will either keep the cell from reproducing or destroy the cell entirely (Geosyntec, 2008). Because UV treatment does not involve chemicals, there are no toxic byproducts or residuals in the treated discharge.

The efficiency of UV radiation greatly depends on the turbidity of the water. Total suspended solids in the water will deflect and absorb the UV energy being emitted so that bacterial cells are not treated. For this reason, UV radiation is not used on wastewaters with high total suspended solids concentrations. UV radiation has, however, been proven to disinfect water from pathogenic bacteria and most viruses with a similar efficiency to chlorine, which was stated above as having greater than a 99% bacterial inactivation rate. Although UV disinfection has a high bacterial inactivation rate, it is necessary to keep in mind that some cells are not fully destroyed during treatment and are able to recover and regenerate downstream of the treatment facility (Droste, 1997).

Most UV units are designed to provide a dosage greater than 30,000  $\mu\text{W}\cdot\text{sec}/\text{cm}^2$  after a full year of operation (Edstrom Industries, 2003). To put this in perspective, a dosage of 7,000  $\mu\text{W}\cdot\text{sec}/\text{cm}^2$  can reduce water's *E. coli* concentration by 99.9%. The unit of dosage is the product of intensity and contact time and varies depending on the bacteria being removed.

In Enartec's discussion of UV radiation in their Preliminary Design Report (2003), low pressure (LP) lamps were recommended over the option of medium pressure (MP) lamps. However, there are both advantages and disadvantages to the two bulb types. See **Table 10** for the lamp comparisons.

**Table 10: MP and LP Lamp Comparison**

Characteristic	Medium Pressure Lamp	Low Pressure Lamp
Footprint	Smaller	Larger
Number of Bulbs	Smaller	Larger
Required Energy	Larger	Smaller
Lamp Life	Shorter	Longer

Before selecting a bulb type, it is recommended that the pros and cons of each option be further considered as related to project-specific criteria (i.e., space availability, energy use, etc.).

At a minimum, processes associated with a UV radiation treatment facility which are likely to require maintenance are described below:

- UV lamps should be cleaned on a regular basis because build-up will decrease the amount of UV radiation reaching and disinfecting the water;
- Because the average life of a low pressure UV bulb is 8,000 to 12,000 hours and the average life of a medium pressure UV bulb is 4,000 to 8,000 hours, regular bulb replacement is necessary (EPA, 2006); and
- Due to the heat associated with this facility type, bacteria growth may occur on facility surfaces (i.e., within UV contact chamber) and should be regularly cleaned.

The sizing of a UV disinfection system is based on the design flow rate, which in this case is 100 gpm. The following estimated size is taken from an Integrated Aqua Systems, Inc. UltraDynamic Series specification pamphlet (2004). For flow between 90 and 100 gpm (65 to 90 percent transmissivity, respectively), the dimension of a UV disinfection unit would be 66" x 8 1/4" x 13" and would consist of 4 low pressure UV lamps. The energy consumption of this unit is estimated to be approximately 9.2 kWh/day.

Because UV radiation disinfects by destroying or altering the DNA of a bacterial cell, it does not remove odors nor increase the clarity of the water. For this reason, turbidity has a negative effect on this treatment option's efficiency. Turbidity data was provided by the City and a dry-weather (April through October) average turbidity value of roughly 7 NTU was calculated (see Appendix F for raw turbidity data). Although the dry weather results indicate a relatively low turbidity, to increase the efficiency of the UV treatment system it is recommended that a sand filter is implemented as pretreatment. One sand filtration option is a 36" diameter mechanical system that can filter up to 140 gpm. This system includes an automatic backwash system, a 3.0 hp pump, and sits on a 48" x 60" x 6" high density polyethylene base (Integrated Aqua, 2009). To maintain this filter system, it would need to be back-flushed with water regularly to alleviate clogging. For example, if back-flushing took place for two minutes each week at a flow rate of 150 gpm, there would be 1,200 gallons of back-flush water per month. Back-flush water can be discharged to the sewer system; however, if there is not sufficient sewer capacity, an equalization tank may be used to release the flows at an acceptable rate.

Public perception of a UV radiation facility would likely be positive due to the absence of chemicals in the treatment method. There would be no storage or transportation of unstable chemicals. The only characteristic of UV radiation that may cause speculation is the fact that the water does not seem "cleaner" post-treatment. Treated water would not appear clearer nor would it smell any different than the UV facility inflow. The potential reduction in beach postings would need to be the public's visual evidence to gain confidence in the treatment method.

### Peracetic Acid

Peracetic acid (PAA) has become a disinfection alternative that is rising in popularity and, although mainly used in the food and beverage industry, can be applied to urban runoff

discharges. The chemical mixture is a combination of glacial acetic acid, hydrogen peroxide, and water (EPA, 1999). Peracetic acid disinfects through oxidation. It deactivates bacteria and virus cells by instigating electron transfer when oxidizing a microorganism's cell wall. When added to water, the peracetic acid degrades into acetic acid, carbon, oxygen and water, all of which are non-toxic, odorless compounds.

Although peracetic acid has a non-toxic disinfection nature, there are some disadvantages to this treatment option. The most significant of which is the explosive nature of the peracetic acid itself. For this reason, the individual liquid reactants, which are combined to create peracetic acid, must be transported onto the facility site separately and must be combined on site, as-needed (EPA, 1999). Because of the instability of the final compound, the maintenance and operation of the treatment facility must be performed with caution and inspections must be thoroughly completed.

Maintenance requirements would likely include the following:

- The facility's emergency sprinkler system should be inspected regularly due to the explosive nature of the chemical agents;
- Feed lines, storage areas, leakage detention equipment, and chemical injectors should also be inspected regularly;
- Chemicals should be stored in a cool, dry, well-ventilated area; and
- The facility building should be made of fire resistant material with a low explosion potential.

Another disadvantage of this treatment options is that, due to the recent emergence of this stormwater disinfectant method, there have not been many cases of peracetic acid selected and implemented for the disinfection of urban runoff. Therefore, there are few examples from which to gain design and technical insight and there is limited information on the efficiency of the process itself. Also, because of the constant transportation of the chemical and on-site storage, there will likely be permitting issues that will increase the time and cost of implementing a treatment facility of this nature (CDM and Geosyntec, 2006).

Due to the explosive nature of peracetic acid's final compound, there is potential that the public will perceive this treatment option as highly volatile and dangerous for a project site adjacent to a popular beach. For this reason and the lack of reliable historical data for the treatment of runoff with peracetic acid, it is not recommended as a viable treatment alternative for the project site.

#### **4.2.2 Natural Treatment System Alternatives**

Described below are two natural dry weather flow treatment options. The most feasible location to install either of these two treatment options is downstream of the existing pump station, to the east of the Laguna Channel where the water would be diverted at the pump station to the

treatment system. The construction wetlands alternative would be located at Chase Palm Park, east of the existing pumping station and the infiltration trench would be located below the beach itself, east of Mission Lagoon.

### Constructed Wetlands

Constructed wetlands are a natural treatment alternative specifically designed to reduce pollution and manage waste. There are two types of wetlands: surface and subsurface flow wetlands. These two types are similar in that they both involve a lined basin or channel to prevent seepage into the ground. They also both consist of emergent vegetation with a root system through which the water is treated via horizontal flow.

The difference between the wetland types is that surface flow wetlands are characterized by lateral flow with a free water surface and subsurface flow wetlands are characterized by a lateral flow through a porous media with a water level lower than the top of the media (EPA, 1993). Due to the potential swampy appearance and the possibility of mosquito breeding, surface flow wetlands are not recommended for implementation at East Beach. For this reason, subsurface flow wetlands (SSF) are discussed below.

There are many advantages to the use of SSF wetlands as a treatment option. Most importantly, SSF wetlands are effective in removing bacterial loads in water. Studies have shown that SSF wetlands can reduce total coliform by greater than 99% (Kadlec and Knight, 1996). In addition to its treatment efficiency, SSF wetlands can provide an aesthetically pleasing environment; the vegetation may be an attractive addition to the surrounding area. Odor control would not likely be a concern as the water surface is below ground level. The combination of these advantages would potentially create a positive public perception due to its “eco-friendly” appearance and nature.

Maintenance may include but is not limited to the following:

- Regularly scheduled inspections for burrows, sediment accumulation, litter, and debris;
- The removal of accumulated sediment in the forebay and a re-grading every 5-7 years when the accumulated sediment volume is greater than 10 percent of the basin volume (California Stormwater Quality Association, 2003); and
- Regularly scheduled inspections (structural integrity and debris cleanout) of inlets, outlets, and monitoring devices (EPA, 1994).

Despite the mentioned advantages, there are some disadvantages to this treatment method. One disadvantage is the large amount of land required. Such a design constraint could ultimately eliminate this as an option if sufficient land area cannot be obtained. Because the most beneficial location for the wetlands facility would be near the outlet of the existing pump house, in Chase Palm Park or on the beach itself, it is likely that such high-value, publicly owned land

would be difficult to obtain if not already City-owned. A significant construction cost is also anticipated due to the large volume of excavation required. Also, because the construction would take place next to the shore, permitting complications could arise (i.e., involvement with the California Coastal Commission). Taking into account these disadvantages, it is recommended that implementing a wetlands treatment facility would not be feasible downstream of the pump house and will not be further evaluated as a treatment alternative for this project site.

#### Subsurface Disposal via Infiltration Trench

Another natural treatment option that can be applied to this study is a subsurface infiltration trench. These facilities would resemble open-bottom infiltration chambers to which water can be diverted. This captured runoff would pass through bottom of the unit and ultimately infiltrate through the existing sand layer below which would act as a filter. Over time, a biofilm layer will form and increasingly remove bacteria in the water as it passes through. The amount of water that can be treated through this system depends on land availability and the infiltration rate of the sand beneath the infiltration chambers (Burchell et. al., 2007).

The advantages of this treatment option include the following:

- The system is completely underground (i.e., out of sight, out of mind);
- The beach's aesthetics would be unaffected; and
- Odor would not be an issue.

One disadvantage of this treatment system is that, because it is a large construction project (excavation for a long trench), a building permit may be required. Also, the location of this system would need to be on the beach and the California Coastal Commission would potentially become involved in the implementation process.

The major disadvantage of this treatment option, which would be a potential roadblock to the implementation of the facility, is the public's perception of how the water is actually being treated (i.e., the misconception that the option would not treat the water but simply dispose of it beneath the sand surface). For this treatment method to actually become feasible on the project site, further discussion with the public would be necessary to increase understanding of the infiltration/treatment process.

The maintenance of the infiltration trench is essential to the efficiency of the treatment system. Every six months or as frequently as necessary, trash, paper, and debris would need to be removed from the filters if no pretreatment were provided. Additional maintenance may include removing and replacing the top 1-3 inches of sand (Burchell et. al., 2007).

The sizing criteria of the subsurface infiltration trench are dependent on the influent flow rate and the infiltration rate of the existing beach sand. A conservative infiltration rate of a sand filter

is 3.5 ft/day (Center for Watershed Protection, 1996). As stated previously in discussions of other treatment alternatives, the design flow rate is 100 gpm. These factors result in an infiltration trench footprint of about 5,600 square feet. A typical infiltration trench is roughly 4 feet wide, which would result in a trench 1,400 feet long. This is a large amount of land to be excavated and to be under construction in a public area. Due to the amount of land necessary for an effective treatment facility and the issues that may arise from public perception, this treatment option will likely not be feasible for the project site and will not be evaluated further.

Although treatment via either constructed wetlands or a subsurface infiltration trench would likely reduce the bacterial loading on receiving waters, both systems require a much larger footprint than the mechanized treatment options described prior, and therefore would not be cost effective for the City to implement.

#### 4.3 Possible Environmental Review and Permitting Requirements

Due to various state and regional requirements, various permits and environmental review processes are likely to be required in order to implement these treatment alternatives. These requirements may include but are not limited to those listed in **Table 11** below.

**Table 11: Potential Environmental Review and Permit Requirements**

Possible Environmental Review or Permit Required	Description
National Pollutant Discharge Elimination System (NPDES) Permit	Required for potential pollutant discharge directly into surface waters. This permit may likely be required for chemical treatment alternatives.
Army Corps of Engineers Nationwide Permit (NWP)	<ul style="list-style-type: none"> <li>• Maintenance of a structure or the removal of accumulated sediments and debris near or within existing structures (NWP 3)</li> <li>• Structural discharges (NWP 25)</li> <li>• Construction of stormwater management facilities (NWP 43)</li> </ul>
City of Santa Barbara Coastal Development Permit	Required for development within the local coastal plan boundary
Regional Water Quality Control Board (RWQCB) Clean Water Act Section 401 Certification	Required for any license or permit used by a federal agency for potential discharge into State waters.

#### 4.4 Treatment Alternatives Cost Analysis

Estimated cost information is presented for ozone disinfection and UV radiation in **Table 12**. Based on project design constraints, these two alternatives were selected as the most feasible and appropriate for the treatment of dry weather flows from the Laguna Channel.

**Table 12: Ozone and UV Cost Comparison**

	Ozone	UV <sup>8</sup>
Primary Treatment Facility	\$75,000 (minimum) <sup>9</sup>	\$22,000 <sup>10</sup>
Pretreatment System	\$11,000 (minimum) <sup>9</sup>	\$11,000 (minimum) <sup>9</sup>
Back Flush Equalization Tank	\$1,200 (minimum) <sup>9</sup>	\$1,200 (minimum) <sup>9</sup>
<b>Total Capital Cost:</b>	<b>\$87,200 (minimum)</b>	<b>\$34,200 (minimum)</b>

The estimated capital cost of the typical ozone treatment facility as described in **Table 9** is a minimum of \$75,000 as quoted by BiOzone, Inc. (Moldzio, 2009). This includes engineering, supervision, and management of the ozonation plant by trained personnel as well as assembly of the unit into a trailer for transport. Unit delivery and installation is not included in the estimate. A coarse strainer is recommended as a pretreatment system for ozone disinfection and was quoted by Hayward Filtration as a minimum of \$11,000. Conservatively assuming no back flush water can be discharged to the sewer facility, an equalization tank for the coarse strainer back flush water would be a minimum of \$1,200.

The above cost for the UV system is a simplified estimate that does not include facility design costs, construction costs, and other implementation costs. This cost is scaled down proportionate to load reduction demand from the Westside SURF Project cost. Per City contact, the UV treatment equipment alone cost roughly \$122,000. This equates to approximately  $1.3 \times 10^5$  MPN/day/project dollars spent. The E. coli load in the Laguna Channel study was estimated as  $2.9 \times 10^9$  MPN/day. Using the load per dollar spent ratio from the SURF project, the cost of the UV treatment system in this study can be estimated at \$22,000. It was reported that the total SURF UV project cost was approximately \$1 million. This includes personnel services, consultant services, and construction (City of Santa Barbara, 2008), none of which are assumed to be included in the \$122,000 capital cost mentioned previously. Using a similar scaling method as above, the entire UV treatment project recommended in this study can be estimated to cost roughly \$330,000.

A vendor quote was also received for the UV treatment system described in Section 4.2.1.3 included the cost of an optional UV monitoring device (shows the UV efficiency of the lamps) for a minimum of \$1,450 and a manual quartz sleeve wiping system which was quoted as a

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<sup>8</sup> The cost for the UV system is a simplified estimate that does not include facility design costs, construction costs, and other implementation costs.

<sup>9</sup> This value was obtained from a vendor and should be viewed as a minimum estimation and should not be used as a direct cost comparison with implemented project costs, which is the basis for the UV primary treatment facility cost estimate.

<sup>10</sup> This value is scaled down from the estimated \$122,000 cost of the UV equipment for the City's SURF Project.

minimum of \$600<sup>9</sup>. Because filtration is necessary for efficient UV disinfection, a sand filter is recommended as a pretreatment option and was quoted by Integrated Aqua as a minimum of \$11,000<sup>7</sup>. The back flush equalization tank was estimated to be the same size as that of the ozone pretreatment system.

The operation and maintenance costs of both systems are derived from the annual energy cost to operate the system in addition to the regular replacement of facility components. For the purpose of this cost estimate, it is assumed that the energy cost is a minimum of \$0.25 per kilowatt-hour and that the facilities are running 24 hours a day, 365 days per year.

Operation and maintenance costs are shown in **Table 13**.

**Table 13: Operation and Maintenance Costs**

Facility Type	O&M Item	Facility Demand	Unit Cost	Annual Cost
UV Disinfection	Energy Consumption	9.2 kWh/day	\$0.25/kWh	\$840
	Annual Maintenance <sup>11</sup>	-	-	\$11,160
	Replacement Bulb Supply (avg. LP bulb life = 10,000 hours)	4 bulbs	\$210/bulb	\$840
	Annual O&M Cost			\$12,840
Ozone Disinfection (Moldzio, 2009)	Energy Consumption	96 kWh/day	\$0.25/kWh	minimum \$8,760 <sup>9</sup>
	Annual Maintenance	\$3,000/year	-	minimum \$3,000 <sup>9</sup>
	Regular Monitoring	15 hours/month	\$15/hour	minimum \$2,700 <sup>9</sup>
	Spare Parts Depot	1/year	\$875/year	minimum \$875 <sup>9</sup>
	Annual O&M Cost			minimum \$15,335 <sup>9</sup>

Implementation costs associated with each treatment alternative may be reduced pending the degree of upstream pollutant/flow removal. For example, diverting flows from the Ortega well to the sanitary sewer system could reduce the total treatment system design flow. However, prior to such a diversion actually being implemented, it is difficult to estimate the exact cost-benefit due to the significant variance and uncertainty in measured flows at specific locations as

<sup>11</sup> The annual maintenance value for UV was derived from the facility maintenance cost of the SURF project. Per City contact, \$18,000 per year is spent on maintenance of the 150 gallons per minute treatment system. This cost was scaled down for the Laguna Channel design flow of 100 gallons per minute and the cost of replacement bulbs was subtracted from the maintenance total and inputted as a separate line item.

well as along the drain network itself. A more accurate estimate of the flow rates in the Channel at the pump station should occur prior to revising the design flow based on proposed upstream diversions. Another example of potential cost savings may result from increasing the level of maintenance on the existing storm drains and channel; this could lead to less sediment accumulation and decaying vegetation, both of which have been noted to contribute to FIB concentrations downstream.

#### **4.5 Treatment Alternatives Decision Matrix**

In order to evaluate the overall feasibility and desirability of each treatment alternative discussed in Section 4.2.1 and 4.2.2, a decision matrix was established as shown in **Table 14**. Each treatment alternative is ranked from 0 (not desirable) to 5 (very desirable) for each of several key selection criteria. Additionally, some criteria are more important than others with respect to feasibility; to address this, the criteria deemed most important have been weighted higher (i.e., each operations and maintenance score was been multiplied by 2).

The decision matrix shown in **Table 14** is broken up into six categories: safety, public perception, land requirements, ecological impacts, capital cost, and operation and maintenance cost. These criteria are listed below with questions that were considered while applying numerical rankings to each alternative:

1. Safety
  - Is there a toxic chemical involved in the treatment process? If so, will this chemical need to be transported to the site?
  - Are their toxic byproducts?
  - Are there other hazards associated with this facility?
  
2. Public Perception
  - Will the public think this facility is safe?
  - Will the public think the facility is effective?
  - Does the treated water “appear” to be cleaner?
  - Does this facility degrade or add aesthetic beauty to the area?
  - Will this facility emit unwanted odor?
  - Will construction be a public nuisance?
  
3. Land Requirements
  - Does this facility require a large plot of land?
  - Is the required land in a costly location?
  
4. Ecological Impacts

- Does the implementation of this facility negatively impact the ecology of the surrounding area?

5. Capital Cost

- What is the capital cost of the facility?
- Will there be significant long-term construction?
- Is there potential for an intensive permitting process?

6. Operation and Maintenance

- How often does equipment need to be replaced?
- How often does this facility need to be cleaned?
- Is the cleaning of this facility labor intensive?
- What is the energy consumption of this facility?
- Does the facility need to be intensely monitored?

As discussed in **Table 14**, based on the specified criteria and weighting system, the most desirable treatment alternatives are disinfection using UV (score = 35) and ozone (score = 28). **Figure 13** illustrates the conceptual design for the recommended UV disinfection system.

**Table 14: Treatment Alternative Decision Matrix**

Treatment Alternatives	Criteria (weight)						Total	Discussion
	Safety (1)	Public Perception (2)	Land Required (1)	Ecological Impacts (1)	Capital Cost (1)	O&M (2)		
<b>Chlorine</b>	0	1	4	1	4	4	<b>19</b>	Chlorine is a toxic compound that leaves residuals that can threaten aquatic life downstream. Treated water would appear cleaner; however, due to residuals and the risks of on-site chemical storage, negative public perception is expected. Compared to the other alternatives, the land requirements, capital cost, and O&M are low. Capital cost includes the treatment tank and initial chlorine supply. O&M consists of regular cleaning of the system and chlorine re-supply.
<b>UV</b>	5	4	4	5	5	4	<b>35</b>	UV is a safer option with no known downstream ecological affects. Public perception would be positive because no chemicals are involved. Because UV does not remove odors or make water visibly clearer, water may appear untreated to the public. The system may be placed in the existing pump house and does not require additional land. The capital cost is low compared to other alternatives. O&M includes regular inspection, cleaning, bulb replacement, and energy supply.
<b>Ozone</b>	4	4	4	4	3	3	<b>28</b>	Ozone involves on-site production of an unstable chemical. Depending on the influent's chemical composition, ozone treatment could produce brominated disinfection by-products. Ozone does not produce disinfection residuals and dissipates when exposed to oxygen. Public perception would be good because treated water would be visibly cleaner; however, because ozone is a chemical, the public may wrongfully assume it is not good for the environment. This facility may be placed in the existing pump house. The capital cost is greater than UV and O&M includes inspection, cleaning, and energy supply.
<b>Peracetic Acid</b>	2	0	4	4	4	4	<b>22</b>	This treatment type is less safe than UV or ozone because of the compound's explosive nature. For this reason and the lack of implementation examples, the public may have a negative response. The footprint, capital cost, and O&M would be similar to that of a chlorine facility due to its comparable configuration.
<b>Subsurface (SSF) Wetlands</b>	5	3	0	5	1	5	<b>27</b>	SSF wetlands are very safe and would likely not impact downstream ecology. Public perception would be good due to its aesthetic character and use of natural processes. However, because a large wetland is necessary and installation includes construction on the beach, the public may dislike disturbances. Capital cost would be large due to land requirement and additional permits. The O&M is less frequent, but potentially more labor intensive.
<b>Infiltration Trench</b>	5	2	0	5	1	5	<b>25</b>	An infiltration trench is safe because it involves natural treatment and is completely underground. Negative public perception may arise because of misconception that the water is secretly discharged instead of treated through natural filtration. The facility would have no odor problems and would not affect the beach's aesthetics or ecology. The footprint and capital cost of this system is undesirable because it requires a large amount of costly land. Despite large initial cost, O&M would require no energy and would need maintenance less frequently than the other mechanical treatment options.

## 5.0 CONCLUSIONS AND RECOMMENDATIONS

A multi-tiered approach is recommended for the treatment of dry weather flows in order to meet the project goal of ultimately reducing the risk of human illness at East Beach by reducing the loading of human fecal contamination into the Mission Lagoon, to which the Laguna Channel discharges.

First, prior to the construction of any treatment facility, source control measures should be implemented where appropriate and feasible.

Recommended source controls include:

- Continued public outreach;
- Investigation into and repairs of potential sewer leaks;
- Restoration of the low flow diversion from the Annex Yard to the El Estero Wastewater Treatment Plant;
- Investigation into and possible diversion of cross connection to the Laguna storm drain line upstream of the Laguna Street and Ortega Street intersection;
- Diversion of flows from the Ortega Park and Corporation Yard wells;
- Routine maintenance and cleanout of the Laguna Channel and catch basins; and
- Supplemental sampling within the lower Laguna Channel and the pond at Chase Palm Park.

Then, if elevated bacteria levels persist despite source control implementation, structural treatment should then be implemented as necessary. Structural treatment design flow rates should be based on the state of the watershed post-implementation of source controls (for instance, the implementation of source controls could also result in reduced upstream bacteria levels, and therefore result in the requirement of a lesser degree of treatment than originally anticipated) but are approximated as 100 gpm. Based on a capital cost, operations and maintenance cost, public perception, land requirements, ecological impacts, and project safety, UV disinfection was determined to be the most in line with the project design goals and site constraints. Project costs are estimated to range from \$22,000 for equipment alone to \$330,000 for project completion including design, construction, and permitting. Operations and maintenance costs of a UV disinfection facility are estimated at \$13,000 per year.

It should also be noted that the disinfected runoff from Laguna Channel is not expected to entirely retain its quality upon comingling with the untreated waters of Mission Creek due to mixing, bird contributions, and re-growth in the lagoon. A 2-log level of treatment (99% removal) of FIB is recommended for the Laguna Channel dry weather flows. Based on

evaluation of FIB data collected from Mission Creek and Laguna Channel between 2001 and 2007, such a level of treatment is estimated to reduce the FIB concentration in the Mission Lagoon by approximately 19-31%. Such a reduction in FIB applied through the AB411 dry season (April – October) was only predicted to reduce the number of beach postings from 22 to 21 (annually) and from 7 to 6 (dry season only). Therefore, the benefits of the project, much like the City's Mission Creek (SURF) project, would be to address human fecal contamination coming from the Laguna Channel and discharging to the Lagoon and the Pacific Ocean, with significant uncertainty associated with the effects of Mission Lagoon.

Although the benefits of the project include reducing the risk of human illness in the Laguna Channel, in the Mission Lagoon, and ultimately at East Beach, there are potential downsides that need to be acknowledged prior to the implementation of these recommendations. One disadvantage is the significant capital cost of project implementation combined with the long-term cost of energy, operation, and maintenance. Other potential problems include the potentially negative public perception of channel flow disinfection and the unknown ecological impacts to the lagoon after discharging disinfected water.

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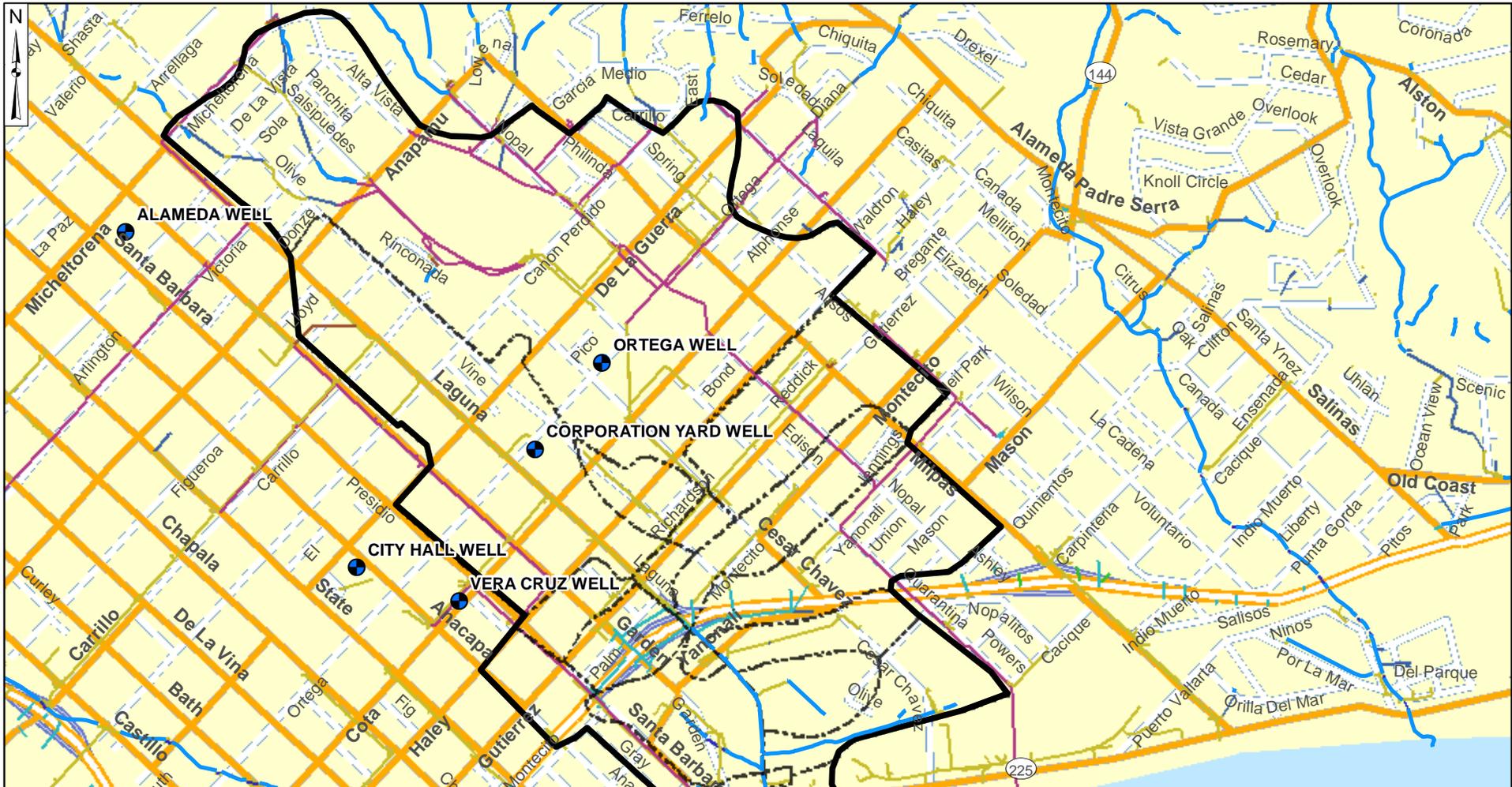
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# FIGURES



<b>Site Location Map</b> Laguna Channel Santa Barbara, CA		<b>Figure</b>  <b>1</b>
Project No. LA0178	March 2009	

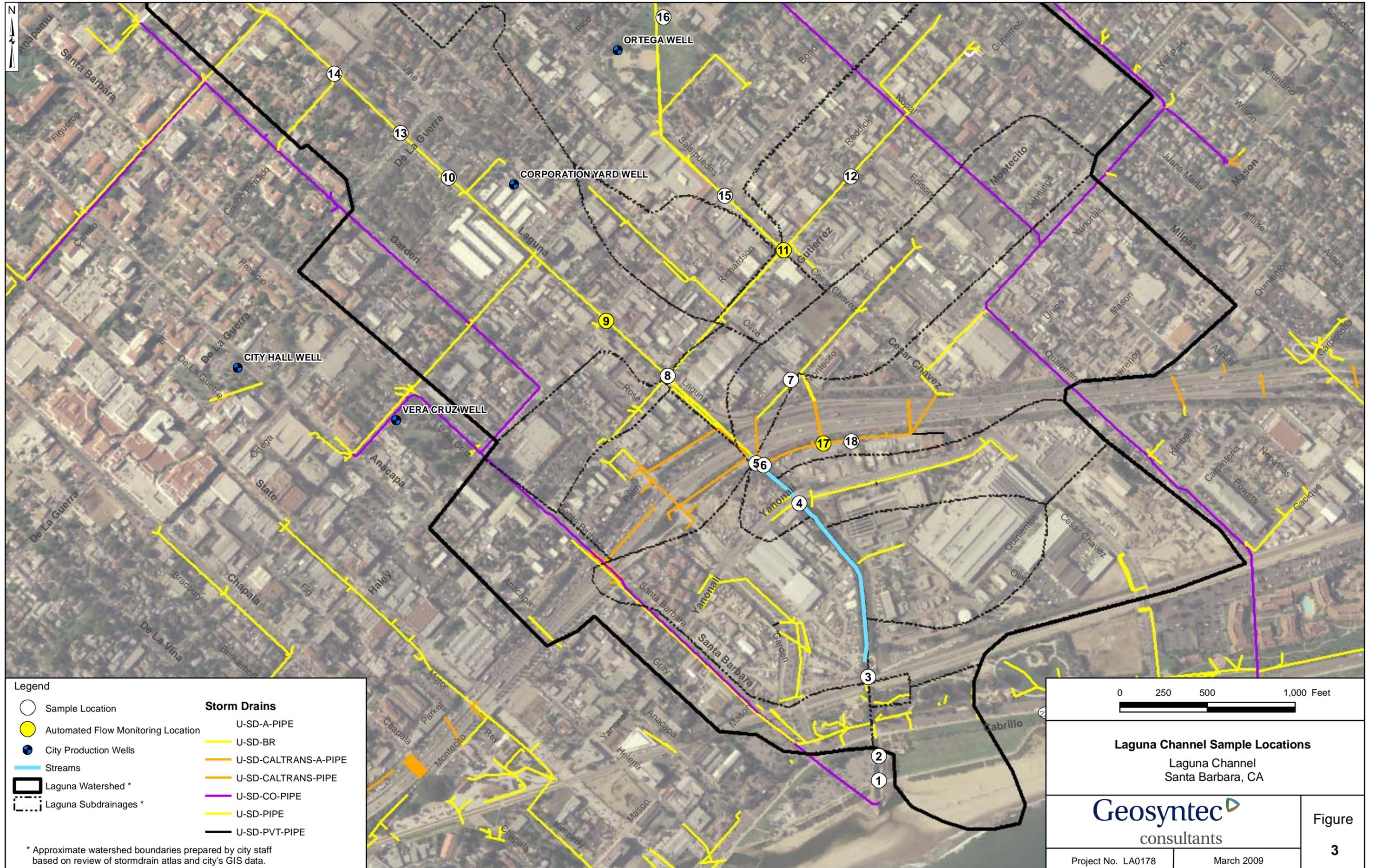


**Legend**

- City Production Wells
- Channel
- Laguna Watershed \*
- Laguna Subdrainages \*
- U-SD-A-PIPE
- U-SD-BR
- U-SD-CALTRANS-A-PIPE
- U-SD-CALTRANS-PIPE
- U-SD-CO-PIPE
- U-SD-PIPE
- U-SD-PVT-PIPE

\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.

<p><b>Laguna Channel Watershed</b></p> <p>Laguna Channel Santa Barbara, CA</p>	
Project No. LA0178	March 2009
<p>Figure <b>2</b></p>	





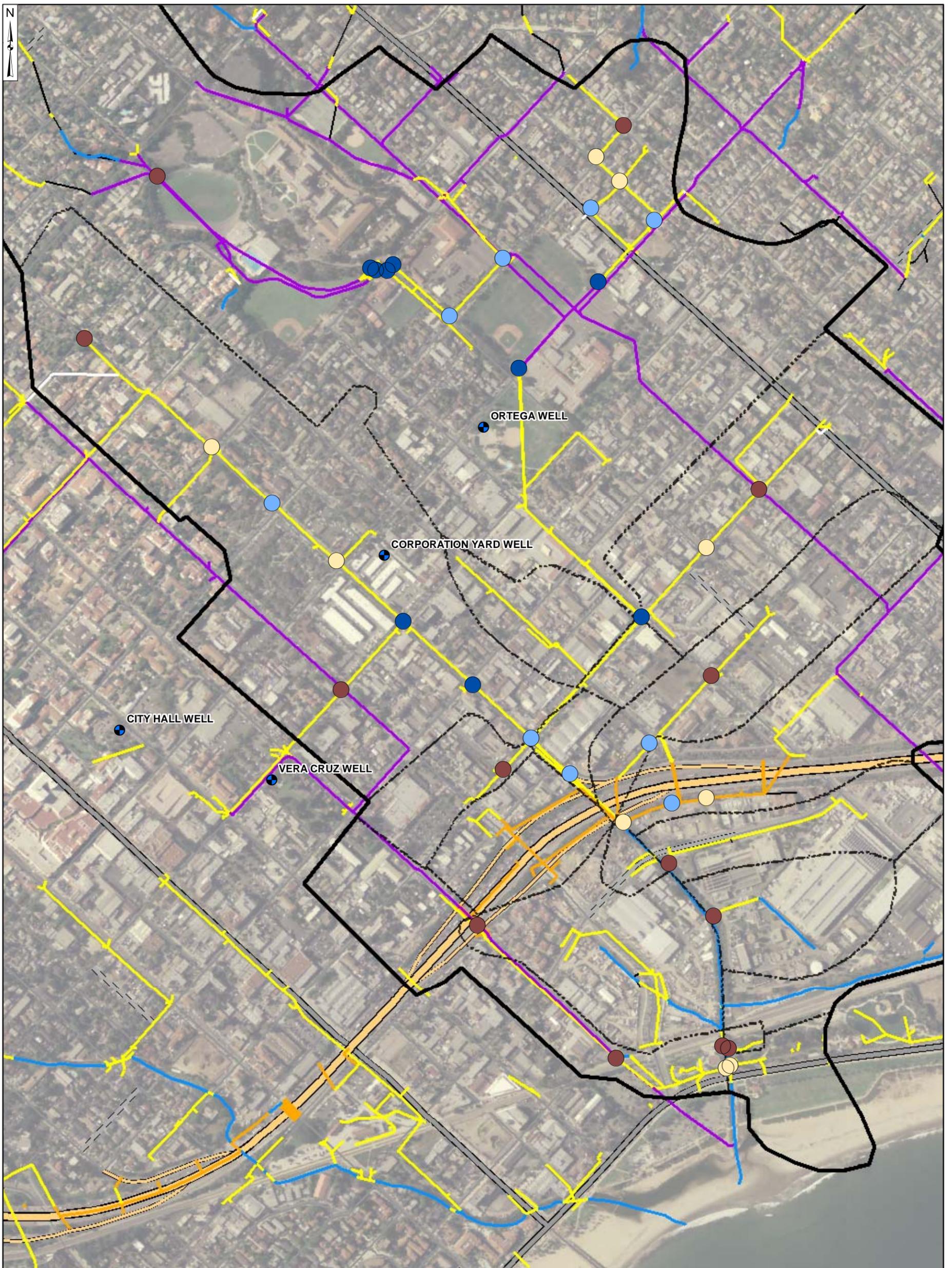
**Pump Station Location Map**  
Laguna Channel  
Santa Barbara, CA



Figure  
**4**

Project No. LA0178

March 2009



**Legend**

<b>Flow Observations</b>	<b>Storm Drains</b>
● Dry	U-SD-A-PIPE
● Wet but not flowing or trickle	U-SD-BR
● Observable flow	U-SD-CALTRANS-A-PIPE
● Significant flow	U-SD-CALTRANS-PIPE
● City Production Wells	U-SD-CO-PIPE
▭ Laguna Watershed *	U-SD-PIPE
▭ Laguna Subdrainages *	U-SD-PVT-PIPE
— Channel	

\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.

**Quantitative Graphical Summary of  
Dry-Weather Flow Stormdrain  
Reconnaissance Findings**  
Laguna Channel  
Santa Barbara, CA

**Geosyntec**  
consultants

Project No. LA0178	March 2009
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Figure  
**5**



A) View looking downstream of groundwater seepage in cracks in the Gutierrez Stormdrain Line at monitoring location 11



B) Drop inlet wall at southern corner of Ortega Park with groundwater trickling into crack in the inlet wall.

**Groundwater Infiltration In Storm Drains**

Laguna Channel  
Santa Barbara, CA

**Geosyntec**   
consultants

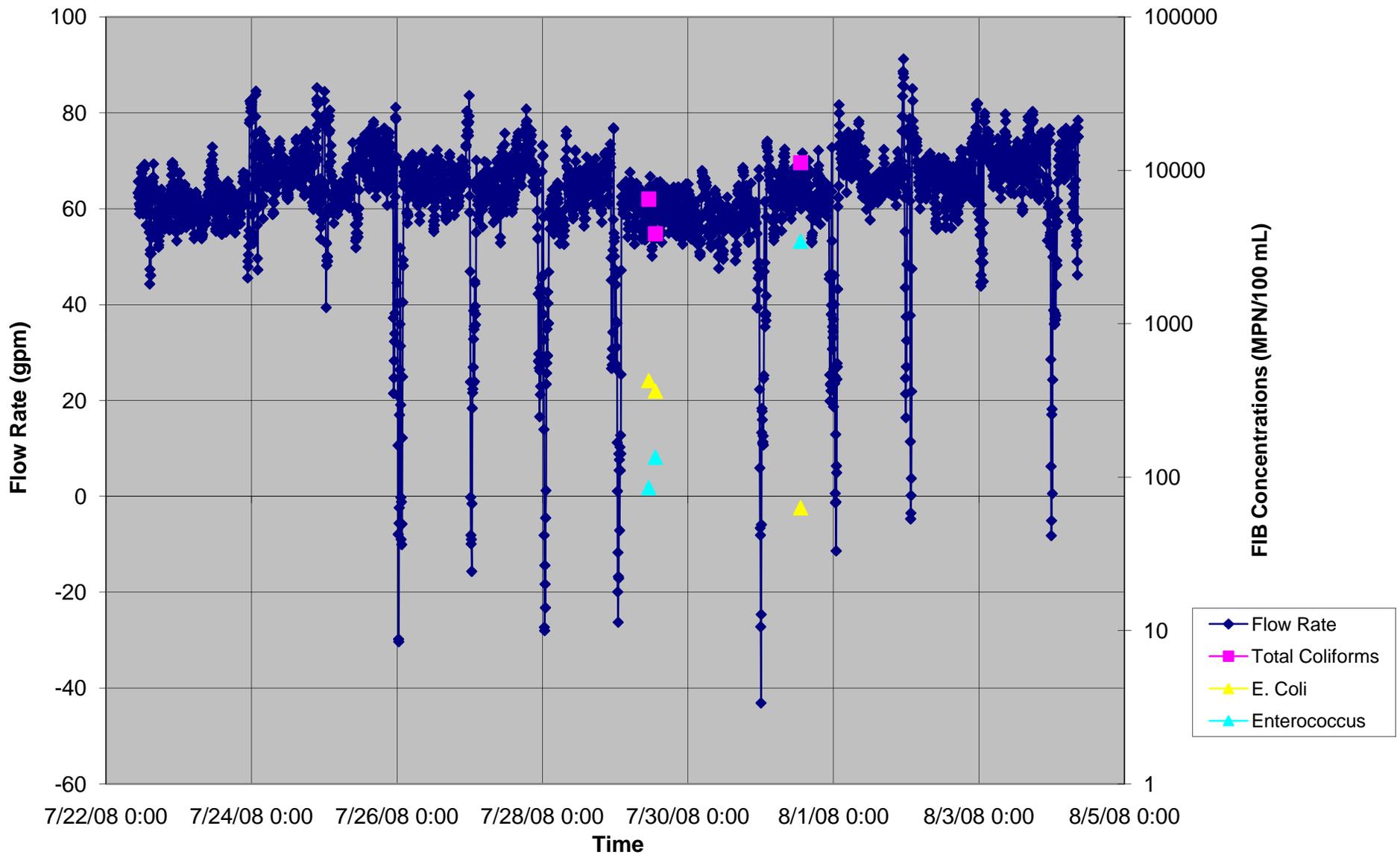
Figure

**6**

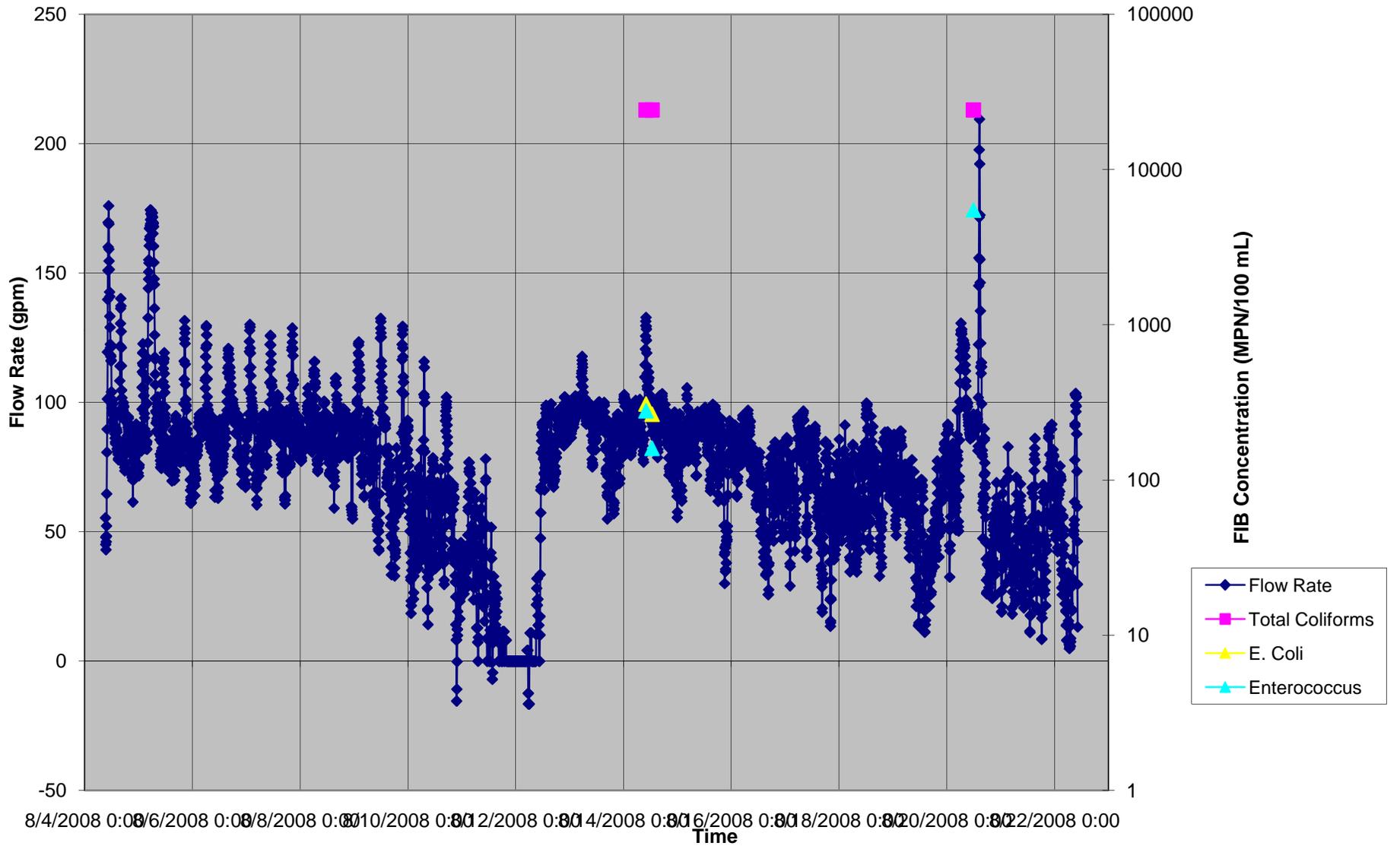
Project No. LA0178

November 2008

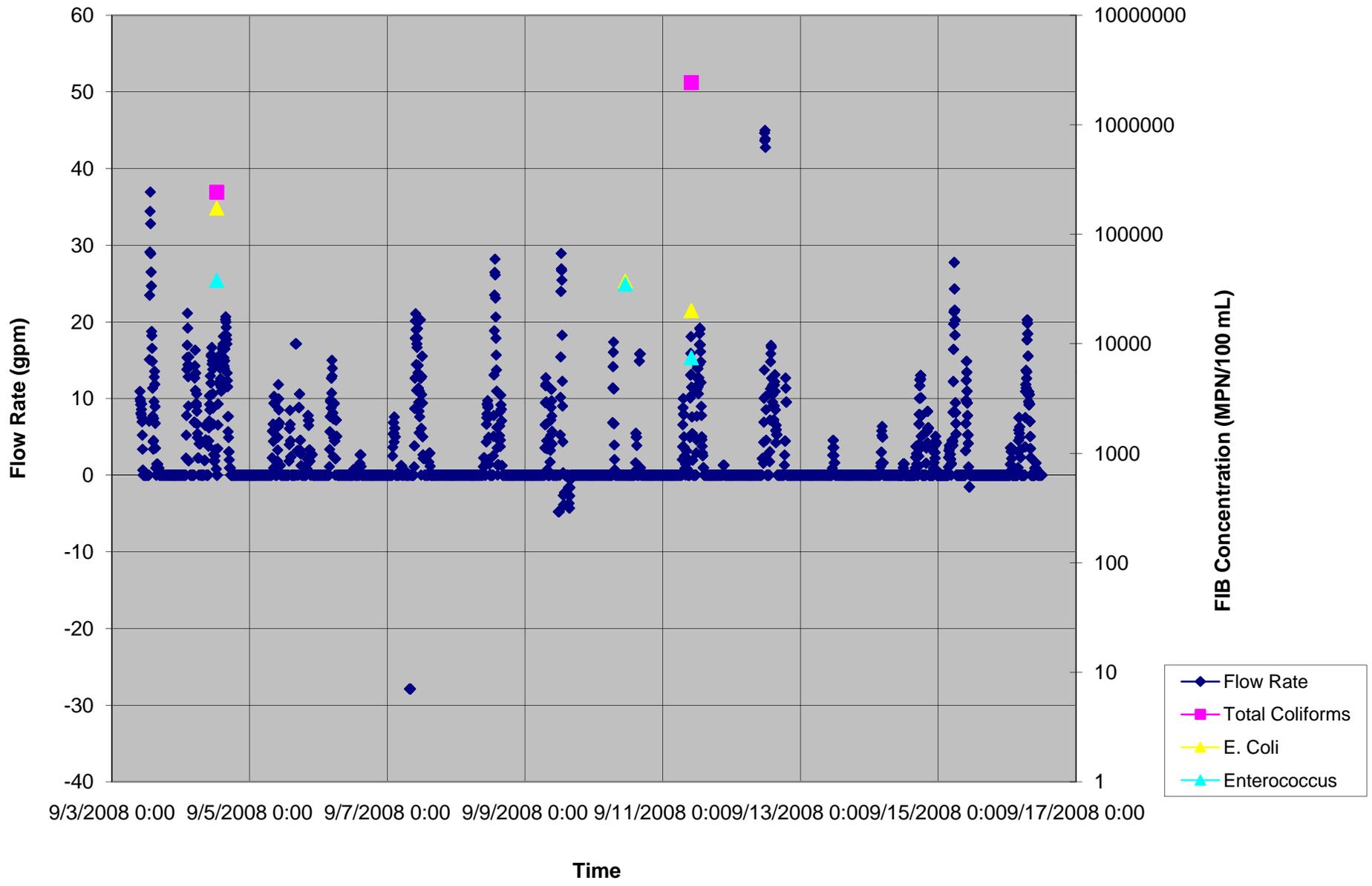
**FIGURE 7**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**  
**Laguna Drain (Sample Location 9)**  
**30-Minute Average Flow Readings and FIB Concentrations, 7/22/08 - 8/4/08**

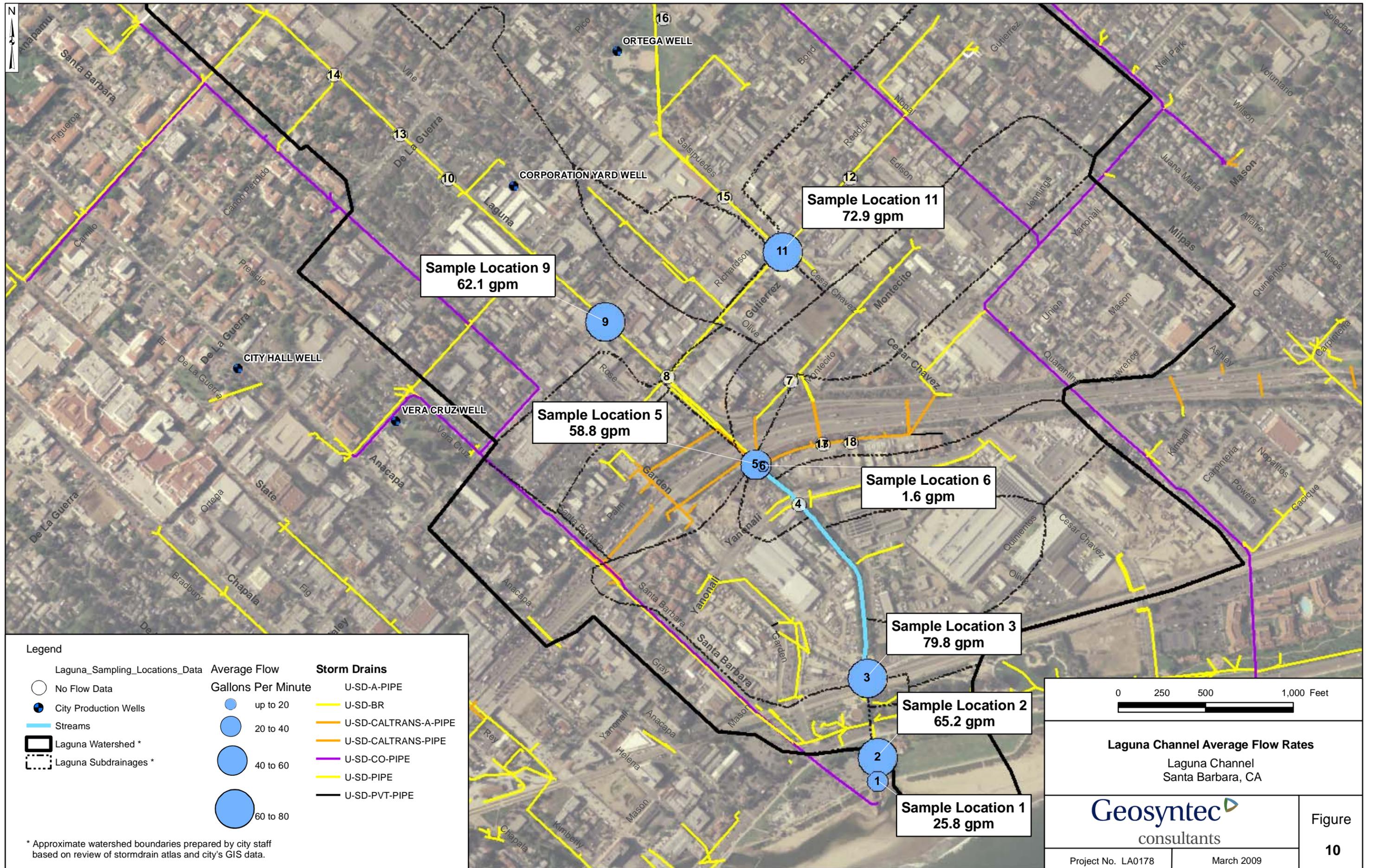


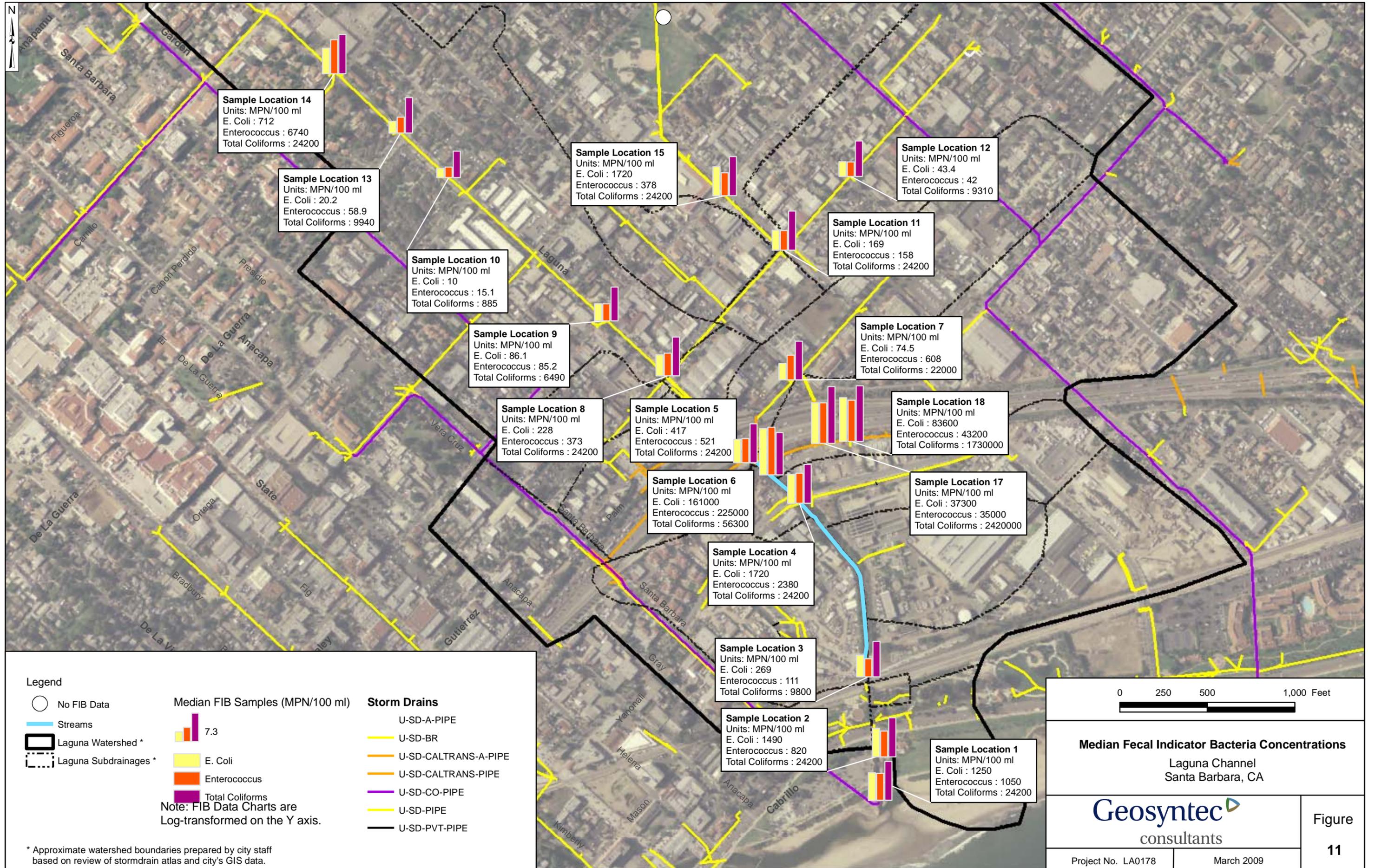
**FIGURE 8**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**  
**Gutierrez Drain (Sample Location 11)**  
**30-Minute Average Flow Readings and FIB Concentrations, 8/4/08 - 8/22/08**



**FIGURE 9**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**  
**Annex Yard Drain (Sample Location 17)**  
**30-Minute Average Flow Readings 9/3/08 - 9/5/08**







**Sample Location 14**  
Units: MPN/100 ml  
E. Coli : 712  
Enterococcus : 6740  
Total Coliforms : 24200

**Sample Location 13**  
Units: MPN/100 ml  
E. Coli : 20.2  
Enterococcus : 58.9  
Total Coliforms : 9940

**Sample Location 10**  
Units: MPN/100 ml  
E. Coli : 10  
Enterococcus : 15.1  
Total Coliforms : 885

**Sample Location 9**  
Units: MPN/100 ml  
E. Coli : 86.1  
Enterococcus : 85.2  
Total Coliforms : 6490

**Sample Location 8**  
Units: MPN/100 ml  
E. Coli : 228  
Enterococcus : 373  
Total Coliforms : 24200

**Sample Location 5**  
Units: MPN/100 ml  
E. Coli : 417  
Enterococcus : 521  
Total Coliforms : 24200

**Sample Location 6**  
Units: MPN/100 ml  
E. Coli : 161000  
Enterococcus : 225000  
Total Coliforms : 56300

**Sample Location 4**  
Units: MPN/100 ml  
E. Coli : 1720  
Enterococcus : 2380  
Total Coliforms : 24200

**Sample Location 3**  
Units: MPN/100 ml  
E. Coli : 269  
Enterococcus : 111  
Total Coliforms : 9800

**Sample Location 2**  
Units: MPN/100 ml  
E. Coli : 1490  
Enterococcus : 820  
Total Coliforms : 24200

**Sample Location 1**  
Units: MPN/100 ml  
E. Coli : 1250  
Enterococcus : 1050  
Total Coliforms : 24200

**Sample Location 15**  
Units: MPN/100 ml  
E. Coli : 1720  
Enterococcus : 378  
Total Coliforms : 24200

**Sample Location 12**  
Units: MPN/100 ml  
E. Coli : 43.4  
Enterococcus : 42  
Total Coliforms : 9310

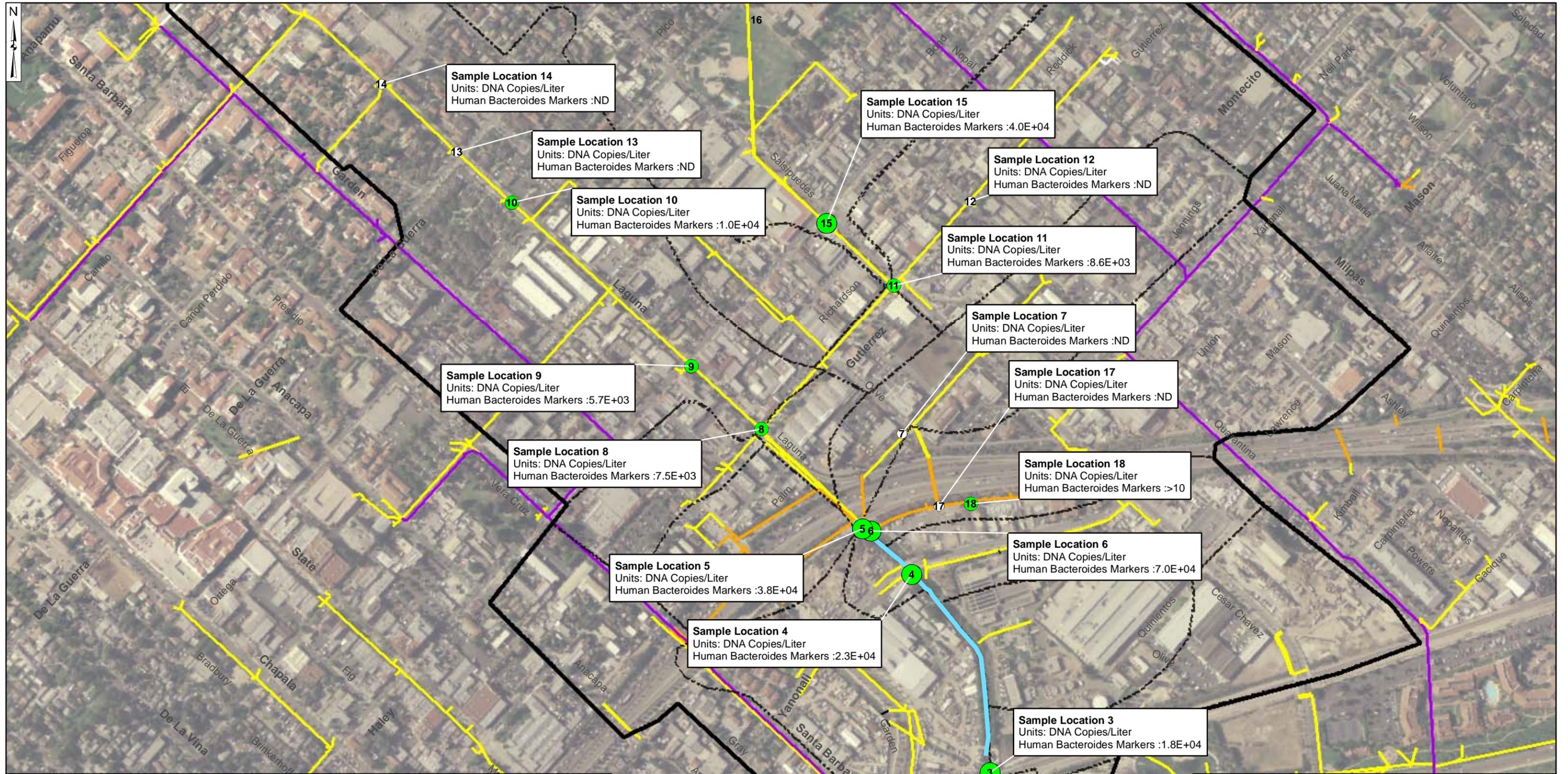
**Sample Location 11**  
Units: MPN/100 ml  
E. Coli : 169  
Enterococcus : 158  
Total Coliforms : 24200

**Sample Location 7**  
Units: MPN/100 ml  
E. Coli : 74.5  
Enterococcus : 608  
Total Coliforms : 22000

**Sample Location 18**  
Units: MPN/100 ml  
E. Coli : 83600  
Enterococcus : 43200  
Total Coliforms : 1730000

**Sample Location 17**  
Units: MPN/100 ml  
E. Coli : 37300  
Enterococcus : 35000  
Total Coliforms : 2420000

\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.



**Legend**

- Streams
- Laguna Watershed \*
- Laguna Subdrainages \*

**Median HBM Samples (DNA copies/liter)**

- Not Detected
- Detected < 1E+03
- Detected > 1E+03 and < 1E+04
- Detected > 1E+04 and < 1E+05

**Storm Drains**

- U-SD-A-PIPE
- U-SD-BR
- U-SD-CALTRANS-A-PIPE
- U-SD-CALTRANS-PIPE
- U-SD-CO-PIPE
- U-SD-PIPE
- U-SD-PVT-PIPE

\* Approximate watershed boundaries prepared by city staff based on review of stormdrain atlas and city's GIS data.

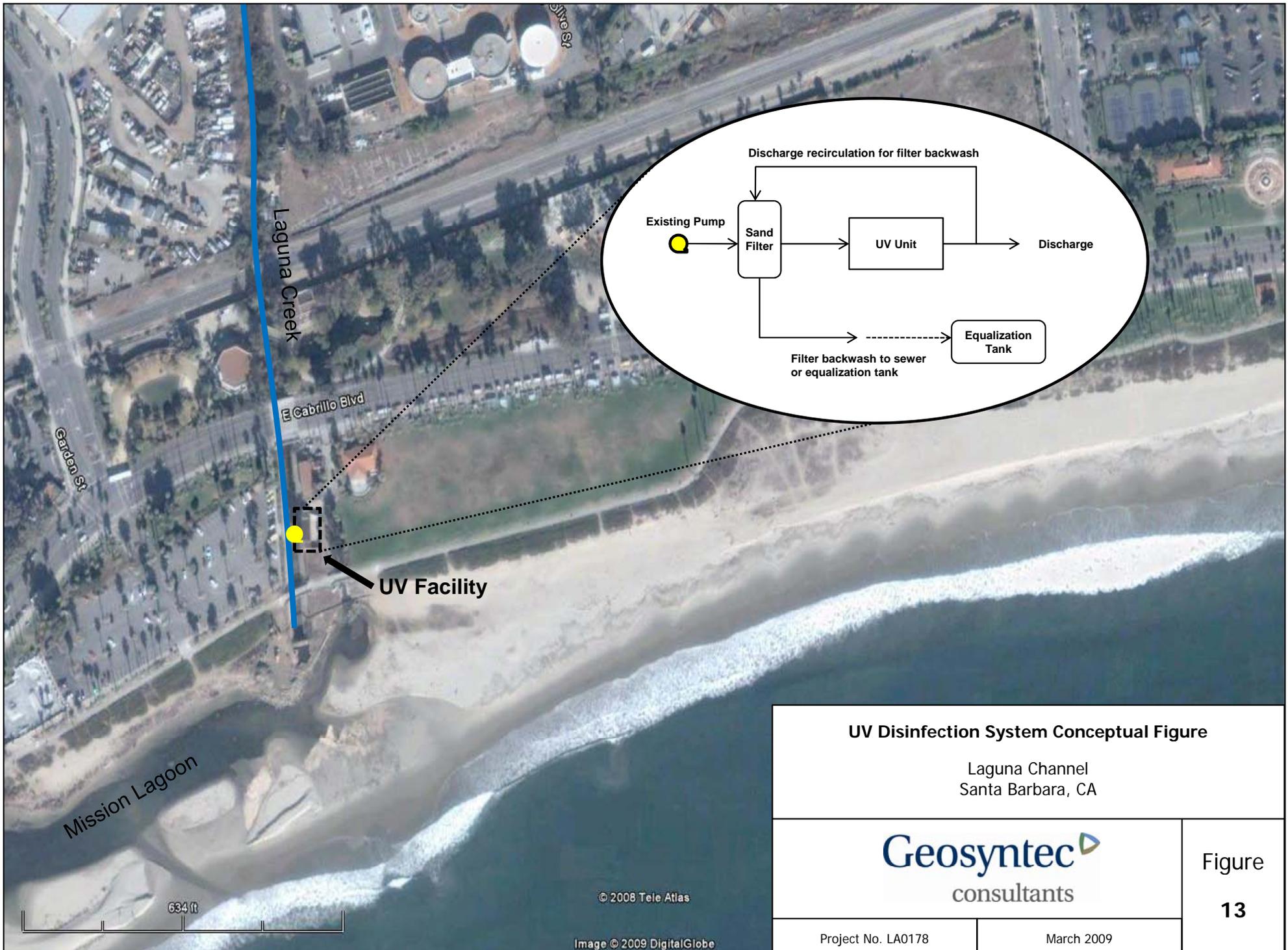
0 250 500 1,000 Feet

**Median Human Bacteroides Marker Concentrations**  
Laguna Channel  
Santa Barbara, CA

**Geosyntec**  
consultants

Project No. LA0178      March 2009

**Figure 12**



**UV Disinfection System Conceptual Figure**

Laguna Channel  
Santa Barbara, CA



Figure  
**13**

Project No. LA0178

March 2009

**Footprint is approximate. Not to scale. Not for construction.**

# TABLES

**Table 2**  
**Sample Timing for Sampling and Flow Data Collection in Laguna Channel Watershed**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**

Location ID*	Date (2008)									
	15-Jul	29-Jul	29-Jul	31-Jul	14-Aug	14-Aug	20-Aug	4-Sep	10-Sep	11-Sep
1	9:07	8:40	13:50	10:04	8:30	13:45	9:20			
2	9:10	8:55	13:45	10:15	8:45	13:30	9:31			
3	10:15	9:40	14:40	10:45	9:05	14:25	10:15			
4	10:45	10:10	15:00	11:22	9:40	14:40	10:40			
5	11:20	10:20	15:20	11:40	10:05	15:00	11:00			
6	11:00	10:30	15:05	11:30	9:50	14:50	10:55			
7		10:06	13:40	13:54						
8		10:43	13:01	13:02		12:25/13:24	11:35			
9		11:02	13:13	13:14	9:46					
10		11:20	13:26	13:25						
11					10:06	12:44	11:51			
12					10:35	13:05	12:05			
13								11:43	10:26	10:36
14								11:30	11:22	10:45
15								10:49	10:00	10:15
17								12:34	10:54	9:53
18								13:18	11:05	10:03

Notes:

\* = Location 16 was not sampled because of the absence of flow

**Table 3  
Flow and FIB Loading Results  
City of Santa Barbara Creeks Division - Laguna Channel Study**

Location	Average Flow Rate		Volume per day		Average FIB Concentration (MPN/100ml)			FIB Load per day (MPN/day)		
	GPM	CFS	Gallons	Cubic Feet	E. Coli	Enterococcus	Total Coliforms	E. Coli	Enterococcus	Total Coliforms
1	26	0.058	37,000	5,000	1.92E+03	2.19E+03	2.42E+04	2.68E+09	3.06E+09	3.39E+10
2*	65	0.14	94,000	13,000	1.49E+03	9.88E+02	2.42E+04	5.30E+09	3.52E+09	8.61E+10
3	80	0.18	120,000	15,000	4.49E+02	2.33E+02	1.22E+04	2.04E+09	1.06E+09	5.53E+10
4	-	-	-	-	4.32E+03	4.67E+03	2.42E+04	-	-	-
5	59	0.13	85,000	11,000	5.93E+02	6.60E+02	2.08E+04	1.91E+09	2.12E+09	8.65E+09
6	-	-	-	-	1.36E+05	4.56E+05	7.38E+05	-	-	-
7	-	-	-	-	1.81E+03	4.53E+03	1.89E+04	-	-	-
8	-	-	-	-	5.97E+02	1.30E+03	1.94E+04	-	-	-
9	62	0.14	89,000	12,000	1.67E+02	5.46E+02	7.06E+03	5.63E+08	1.84E+09	2.38E+10
10	-	-	-	-	7.28E+01	1.27E+02	5.52E+03	-	-	-
11	72	0.16	100,000	14,000	1.02E+03	1.03E+03	2.42E+04	3.88E+09	3.88E+09	9.16E+10
12	-	-	-	-	1.43E+02	4.48E+02	1.23E+04	-	-	-
13	-	-	-	-	4.06E+03	1.19E+02	1.31E+04	-	-	-
14	-	-	-	-	8.42E+03	1.21E+04	6.79E+04	-	-	-
15	-	-	-	-	3.15E+03	5.08E+02	2.42E+04	-	-	-
17	1.6	0.0036	2,300	310	7.69E+04	2.68E+04	1.69E+06	6.70E+09	2.33E+09	1.47E+11
18	-	-	-	-	1.01E+05	4.73E+04	1.46E+06	-	-	-
<b>Total Input</b> <sup>1</sup>	136	0.30	190,000	26,000				1.11E+10	8.05E+09	2.63E+11
<b>Total Output</b> <sup>2</sup>	65	0.14	94,000	13,000				5.30E+09	3.52E+09	8.61E+10
<b>Difference</b> <sup>3</sup>	71	0.16	140,000	13,000				5.84E+09	4.54E+09	1.77E+11

**Notes:**

\* Location 2 is the most downstream monitoring location in the Laguna Channel.

<sup>1</sup> Total Input values include flows and loads from from the upper watershed monitoring locations 9, 11, and 17, which discharge in the Laguna Channel.

<sup>2</sup> Total Output values include flows and loads are those of location 2 (discharge point from Laguna Channel), and are shown here for budget comparison purposes.

<sup>3</sup> The difference is the total input values minus the total output values.

GPM Gallons per minute

CFS Cubic feet per second

MPN Most probable number

FIB Fecal Indicator Bacteria

Average FIB The arithmetic average concentration of FIB constituents for all samples at each location was used for loading calculations.

Some FIB single sample results indicated elevated upper detection limits based on the dilution factor.

**Table 3B**  
**Manual Flow Measurements**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**

Date	Location 1		Location 2		Location 3		Location 5	
	Time	Flow Rate (GPM)						
4/24/08	-	-	-	-	10:20	100	-	-
7/1/08	-	-	-	-	14:00	84	11:00	119
7/11/08	10:30	13	11:00	62	-	-	-	-
7/15/08	9:20	17	9:37	62	10:15	94	11:30	65
7/29/08	8:50	35	9:00	65	9:45	84	11:30	51
7/29/08	2:00	21	1:35	68	2:15	55	3:30	52
7/29/08	-	-	-	-	2:20	78	-	-
7/31/08	10:08	36	10:15	49	10:45	82	11:55	51
8/14/08	8:30	27	8:45	62	9:05	59	10:20	52
8/14/08	13:45	30	13:35	68	9:05	71	15:20	44
8/14/08	-	-	-	-	14:15	62	-	-
8/14/08	-	-	-	-	14:25	63	-	-
8/20/08	9:20	27	9:31	86	10:15	125	10:40	36
<b>Average</b>		26		65		80		59
<b>Range</b>		13 - 36		49 - 86		59 - 125		36 - 119

**Notes:**

GPM gallons per minute

**Table 4**  
**FIB Sample Results (MPN/100mL)**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**  
**April - September 2008**

Constituent	Sample Date / Time	Sample Location																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18
Total Coliform	4/16/08	-	-	-	-	17329	-	10462	9804	6867	19863	>24192	10462	<10	-	-	-	-
	4/22/08	-	-	-	-	5794	-	24192	2282	1860	650	>24192	>24192	<10	-	-	-	-
	4/30/08	-	-	-	-	17329	-	10462	>24196	12997	11199	>24196	>24196	20	-	-	-	-
	7/15/08	>24196	>24196	6867	>24196	>24196	>241960	-	-	-	-	-	-	-	-	-	-	-
	7/29/2008 AM	>24196	>24196	9803.9	>24196	>24196	>241960	19862.9	>24196	6488.2	132.3	-	-	-	-	-	-	-
	7/29/2008 PM	>24196	>24196	7701	>24196	>24196	>241960	>24196.0	>24196.0	3873.2	159.6	-	-	-	-	-	-	-
	7/31/08	>24196	>24196	24196	>24196	>24196	>2419600	>24196	17328.9	11198.7	1119.9	-	-	-	-	-	-	-
	8/14/2008 AM	>24196	>24196	12996.5	>24196	>24196	195590	-	-	6131.4	-	>24196	8164.1	-	-	-	-	-
	8/14/2008 PM	>24196	>24196	15531.2	>24196	>24196	>2419600	-	>24196	-	-	>24196	4351.7	-	-	-	-	-
	8/14/2008 PM	-	-	-	-	-	-	-	>24196	-	-	>24196	-	-	-	-	-	-
	8/20/08	>24196	>24196	8164.1	>24196	22029.3	56330	-	>24196	-	-	>24196	>2419.6	-	-	-	-	-
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	19862.9	>24196	>24196	>241960	>241960
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	34547	155312	>24196	>2419600	1732890
9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	>24196	>24196	>24196	2419570	>2419600	
<b>Median</b>	>24196	>24196	9804	>24196	>24196	56330	22027	>24196	6488	885	>24196	9313	9941	>24196	>24196	2419570	1732890	
E. Coli	4/16/08	-	-	-	-	145	-	<10	122	10	354	<10	41	<10	-	-	-	
	4/22/08	-	-	-	-	86	-	10	74	<10	<10	<10	197	<10	-	-	-	
	4/30/08	-	-	-	-	106	-	272	228	109	52	73	545	<10	-	-	-	
	7/15/08	2603	450	163	754	520	141360	-	-	-	-	-	-	-	-	-	-	
	7/29/2008 AM	1151.3	1497.2	1354	12996.5	959	241957	41.3	696.8	425.7	<10	-	-	-	-	-	-	
	7/29/2008 PM	706.8	1724.7	389.3	9803.9	555.5	241957	74.5	1667.7	364.1	<10	-	-	-	-	-	-	
	7/31/08	3436.2	3130.1	603.4	1723.3	1049.7	161110	8664.4	503.9	63.2	<1	-	-	-	-	-	-	
	8/14/2008 AM	1182	858.6	201.1	1334.4	314.5	1000	-	-	30.6	-	308.9	10	-	-	-	-	
	8/14/2008 PM	1254.2	840.9	161.3	749.1	1934.9	162420	-	110	-	-	265.3	20.2	-	-	-	-	
	8/14/2008 PM	-	-	-	-	-	-	-	186.9	-	-	-	-	-	-	-	-	
	8/20/08	3075.9	1917.9	268.6	2851	259.9	1000	-	1782.1	-	-	5475	45.7	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	85.2	>24196	7269.9	173289	173289
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	30.4	342.3	1723.3	37340	45000
9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	24196	711.6	467.1	20110	83610	
<b>Median</b>	1254	1497	269	1723	417	161110	75	228	86	10	169	43	20	712	1723	37340	83610	
Enterococcus	4/16/08	-	-	-	-	121	-	121	41	30	669	156	74	<10	-	-	-	
	4/22/08	-	-	-	-	20	-	131	20	31	<10	20	<10	<10	-	-	-	
	4/30/08	-	-	-	-	171	-	145	122	10	52	63	166	<10	-	-	-	
	7/15/08	1153	305	111	309	1414	57940	-	-	-	-	-	-	-	-	-	-	
	7/29/2008 AM	1534	1280.9	135	12996.5	732.8	>241960.0	1528.6	387.7	85.2	20.2	-	-	-	-	-	-	
	7/29/2008 AM	234.4	369.2	73.1	10462.4	601.5	>241960.0	1071.2	373.4	135	<10	-	-	-	-	-	-	
	7/31/08	988.1	2602.5	1030	4611.1	880.3	>2419600	>24196.0	771.2	3448	3.3	-	-	-	-	-	-	
	8/14/2008 AM	1049.7	820.2	74.5	2382.2	441.2	2020	-	-	86	-	278.5	10	-	-	-	-	
	8/14/2008 PM	537.1	281.6	132.3	573.1	1860	224680	-	299.2	-	-	159.6	<10	-	-	-	-	
	8/14/2008 PM	-	-	-	-	-	-	-	503.6	-	-	-	-	-	-	-	-	
	8/20/08	9803.9	1259.1	74.5	1332.7	361.6	1000	-	9208.4	-	-	5475	>2419.6	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	428.4	>24196	336.1	37844	86644
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	107.8	6739.85	808.8	34980	43210
9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	148	5475	378.6	7450	12110	
<b>Median</b>	1050	820	111	2382	521	224680	608	373	85	15	158	42	59	6740	379	34980	43210	

**Notes:**  
 Results shown in **bold** are above the State of California Health Services single sample standard for ocean beaches for the specific fecal indicator bacteria.  
 Median concentrations were calculated using the FIB data. Results published by USCB Laboratory of Dr. Patricia Holden in report included as **Appendix D**.  
 FIB Fecal Indicator Bacteria  
 MPN/100 mL Most probable number of bacteria per one hundred milliliter units of sample water.  
 >24196 The greater than symbol indicates the result is above the upper detection limit for the dilution factor used for that sample.  
 <10 The less than symbol indicates the result is below the lower detection limit for the dilution factor used for that sample

**Table 5**  
**Water Quality Parameters**  
**City of Santa Barbara Creeks and Water Quality Division - Laguna Channel Study**

Constituent	Sample Date / Time	Sample Location																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	
Conductivity (mS)	7/29/2008 AM	10.4	2.4	1.6	1.6	1.5	1.2	0	1.4	0.02	0.01	-	-	-	-	-	-	-	
	7/29/2008 PM	9.4	2	1.6	1.6	1.6	1.2	0.0054	1.92	0.02	0.0083	-	-	-	-	-	-	-	
	7/31/2008	13.06	0.019	0.73	0.74	1.42	0.33	0.87	1.4	0.52	0.006	-	-	-	-	-	-	-	
	8/14/2008 AM	13.3	2.7	1.9	1.6	1.6	1.4	-	-	0.49	-	1.87	0.012	-	-	-	-	-	
	8/14/2008 PM	11.7	2.4	1.9	1.6	1.6	1	-	1.88	-	-	0.031	0.63	-	-	-	-	-	
	8/20/2008	10.8	2.2	1.6	1.6	1.2	1.2	-	0.68	-	-	0.99	0.73	-	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	1.6	1.3	1	0.7	0.5	
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	1.7	0.5	1	0.01	0.05	
	9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	1.8	0.8	1.4	1.5	1.2	
<b>Average</b>		11.4	2.0	1.6	1.5	1.5	1.1	0.3	1.5	0.3	0.0	1.0	0.5	1.7	0.9	1.1	0.7	0.6	
Salinity (ppm)	7/29/2008 AM	5.8	1.4	0.8	0.8	0.8	0.6	0	1	0	0	-	-	-	-	-	-	-	
	7/29/2008 PM	5.1	1	0.8	0.8	0.8	0.6	0	-	-	-	-	-	-	-	-	-	-	
	7/31/2008	7.9	0	0.4	0.4	0.8	0.5	0.4	0.8	0.2	0	-	-	-	-	-	-	-	
	8/14/2008 AM	7.7	1.4	1	0.8	0.8	0.7	-	-	0.3	-	1	0	-	-	-	-	-	
	8/14/2008 PM	6.6	1.2	1	0.8	0.8	0.5	-	1.1	-	-	0	0.3	-	-	-	-	-	
	8/20/2008	6.4	1.7	0.9	0.9	0.6	0.9	-	0.3	-	-	0.5	0.3	-	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.7	0.5	0.3	0.2	
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.3	0.5	0	0	
	9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	0.9	0.4	0.7	0.8	0.6	
<b>Average</b>		6.6	1.1	0.8	0.8	0.8	0.6	0.1	0.8	0.2	0.0	0.5	0.2	0.9	0.5	0.6	0.4	0.3	
Temperature (dC)	7/29/2008 AM	21.4	19.4	19.4	20.4	21.5	18.7	22.5	22	21.9	22.8	-	-	-	-	-	-	-	
	7/29/2008 PM	26.6	21.6	19.8	21.4	21.7	19.8	29.3	22.4	22.9	29.2	-	-	-	-	-	-	-	
	7/31/2008	22.4	19.8	19.5	20.6	21.6	19.1	26.9	22	24.1	27.6	-	-	-	-	-	-	-	
	8/14/2008 AM	21.7	19.8	19.7	20.6	22	19.2	-	-	22.2	-	22.5	23.3	-	-	-	-	-	
	8/14/2008 PM	26.5	22	20.2	22.4	21.9	21.3	-	22.7	-	-	22.8	25.2	-	-	-	-	-	
	8/20/2008	22.5	20.1	20.3	21.7	22.3	20.1	-	22.9	-	-	22.8	25.8	-	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	25.3	22.2	22.4	23.6	25.6	
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	22.4	21	22	22.5	20.2	
	9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	22.4	20.7	22	19.9	20.2	
<b>Average</b>		23.5	20.5	19.8	21.2	21.8	19.7	26.2	22.4	22.8	26.5	22.7	24.8	23.4	21.3	22.1	22.0	22.0	
Dissolved Oxygen (mg/L)	7/29/2008 AM	-	-	-	-	-	-	6.02	5.48	5.38	3.4	-	-	-	-	-	-	-	
	7/29/2008 PM	-	-	-	-	-	-	3.66	4.81	5.02	2.15	-	-	-	-	-	-	-	
	7/31/2008	-	6.7	2.7	4.5	5.66	3.8	5.44	6.11	5.9	2.82	-	-	-	-	-	-	-	
	8/14/2008 AM	-	-	-	-	-	-	-	-	7.11	-	6.39	2.07	-	-	-	-	-	
	8/14/2008 PM	-	-	-	-	-	-	-	6.06	-	-	5.76	5.25	-	-	-	-	-	
	8/20/2008	6.7	6.7	6.9	6.7	7.44	6.8	-	7.05	-	-	6.05	7.1	-	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	4.61	1.03	7.27	3.2	3.99	
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	8.8	3.4	7.66	4.63	2.33	
	9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	5.92	2.16	5.79	5.32	3.87	
<b>Average</b>		6.7	6.7	4.8	5.6	6.6	5.3	5.0	5.9	5.9	2.8	6.1	4.8	6.4	2.2	6.9	4.4	3.4	
Dissolved Oxygen (%)	7/29/2008 AM	-	-	-	-	-	-	68.3	60.3	61.5	40	-	-	-	-	-	-	-	
	7/29/2008 PM	-	-	-	-	-	-	46.9	58.2	57.9	38.8	-	-	-	-	-	-	-	
	7/31/2008	-	55.6	30.5	50.3	65.4	42.6	64.5	65.4	66.7	38.5	-	-	-	-	-	-	-	
	8/14/2008 AM	-	-	-	-	-	-	-	-	82.1	-	71	25	-	-	-	-	-	
	8/14/2008 PM	-	-	-	-	-	-	-	70.7	-	-	66.7	61.4	-	-	-	-	-	
	8/20/2008	84	73	77	77.7	85	67.8	-	82	-	-	68.7	84	-	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	55	11.5	83.7	34.5	43.2	
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	100	38	82.9	54.4	26.6	
	9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	67.7	24.4	60.4	56	39.6	
<b>Average</b>		84.0	64.3	53.8	64.0	75.2	55.2	59.9	67.3	67.1	39.1	68.8	56.8	74.2	24.6	75.7	48.3	36.5	
pH	7/29/2008 AM	7.95	7.82	7.74	7.8	8.03	7.87	7.79	8.04	7.78	7.62	-	-	-	-	-	-	-	
	7/29/2008 PM	8.42	7.9	7.75	7.95	8.03	7.93	7.96	8.05	7.83	7.58	-	-	-	-	-	-	-	
	7/31/2008	7.98	7.7	7.69	7.8	7.89	7.55	7.62	7.75	7.62	7.43	-	-	-	-	-	-	-	
	8/14/2008 AM	7.86	7.85	7.87	7.91	8.14	8	-	-	8.06	-	8.04	8.18	-	-	-	-	-	
	8/14/2008 PM	8.24	7.84	7.84	8.03	8.04	7.8	-	7.9	-	-	8.02	8.09	-	-	-	-	-	
	8/20/2008	7.95	7.92	7.8	7.95	7.98	8.21	-	7.95	-	-	8.09	7.94	-	-	-	-	-	
	9/4/2008	-	-	-	-	-	-	-	-	-	-	-	-	7.45	7.44	8.01	7.91	9.17	
	9/10/2008	-	-	-	-	-	-	-	-	-	-	-	-	7.7	7.71	8.09	8.17	8.08	
	9/11/2008	-	-	-	-	-	-	-	-	-	-	-	-	7.54	7.59	7.88	8.01	7.78	
<b>Average</b>		8.1	7.8	7.8	7.9	8.0	7.9	7.8	7.9	7.8	7.5	8.1	8.1	7.6	7.6	8.0	8.0	8.3	

**Notes:**  
mS microsiemens per second  
ppm parts per million  
dC degrees Centigrade  
mg/L milligrams per liter  
% percent  
- Data not collected this sampling event

**Table 6**  
**Human Bacteroides DNA Markers (copies/L)**  
**City of Santa Barbara Creeks Division - Laguna Channel Study**

Site	7/15/2008	7/29/2008		7/31/2008	8/14/2008			8/20/2008	9/4/2008	9/10/2008	9/11/2008	Median
		AM	PM		AM	PM	PM					
1	ND	ND	ND	8.4E+03	>10	ND		5.96E+04				3.4E+04
2	>10	1.2E+04	1.7E+04	3.47E+04	3.01E+04	ND		ND				2.3E+04
3	ND	ND	1.8E+04	>10	ND	ND		>10				1.8E+04
4	2.3E+04	3.6E+04	1.9E+04	1.3E+04	4.8E+04	1.6E+04		3.8E+04				2.3E+04
5	>10	4.0E+04	3.49E+04	5.1E+05	4.6E+04	8.4E+03		4.7E+03				3.8E+04
6	ND	ND	6.99E+04	ND	ND	ND		ND				7.0E+04
7		ND	ND	ND								ND
8		ND	5.91E+04	3.7E+03		1.0E+04	4.67E+03	ND				7.5E+03
9		ND	>10	ND	5.7E+03							5.7E+03
10		ND	7.64E+03	1.25E+04								1.0E+04
11					5.3E+04	8.6E+03		7.6E+03				8.6E+03
12					ND	ND		ND				ND
13									ND	ND	ND	ND
14									ND	ND	ND	ND
15									8.8E+03	4.76E+04	4.01E+04	4.0E+04
17									ND	ND	ND	ND
18									>10	>10	ND	>10

**Notes:**

- >10 below level of detection but not non-detect.
- blank no sample taken
- ND non detect

# APPENDICES

## **APPENDIX B**

Fecal indicator bacteria and human fecal pollution in the Laguna Watershed-Phase I

# **Fecal indicator bacteria and human fecal pollution in the Laguna Watershed – final report**

Report date: November 18, 2008

Performed by the Laboratory of Patricia Holden, Donald Bren School of Environmental Science & Management, UC Santa Barbara ([holden@bren.ucsb.edu](mailto:holden@bren.ucsb.edu), (805) 893-3195).  
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## 1. Project summary

The Laguna watershed is entirely urban and the flow is conveyed by storm drains to a 1/2 mile open channel, where it is then pumped around tide gates to East Beach at the Laguna Pump Station. Due to its proximity to downtown and beach hotels, East Beach receives in excess of one million visitors per year. Since microbiological testing in the ocean surf zone began in 1999, East Beach was posted an average of 19% of the test days. These postings are due in part to contributions from Laguna Channel, next to contributions from Mission Creek which also discharges into the ocean at East Beach. Previous microbial DNA testing by the Holden lab has found Laguna Channel to contain human fecal markers. To prevent beach closures associated with the dry weather discharge from the pump station, the City of Santa Barbara is considering the option of disinfecting this discharge.

This project by the City of Santa Barbara, in collaboration with UCSB and Geosyntec consultants, is the 1st phase of a 2 phase implementation project to eliminate bacteria from the Laguna Channel prior to discharge at East Beach. The first phase of the project includes a watershed assessment to quantify sources of flow to Laguna Channel, and microbial source tracking to confirm the presence and temporal variability of human waste in the channel. The work performed by UCSB included study and water sampling design, water sampling during summer 2008 (including manual flow measurements), sample analysis (fecal indicator bacteria, qPCR for human-specific *Bacteroides*, PCR for human-specific *Methanobrevibacter smithii nifH* gene), and microbial data analysis. Part of the data generated by UCSB will be used by GeoSyntec consultants for calculations of microbial fluxes in the Laguna drains, as is detailed in an accompanying report.

## 2. Description of field sites and timing

Sixteen field sites were chosen, in coordination with the City of Santa Barbara and GeoSyntec. The selection of the sampling locations (Fig. 1, Table 1) was based on field visits, visual flow assessments and preliminary fecal indicator bacteria (FIB) concentrations obtained by the City of Santa Barbara. Sampling timing is summarized in Table 2.

Field site selection criteria were:

- Fecal sources are expected to be present between two sampling points for the Laguna Channel locations
- There should be observable flow for the drain locations
- FIB are present in significant amounts (*E. coli* and *Enterococcus* > 10 MPN/100ml)

Table 1. ID and description of Laguna Watershed sampling locations

<b>ID</b>	<b>Location</b>
1	Laguna Channel, Mission Lagoon overflow
2	Laguna Channel, just upstream of pump station
3	Laguna Channel, upstream of railroad tracks
4	Laguna Channel, Yanonali bridge
5	Laguna Drain, under US101 onramp
6	Drain from annex yard, outfall
7	Drain Montecito @ Olive
8	Drain Laguna @ Gutierrez
9	Drain Laguna @ Haley
10	Laguna drain lateral
11	Drain Gutierrez @ Salsipuedes
12	Drain Gutierrez @ Quarantina
13	Drain Laguna @ De La Guerra
14	Drain Laguna @ Canon Perdido
15	Drain Salsipuedes @ Haley
16	Drain upstream Ortega Park
17	Drain from annex yard, middle
18	Drain from annex yard, upper



Table 2. Sample dates and times for Laguna Watershed samples

ID*	Date									
	07/15	07/29	07/29	07/31	08/14	08/14	08/20	09/04	09/10	09/11
1	9:07	8:40	13:50	10:04	8:30	13:45	9:20			
2	9:10	8:55	13:45	10:15	8:45	13:30	9:31			
3	10:15	9:40	14:40	10:45	9:05	14:25	10:15			
4	10:45	10:10	15:00	11:22	9:40	14:40	10:40			
5	11:20	10:20	15:20	11:40	10:05	15:00	11:00			
6	11:00	10:30	15:05	11:30	9:50	14:50	10:55			
7		10:06	13:40	13:54						
8		10:43	13:01	13:02		12:25	11:35			
						13:24				
9		11:02	13:13	13:14	9:46					
10		11:20	13:26	13:25						
11					10:06	12:44	11:51			
12					10:35	13:05	12:05			
13								11:43	10:26	10:36
14								11:30	11:22	10:45
15								10:49	10:00	10:15
17								12:34	10:54	9:53
18								13:18	11:05	10:03

\*location 16 was not sampled because of the absence of flow

### 3. Materials and Methods

#### 3.1 Sampling and field measurements

Water samples (approximately 2 L) were grabbed using a sterile beaker, passed through 25 µm pore size Miracloth (Calbiochem, San Diego, CA), and stored on ice until processing (within 6 hours). Dissolved oxygen, temperature, and salinity were measured in the field with a YSI Model 85 meter (YSI Inc., Yellow Springs, OH), and pH was measured in the lab with a Corning pH meter 430 (Corning, NY). Manual flow measurements in open channel were performed using the velocity-area method, using a Flo-Mate Model 2000 velocity probe (Marsh-McBirney, MD, USA). At least six depth-velocity measurements were collected at each location, depending on the width and geometry of the channel. For locations 1 and 2 (see Table 1), sand bags were used to narrow the channel width so that sufficient velocity (> 0.01 m/s) and water depth (> 0.02 m) was obtained. Automated continuous flow measurements in drains were performed as detailed in an accompanying report by GeoSyntec.

#### 3.2 Fecal indicator bacteria

FIB (total coliform, *E. coli* and enterococci) most probable numbers (MPNs) were quantified using the Quanti-Tray®/2000 method, according to manufacturer's instructions (IDEXX Laboratories, Westbrook, MA). Water samples were diluted in

sterile Nanopure water prior to analysis (between 1:1 and 1:1000, depending on the sample).

### 3.3 DNA extraction

The UltraClean Water DNA Kit (MoBio Laboratories, Carlsbad, CA) was used to extract the DNA from water samples. Water samples were vacuum filtered through 0.22 µm filters until either the collected volume was filtered or the point of refusal. Filters were stored at -20 °C until extraction. DNA was extracted according to the manufacturer's protocols, followed by ethanol precipitation. Total DNA was quantified using the Quant-iT PicoGreen® dsDNA kit (Molecular Probes/Invitrogen).

### 3.4 Quantitative real-time PCR for human-specific *Bacteroides*

The human-specific HF 183 *Bacteroides* 16S rRNA marker was quantified using SYBR® Green I detection, as in Seurinck et al. (Seurinck, Defoirdt et al. 2005), with the addition of fluorescein (Eurogentec, Belgium) in an iQ5 thermocycler (Bio-Rad, Hercules, CA). Reactions were run in 25 µl volumes, with 10 and 2 ng of sample DNA. Samples were diluted with molecular biology grade water (Sigma-Aldrich, St. Louis, MO). Samples were run in triplicate, including standards ( $5.6 \times 10^1$  to  $5.6 \times 10^7$  targets) and non-template control. Standards were created by diluting purified PCR amplicons from raw sewage DNA extracts, using the human-specific HF 183 *Bacteroides* primers (Bernhard and Field 2000). Standard amplicon concentrations were quantified using the Quant-iT PicoGreen® dsDNA kit (Molecular Probes/Invitrogen). Any qPCR well replicate that did not amplify, or that produced a threshold cycle value higher than the lowest standard, was treated as a zero value. Moreover, all samples having only 1 analytical replicate amplify were treated as “detectable but non quantifiable”. To ensure correct target amplification, a melt curve was verified for each sample. Reaction inhibition was examined by comparing the human-specific *Bacteroides* concentrations obtained with 10 and 2 ng of template DNA for each sample. Inhibition occurred if the HBM concentrations (as HBM copies/L) using 2 ng of template were higher than those obtained using 10 ng template. In that case, the HBM concentrations of the reactions using 2 ng template were used as final values. Otherwise the HBM concentrations of the 10 ng template reactions were considered as the final values.

### 3.5 PCR for the *nifH* gene of *Methanobrevibacter smithii*

The PCR method for detection of *nifH* genes of *Methanobrevibacter smithii* (Mnif assay) was based on the method of Ufnar et al. (Ufnar, Wang et al. 2006), using the primers Mnif-342f and Mnif-363r. We modified the method into a 2 step PCR method, where the PCR product of round 1 was diluted and amplified again with the same protocol in a 2<sup>nd</sup> round of PCR. The addition of a 2<sup>nd</sup> PCR round improved the sensitivity of the method. PCR reactions were performed in 25 µl reactions using the Taq PCR Core Kit (Qiagen, Valencia, CA). Each reaction contained 1X PCR buffer, 1X Q-solution, 0.2 mg/ml bovine serum albumin, 0.2 mM of each dNTP, 500 nM of each primer, and 1.25 U of Taq Polymerase. In round 1, 1 µl of DNA template was added, while in round 2, 5 µl of 1/100

diluted PCR product from round 1 was added. For each reaction, a non-template control was analyzed. In round 2, the PCR product of the round 1 non-template control was also analyzed. Thermocycling conditions were 3 min at 92 °C for initial denaturation, 30 cycles of 1 min at 92 °C, 30 sec at 55.1 °C and 1 min at 72 °C, and final extension for 6 min at 72 °C. PCR products were visualized by running 5 µl PCR product in a 2 % agarose gel stained with 0.5 mg/ml ethidium bromide. Electrophoresis was run at 100 V for approximately 30 minutes.

#### 4. Results

##### 4.1 Laguna Channel flow

Manual flow measurements indicate that flows are relatively constant in time for locations 1, 2, 3 and 5 (Table 3). Paired t-tests do not reveal any significant differences in flow between locations 2-3 ( $P = 0.08$ ) and 3-5 ( $P = 0.11$ ). Therefore, the changes in bacterial fluxes transported from location 5 to 2 in Laguna Channel are proportional to the changes in bacterial concentrations. The average flow to the Laguna pumping station is 70% Laguna Channel flow and 30% Mission Lagoon overflow.

Table 3. Summary of manual flow measurements (L/s) for locations 1, 2, 3, and 5. Flows indicated by similar superscripts are duplicate measurements taken about 1 m apart.

Date	Location							
	1		2		3		5	
	Time	Flow	Time	Flow	Time	Flow	Time	Flow
07/01					14:00	5.3	11:00	7.5
07/11	10:30	0.8	11:00	3.9				
07/15	9:20	1.1	9:37	3.9	10:15	5.9	11:30	4.1
07/29	8:50	2.2	9:00	4.1	9:45	5.3	11:30	3.2
	14:00	1.3	13:35	4.3	14:15	3.5 <sup>a</sup>	15:30	3.3
					14:15	4.9 <sup>a</sup>		
07/31	10:08	2.3	10:15	3.1	10:45	5.2	11:55	3.2
08/14	8:30	1.7	8:45	3.9	9:05	3.7 <sup>b</sup>	10:20	3.3
	13:45	1.9	13:35	4.3	9:05	4.5 <sup>b</sup>	15:20	2.8
					14:15	3.9 <sup>c</sup>		
					14:15	4.0 <sup>c</sup>		
08/20	9:20	1.7	9:31	5.4	10:15	7.9	10:40	2.3
<b>Mean</b>		1.6		4.1		4.9		3.7
<b>SE</b>		0.2		0.2		0.4		0.6

## 4.2 FIB concentrations

All FIB concentrations and 95 % confidence intervals are shown in Table 4, and results are summarized for EC and ENT in Fig. 2. Total coliform data are not shown in a graph because most of the data are out of range.

The highest EC and ENT concentrations were found at locations 6, 17 and 18. Very high concentrations, exceeding  $1 \times 10^5$  MPN/100 ml were found for both FIB. Locations 10 and 12 generally had the lowest EC concentrations, although one spike in ENT concentration was observed on 08/20 at loc. 12. Also for loc. 7, a spike in both EC and ENT was observed on one sampling day (07/31/08 AM).

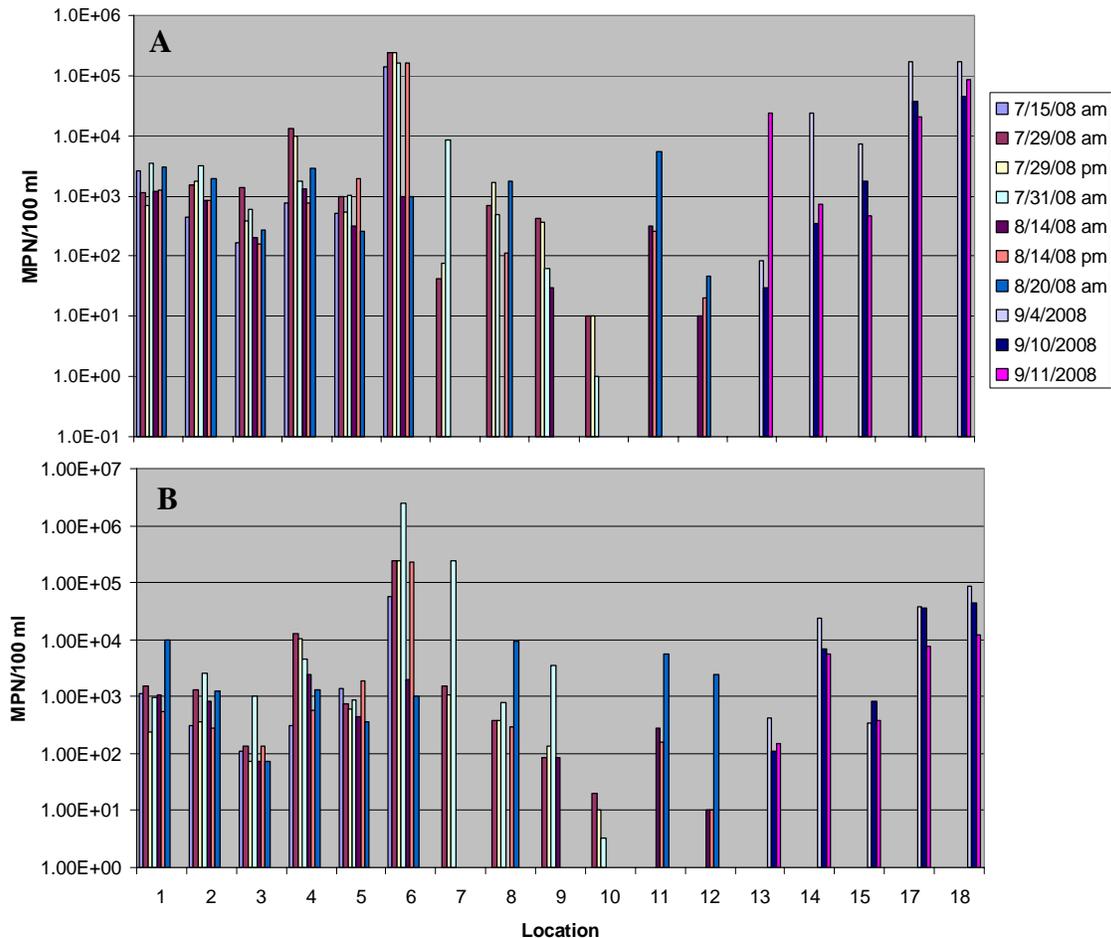


Fig. 2. Summary of EC (A) and ENT (B) concentrations in Laguna Watershed.

Similar longitudinal concentration profiles were observed in the Laguna Watershed for EC and ENT. In the Gutierrez drain, FIB mostly originated from the Salspuedes drain (loc. 15), and not from the upstream reaches of the Gutierrez drain (loc. 12). In the Laguna drain, the FIB concentrations usually decrease from loc. 14 to 13, but remain fairly constant afterwards. Comparison between loc. 13 and loc. 9 is difficult, because samples were never taken on the same day. The FIB concentrations in Laguna drain (loc.

9) and the Gutierrez drain (loc. 11) are similar, so each drain segment may similarly contribute to FIB concentrations downstream in Laguna drain (loc. 8). Load calculations should be performed to determine the most influential drain sections on water quality in the downstream Laguna drain section (see GeoSyntec study).

In the open Laguna Channel, FIB concentrations generally increase from loc. 5 to 4, decrease from loc. 4 to 3, but increase again from loc. 3 to 2, suggesting FIB sources between loc. 5 and 4, and between loc. 3 and 2. Very high FIB concentrations consistently originate from the City Annex Yard (loc. 6, 17, 18), which may therefore be the source of FIB between loc. 5 and 4.

Since no statistical differences were observed in flow between Laguna Channel sampling locations, FIB fluxes (Fig. 3) are almost proportional to the FIB concentrations, and similar longitudinal patterns are found for FIB fluxes and concentrations. The FIB fluxes from Laguna Channel (location 2) and the Mission Lagoon overflow (location 1) flowing towards the pumping station were usually similar on a given sampling day, but some day-to-day variation was observed. The latter fluxes usually ranged between  $10^4$  and  $10^5$  FIB/s.

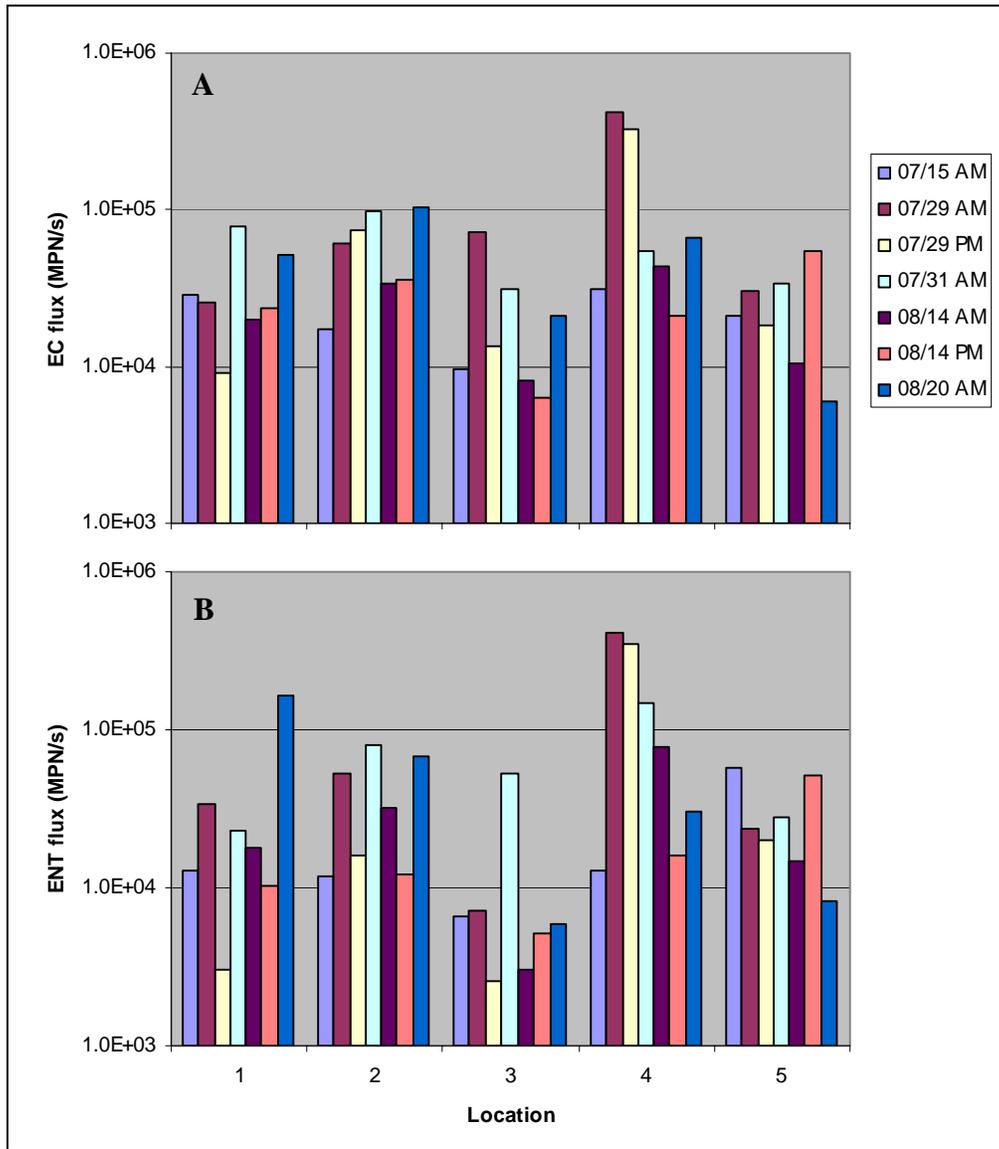


Fig. 3. Fluxes of EC (A) and ENT (B) in Laguna Channel.

Table 4. Summary of FIB concentrations (MPN/100 ml) for all samples, including lower (LCI) and upper (UCI) 95% confidence intervals. Analytical replicates are indicated by \*.

	Time	Conc.	TC LCI	UCI	Conc.	EC LCI	UCI	Conc.	ENT LCI	UCI
<b>07/15</b>										
1	9:07	>24196	14395	∞	2603	1754	3652	1153	822	1581
2	9:10	>24196	14395	∞	450	312	630	305	194	448
3	10:15	6867	4493	9744	109	56	195	86	45	169
3*		6867	4493	9744	216	129	337	135	78	234
4	10:45	24196	16304	47161	754	538	1026	309	196	455
5	11:20	>24196	14395	∞	520	361	722	1414	1035	1878
6	11:00	>241960	143950	∞	141360	92490	210160	57940	37910	84720
<b>07/29</b>										
1	8:40	>24196	14395	∞	1067	781.5	1403.8	1515.2	1080.1	2078.1
1*		>24196	14395	∞	1235.6	856.7	1701.3	1552.5	1076.5	2187.2
2	8:55	>24196	14395	∞	1497.2	1096.6	1989.6	1280.9	938.1	1721.1
3	9:40	9803.9	6606.1	14101.6	1354	965.3	1840.1	135	77.8	234
4	10:10	>24196	14395	∞	12996.5	8503.6	18965.6	12996.5	8503.6	18965.6
5	10:20	>24196	14395	∞	959	702.4	1289.3	732.8	522.4	1001.3
6	10:30	>241960	143950	∞	241957	163037	471610	>241960	143950	∞
7	10:06	19862.9	12220.3	33002.3	41.3	16.5	95.2	1528.6	1119.6	2062.1
8	10:43	>24196	14395	∞	696.8	496.8	952.5	387.7	261.2	547.4
9	11:02	6488.2	4245.2	9414.6	425.7	286.9	606.9	85.2	39.1	156.4
10	11:20	132.3	71	220.1	<10	0	36.7	20.2	2.6	71.3
<b>07/29</b>										
1	13:50	24195.7	16303.7	47161	738	526.1	995.2	171.2	98.7	273.9
1*		>24196.0	14395	∞	675.6	494.8	915.9	297.6	194.7	434.3
2	13:45	>24196.0	14395	∞	1724.7	1229.6	2355	369.2	248.8	536.6
3	14:40	7701	5490	10940.3	389.3	262.4	558.9	73.1	29.3	138.9
4	15:00	>24196	14395	∞	9803.9	6606.1	14101.6	10462.4	7049.9	15090.3
5	15:20	>24196	14395	∞	555.5	385.2	771.7	601.5	428.8	829.7
6	15:05	>241960	143950	∞	241957	163037	471610	>241960	143950	∞
7	13:40	>24196	14395	∞	74.5	35.6	148.7	1071.2	784.6	1426.6
8	13:01	>24196	14395	∞	1667.7	1384.3	2004.1	373.4	251.6	532.8
9	13:13	3873.2	2458.6	5670.4	364.1	238.2	525.5	135	77.8	234
10	13:26	159.6	91.9	263.7	<10	0	36.7	<10	0	36.7
<b>07/31</b>										
1	10:04	>24196	14395	∞	3436.2	2516.8	4462.1	988.1	704.4	1353.4
2	10:15	>24196	14395	∞	3130.1	2170.3	4394.9	2602.5	1753.6	3651.9
3	10:45	>24196	14395	∞	763.4	559.2	1011.6	882.3	629	1202.4
3*		>24196	14395	∞	443.4	307.4	618.4	1177.6	862.5	1582.3
4	11:22	>24196	14395	∞	1723.3	1194.9	2422.2	4611.1	2927	6878.8
5	11:40	>24196	14395	∞	1049.7	748.3	1438.8	880.3	627.6	1210
6	11:30	>2419600	1439500	∞	161110	292700	687880	>2419600	1439500	∞
7	13:54	>24196	14395	∞	8664.4	5838.3	12453.8	>24196.0	14395	∞
8	13:02	17328.9	11676.7	27094.7	503.9	349.4	700.8	771.2	549.8	1057.4
9	13:14	11198.7	7546	16140	63.2	29	137.1	3448	2188.7	5206.6
10	13:25	1119.9	754.6	1614	<1	0	3.7	3.3	0.7	8.1
<b>08/14</b>										
1	8:30	>24196	14395	∞	1182	888.8	1539.5	1049.7	748.3	1438.8
2	8:45	>24196	14395	∞	958.3	701.9	1260.3	907.5	664.7	1231.3
2*		>24196	14395	∞	758.9	541	1013.5	732.8	522.4	1001.3
3	9:05	12996.5	8503.6	18965.6	201.1	123.7	318.4	74.5	35.6	148.7
4	9:40	>24196	14395	∞	1334.4	951.3	1779.1	2382.2	1651.8	3407.8
5	10:05	>24196	14395	∞	314.5	205.8	456.8	441.2	305.9	625.2

6	9:50	195590	127980	292870	1000	50	5490	2020	260	7130
9	9:46	6131.4	4011.8	8792.1	30.6	6.9	89.4	86	44.5	168.7
11	10:06	>24196	14395	∞	308.9	196.1	454.6	278.5	182.2	412.6
12	10:35	8164.1	5501.2	11745.9	10	0.5	54.9	10	0.5	54.9
<b>08/14</b>										
1	13:45	>24196	14395	∞	1254.2	943.1	1637.2	537.1	382.9	739.7
2	13:30	>24196	14395	∞	849.8	639	1110.8	349.8	228.8	511.8
2*		>24196	14395	∞	831.9	625.5	1083.3	213.3	127.1	326
3	14:25	15531.2	10162	23530.6	161.3	92.9	268.1	132.3	71	220.1
4	14:40	>24196	14395	∞	749.1	534	1028.8	573.1	397.4	790.8
5	15:00	>24196	14395	∞	1934.9	1303.8	2794.5	1860	1253.3	2687.6
6	14:50	>2419600	1439500	∞	162420	118960	215670	224680	147010	343530
8	12:25	>24196	14395	∞	110	56.9	200.8	299.2	189.9	444.3
8	13:24	>24196	14395	∞	186.9	107.7	299.8	503.6	339.4	709.2
11	12:44	>24196	14395	∞	265.3	168.4	400.8	159.6	91.9	2637
12	13:05	4351.7	2762.3	6500.4	20.2	2.6	71.3	<10	0	36.7
<b>08/20</b>										
1	9:20	>24196	14395	∞	3075.9	1952.5	4712.3	9803.9	6606.1	14101.6
2	9:31	>24196	14395	∞	1917.9	1367.3	2644.9	1259.1	922.2	1719.6
3	10:15	8164.1	5501.2	11745.9	268.6	170.5	398.1	74.5	35.6	148.7
4	10:40	>24196	14395	∞	2851	1976.8	3987.6	1332.7	924	1869.1
5	11:00	19862.9	12220.3	33002.3	291.7	185.2	430.9	349.8	228.8	511.8
5*		24195.7	16303.7	47161	228.1	140.3	350.4	373.4	251.6	532.8
6	10:55	56330	39060	77550	1000	50	5490	1000	50	5490
8	11:35	>24196	14395	∞	1782.1	1305.3	2431.3	9208.4	6204.9	12820
11	11:51	>24196	14395	∞	5475	3582.3	8044.5	5475	3582.3	8044.5
12	12:05	>2419.6	1439.5	∞	45.7	30.8	63.8	>2419.6	1439.5	∞
<b>09/04</b>										
13	11:30	19862.9	12220.3	33002.3	85.2	39.1	156.4	428.4	297	600.8
14	11:43	>24196	14395	∞	>24196	14395	∞	>24196	14395	∞
14*		>24196	14395	∞	>24196	14395	∞	>24196	14395	∞
15	10:49	>24196	14395	∞	7269.9	4756.7	10488.7	336.1	219.9	487.7
17	12:34	>241960	143950	∞	173289	116767	270947	37844	26240	52615
18	13:18	>241960	143950	∞	173289	116767	270947	86644	58383	124538
<b>09/10</b>										
13	10:26	>24196	14395	∞	30.4	6.8	73.7	107.8	51.6	186.1
13*		32554	20664	49808	<100	0	367	100	5	549
13*		36540	23194	55545	<100	0	367	<100	0	367
14	11:22	>24196	14395	∞	378.6	255.1	540.4	11198.7	7546	16140
14*		155312	101620	235306	306	69	894	2281	1403	3504
15	10:00	>24196	14395	∞	1723.3	1194.9	2422.2	808.8	576.6	1116.9
17	10:54	>2419600	1439500	∞	37340	25160	53280	34980	23570	50340
18	11:05	1732890	1167670	2706470	45000	31200	62980	43210	29120	61380
<b>09/11</b>										
13	10:36	>24196	14395	∞	24195.7	16303.7	47161	148	85.3	250.6
14	10:45	>24196	14395	∞	711.6	507.3	983	5475	3582.3	8044.5
15	10:15	>24196	14395	∞	398.6	268.6	565	378.6	255.1	540.4
15*		>24196	14395	∞	419.5	282.7	597			
15*		>24196	14395	∞	583.3	404.5	806			
17	9:53	2419570	1630370	4716100	20110	12370	31840	7450	3560	14870
18	10:03	>2419600	1439500	∞	83610	59610	113830	12110	6500	21090

### 4.3 Human-specific *Bacteroides* concentrations

Human-specific *Bacteroides* (HBM) markers were found very consistently at locations 2, 4, 5, 8, 11, and 15 (Fig. 4, Table 5). HBM were sporadically detected in Laguna Channel locations 1 and 3 and drains 6, 9, 10 and 18. In some cases, HBM targets were classified as “detectable but non-quantifiable”, e.g. for location 3, where HBM were detected on 3 occasions, but only quantifiable once. No HBM were detected in drains 7, 12, 13, 14 and 17. When detected, HBM usually were present in the order of  $10^4$  copies/L. Concentrations exceeding  $10^5$  copies/L were only observed at location 5. Drain 9 exhibited concentrations consistently below  $10^4$  copies/L.

The data indicate that HBM originate from 2 drain sections. The highest concentrations of HBM originate from the drain in Salsipuedes St. (loc. 15), flowing into the Gutierrez drain (loc. 11) and finally to the Laguna-Gutierrez confluent (loc. 8). Some HBM originate from a Laguna drain lateral (loc. 10), flowing in Laguna drain (loc. 9) and to the Laguna-Gutierrez confluent (loc. 8). Downstream of the Laguna-Gutierrez confluent, HBM sources occur between 8 and 5, and between 3 and 2. The HBM concentrations changes between locations 5 and 4 were inconsistent, with order of magnitude decreases on some days, but also increases on other days.

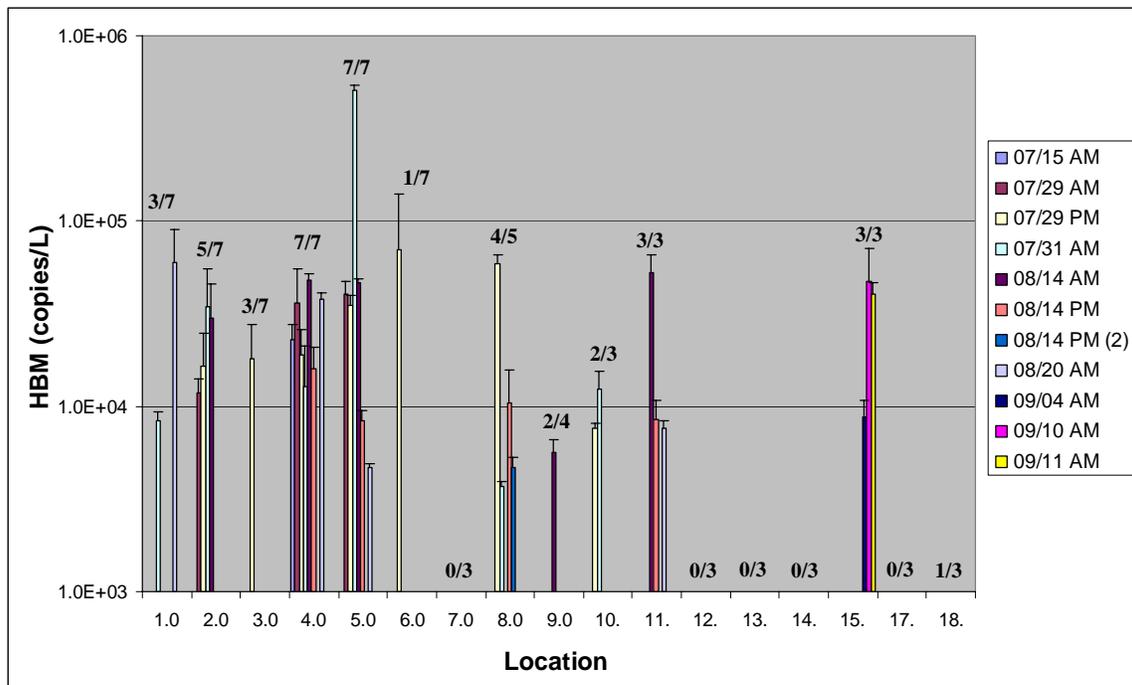


Fig. 4. Human-specific *Bacteroides* (HBM) concentrations at all Laguna Watershed sampling locations. Error bars indicate analytical standard error. The numbers indicate the fraction of samples where HBM were detected. Samples for which only one replicate was above the detection limit were not shown by bars, but were considered as “detectable but non-quantifiable”.

Because the flow in Laguna Channel is constant between locations 5 and 2, the fluxes of HBM (Fig. 5) show similar trends as the concentrations (Fig. 4). To determine the relative magnitudes of the HBM fluxes originating from locations 9 and 11, flow measurements (performed by GeoSyntec) need to be considered.

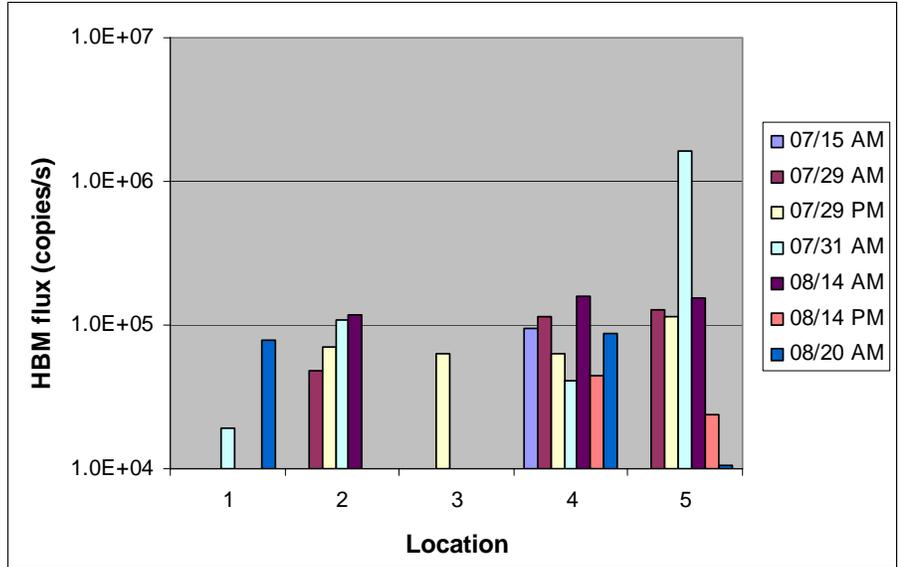


Fig. 5. Fluxes of HBM in Laguna Channel.

#### 4.4 Correlations between FIB and HBM

Scatter plots of log-transformed FIB and HBM concentrations indicate a good correlation between log-transformed EC and ENT concentrations (Fig. 6), with a Spearman's rho ( $\rho$ ) of 0.74 ( $P < 0.001$ ). Also HBM concentrations were correlated with EC ( $P = 0.046$ ,  $\rho = 0.34$ ) and ENT ( $P = 0.038$ ,  $\rho = 0.35$ ). However, those correlation analyses were performed using only quantifiable concentrations ( $n = 35$ ). By including the samples with non-detectable ( $\log(\text{HBM}) = 1$  in Fig. 6) and detectable but non-quantifiable ( $\log(\text{HBM}) = 2$  in Fig. 6) concentrations, the latter correlations disappeared, as indicated in Fig. 6. HBM concentrations for non-quantifiable samples exhibited FIB concentrations ranging between less than 10 to over  $10^6$  FIB/100ml.

A few samples had a rather consistent disconnect between FIB and HBM concentrations. All locations that had FIB exceeding  $3 \times 10^4$  MPN/100 ml, but had no quantifiable HBM, belonged to the drain from the City Annex yard (locations 6, 17, and 18, circled in green in Fig. 6). Conversely, on 2 out of 3 occasions, location 10 (Laguna drain lateral) exhibited  $< 10$  FIB/100 ml, but significant HBM concentrations (circled in red in Fig. 6).

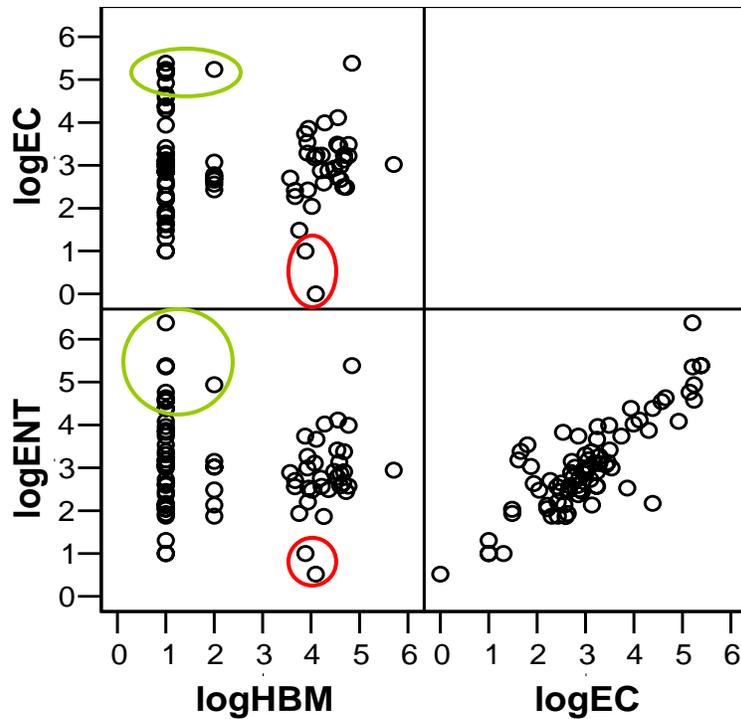


Fig. 6. Scatter plots showing the correlation between log-transformed HBM, EC, and ENT concentrations. For visualization purposes, samples having detectable but non-quantifiable HBM concentrations were assigned a value  $\log(\text{HBM}) = 2$ , samples with HBM concentrations below the detection limit were assigned a value  $\log(\text{HBM}) = 1$ .

#### 4.5 *Methanobrevibacter smithii nifH* gene detection

For initial method testing, *Methanobrevibacter smithii nifH* gene (Mnif) PCR was run on archived DNA extracts from septage, sewage, human feces, dog feces and raccoon feces (Fig. 7). After the first round of PCR, septage and sewage samples showed a band at the same position (222 bp) of the positive control (*Methanobrevibacter smithii* DNA). After the second round of PCR, the same samples as in round 1 showed positive results. For some samples, additional bands were present besides the 222 bp target band. Those additional bands were not considered as positive signals, and their appearance did not inhibit the amplification of the 222 bp band, as was seen for sample W2.

Our results confirmed the specificity of the 222 bp *Methanobrevibacter smithii nifH* gene fragment for human fecal waste. The fragment was not detected in other animals' fecal samples. Therefore, the Mnif assay was used to determine the presence of sewage pollution for all Laguna Watershed samples.

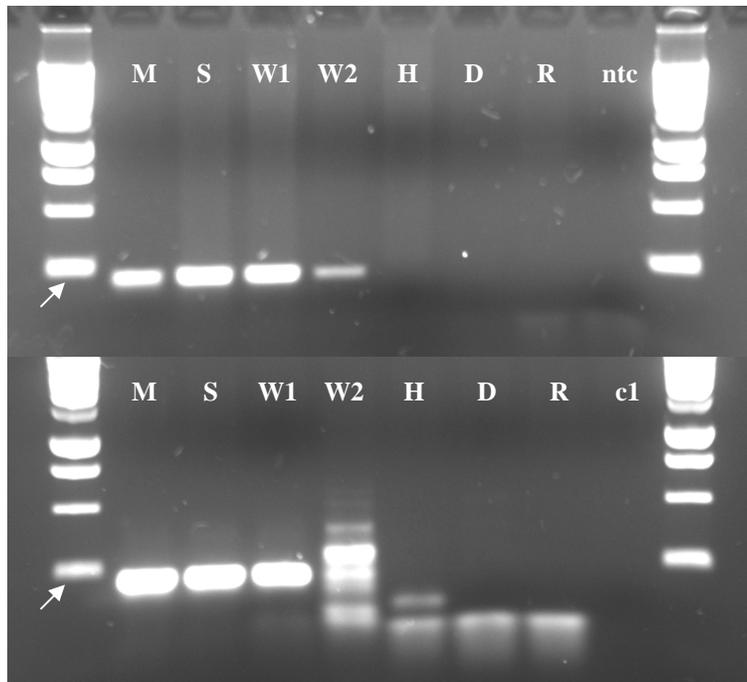


Fig. 7. Agarose gels of round 1 and round 2 Mnif PCR products of *Methanobrevibacter smithii* (M), septage (S), sewage (W1, W2), human feces (H), dog feces (D), and raccoon feces (R). Size ladders at both ends of the gel were 1 kb ladders, an arrow indicating the position of the 250 bp band. A non-template control (ntc) was included in each run. In the second PCR round, the ntc of round 1 was included (c1).

Two rounds of Mnif PCR were performed on all Laguna Watershed samples (Fig. 8, Fig. 9, Table 5). After the first PCR round, none of the samples exhibited amplification of the *Methanobrevibacter smithii nifH* gene (Fig. 8), while the positive control always showed a band of the expected length (222 bp). After the second round of Mnif PCR, several samples showed amplification of the *M. smithii nifH* gene (Fig. 9, Table 5). Some presumed positives were rerun on a second gel to ensure accurate identification of the size of the amplicon. In total, 23 out of 79 samples were considered to be positive for the *M. smithii nifH* gene.

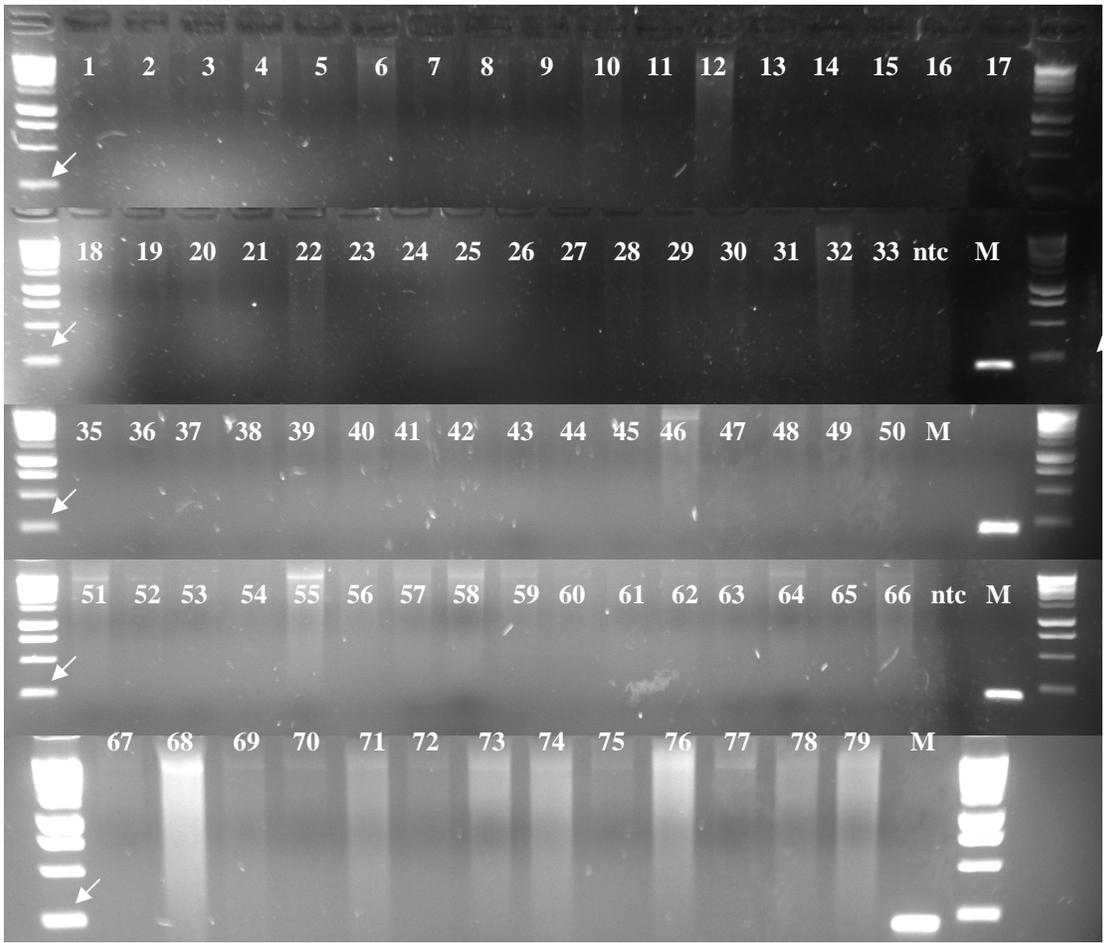


Fig. 8. Agarose gels of round 1 Mnif PCR products of all Laguna samples (1 to 79), including non-template controls (ntc) and *Methanobrevibacter smithii* positive controls (M). Size ladders at both ends of the gel were 1 kb ladders, an arrow indicating the position of the 250 bp band.

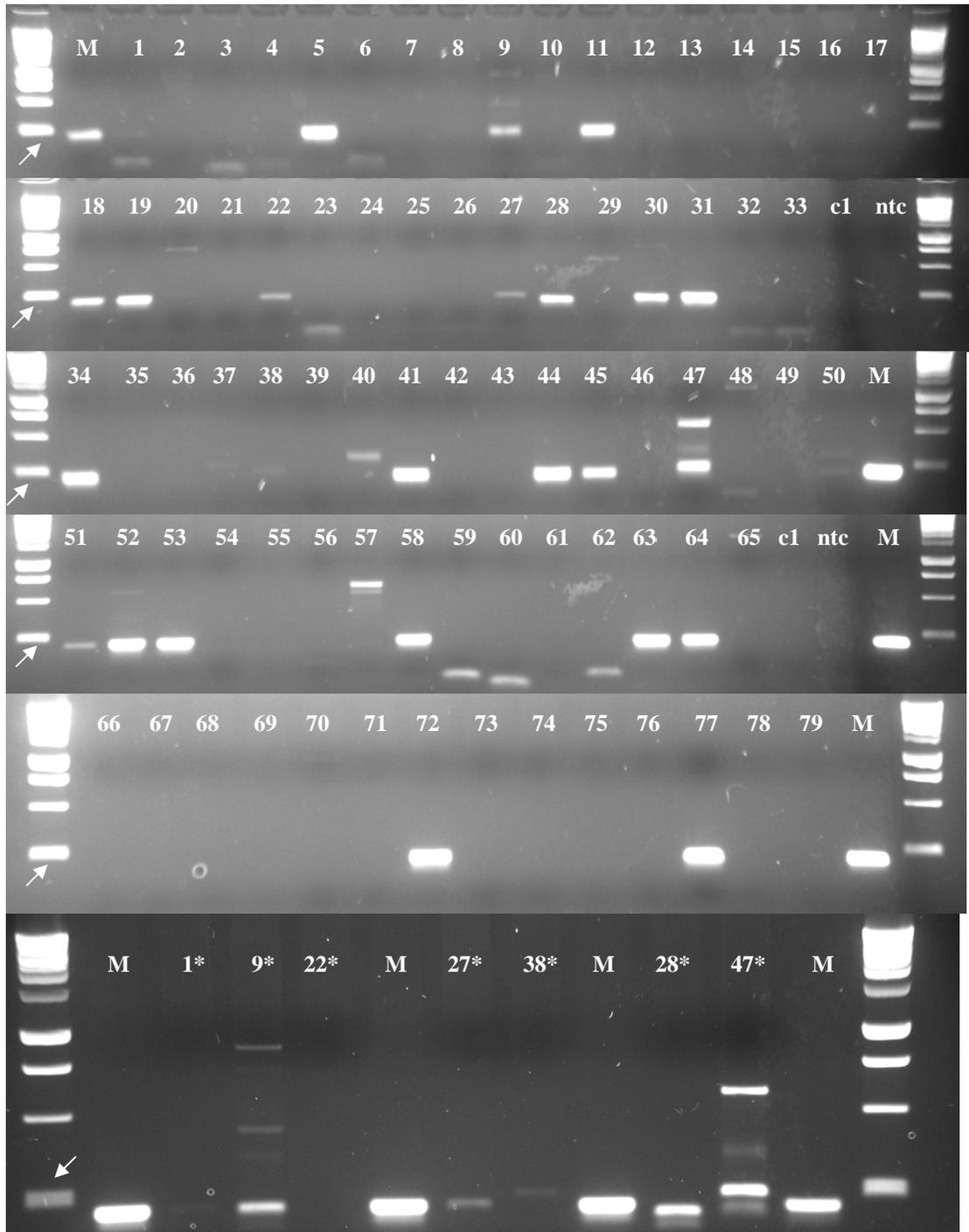


Fig. 9. Agarose gels of round 2 Mnif PCR products of all Laguna samples (1 to 79), including non-template controls for round 1 (c1) and round 2 (ntc), and *Methanobrevibacter smithii* positive controls (M). Size ladders at both ends of the gel were 1 kb ladders, an arrow indicating the position of the 250 bp band. Samples indicated with \* were run a second time to verify the presence and size of the amplicon.

Table 5. Overview of sample IDs and *Methanobrevibacter smithii NifH* gene PCR results for Laguna Watershed samples. Positive Mnif samples are indicated with + (faint band), ++ (medium band) or +++ (intense band). The HBM concentrations (copies/L) are included for comparison.

ID	sample	Mnif	HBM	ID	sample	Mnif	HBM
1	L0715-1			41	L0814-5 AM	+++	4.6E4
2	L0715-2		*	42	L0814-6 AM		
3	L0715-3			43	L0814-9 AM		5.7E3
4	L0715-4		2.3E4	44	L0814-11 AM	+++	5.3E4
5	L0715-5	+++	*	45	L0814-12 AM	+++	
6	L0715-6			46	L0814-1 PM		
7	L0729-1 AM			47	L0814-2 PM	+	
8	L0729-2 AM		1.2E4	48	L0814-3 PM		
9	L0729-3 AM	++		49	L0814-4 PM		1.6E4
10	L0729-4 AM		3.6E4	50	L0814-5 PM	+	8.4E3
11	L0729-5 AM	+++	4.0E4	51	L0814-6 PM	++	
12	L0729-6 AM			52	L0814-8 PM	+++	1.0E4
13	L0729-7 AM			53	L0814-8b PM	+++	4.7E3
14	L0729-8 AM			54	L0814-11 PM		8.6E3
15	L0729-9 AM			55	L0814-12 PM		
16	L0729-10 AM			56	L0820-1		6.0E4
17	L0729-1 PM			57	L0820-2		
18	L0729-2 PM	+++	1.7E4	58	L0820-3	+++	*
19	L0729-3 PM	+++	1.8E4	59	L0820-4		3.8E4
20	L0729-4 PM		1.9E4	60	L0820-5		4.7E3
21	L0729-5 PM		3.5E4	61	L0820-6		
22	L0729-6 PM		7.0E4	62	L0820-8		
23	L0729-7 PM			63	L0820-11	+++	7.6E3
24	L0729-8 PM		5.9E4	64	L0820-12	+++	
25	L0729-9 PM		*	65	L0904-13		
26	L0729-10 PM		7.6E3	66	L0904-14		
27	L0731-1	++	8.4E3	67	L0904-15		8.8E3
28	L0731-2	+++	3.5E4	68	L0904-17		
29	L0731-3		*	69	L0904-18		*
30	L0731-4	+++	1.3E4	70	L0910-13		
31	L0731-5	+++	5.1E5	71	L0910-14		
32	L0731-6			72	L0910-15	+++	4.8E4
33	L0731-7			73	L0910-17		
34	L0731-8	+++	3.7E3	74	L0910-18		
35	L0731-9			75	L0911-13		
36	L0731-10		1.2E4	76	L0911-14		
37	L0814-1 AM		*	77	L0911-15	+++	4.0E4
38	L0814-2 AM		3.0E4	78	L0911-17		
39	L0814-3 AM			79	L0911-18		
40	L0814-4 AM		4.8E4				

#### 4.6 Comparison of HBM and Mnif

For an overall comparison of the detection of human-associated waste using HBM and Mnif markers, we calculated the sensitivity ( $r$ ) and specificity ( $s$ ) of the Mnif assay compared to the HBM assay, using the formulas  $r = a/(a+c)$  and  $s = d/(b+d)$  (Gawler, Beecher et al. 2007), where  $a$  is the number of true positives,  $b$  is the number of false positives,  $c$  is the number of false negatives, and  $d$  is the number of true negatives. Using

the data as shown in Table 6, we find a sensitivity of 43% and a specificity of 87%. While the specificity is high, the sensitivity is rather low. This is likely related to the lower detection limits of qPCR compared to regular PCR (even when running 2 rounds of PCR).

Table 6. Total number of positive (+) and negative (-) samples for the HBM and Mnif assays.

		HBM		sum
		+	-	
Mnif	+	18	5	23
	-	23	33	56
sum		41	38	79

The general trends observed using HBM were confirmed with the Mnif assay (Table 7). Based on the averaged results of both assays, the locations that appeared polluted with human fecal waste were: 2, 4, 5, 8, 11, and 15 (Fig. 10).

The most important differences between the results of the Mnif and HBM assays were (i) Mnif PCR product was detected twice at location 12, where HBM were not detected at all, and (ii) Mnif were detected only once at location 4, while this site had 100 % detection of HBM.

Table 7. Comparison of the fraction of positive samples for the HBM and Mnif assays for all Laguna Watershed sampling locations, and averaged percentage detection of human waste.

Location	# samples	# HBM +	# Mnif +	% HBM +	% Mnif +	% avg
1	7	3	1	43	14	29
2	7	5	3	71	43	57
3	7	3	3	43	43	43
4	7	7	1	100	14	57
5	7	7	5	100	71	86
6	7	1	1	14	14	14
7	3	0	0	0	0	0
8	6	4	3	67	50	58
9	4	2	0	50	0	25
10	3	2	0	67	0	33
11	3	3	2	100	67	83
12	3	0	2	0	67	33
13	3	0	0	0	0	0
14	3	0	0	0	0	0
15	3	3	2	100	67	83
17	3	0	0	0	0	0
18	3	1	0	33	0	17

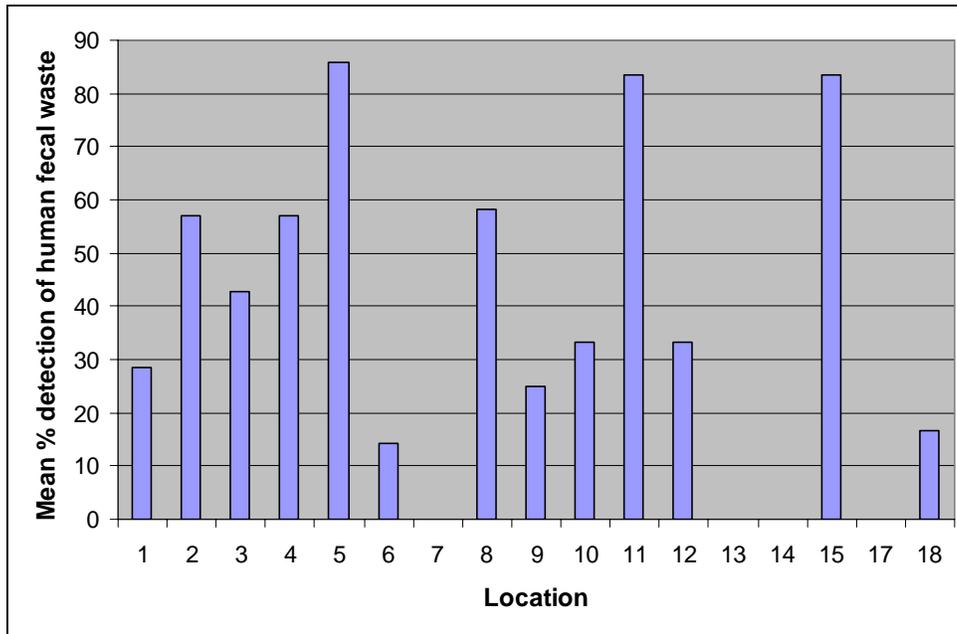


Fig. 10. Mean % detection of human fecal markers using the HBM and Mnif assays for all Laguna Watershed locations.

## 5. Conclusions

This study investigated the temporal variability of concentrations and fluxes of fecal indicator bacteria and DNA markers specific for human waste for the Laguna Watershed in Santa Barbara, CA, during the summer of 2008. Concentrations of FIB and human-specific DNA markers in the entire watershed, and flow in the lower Laguna Watershed (open channel) were determined by UCSB within the Holden Lab, and described in this report. The dry weather flow in the upper watershed (subsurface drains) was investigated by GeoSyntec, and is described in an accompanying report, as are the results concerning microbial fluxes in the upper watershed.

Flow measurements in the Laguna Channel were relatively constant between the most upstream and downstream locations. Consequently, microbial concentrations and fluxes in Laguna Channel were considered proportional to one another. The average flow received by the pump station from Laguna Channel was 4.1 L/s. The average microbial fluxes were  $6.0 \times 10^4$  MPN/s (EC),  $3.9 \times 10^4$  MPN/s (ENT), and  $8.6 \times 10^4$  HBM copies/s.

For FIB, similar fluxes were found entering and exiting the Laguna Channel, but variations occurred as the water traveled through the Channel, indicating possible FIB decay and additional FIB sources within the open channel. A first likely source of FIB within the channel was the City Annex Yard, as its drain flowing into the channel contained very high FIB concentrations. Additional sources of FIB were present between the railroad tracks and the pump station. These sources could not be identified, although likely sources are birds and wildlife that were frequently observed during this study in or near the lower channel.

In the drains of the upper Laguna Watershed, similar FIB concentrations were originating from the Gutierrez and Laguna drain sections, and continuous flow measurements will be able to determine fluxes from each section.

The HBM fluxes in Laguna Channel were somewhat different than those of FIB. The City Annex Yard was not a significant source of HBM to the open channel. Possibly, the proximity of the labor line could increase HBM fluxes in the channel, but such increases were only found sporadically. However, an increase in HBM flux was consistently found between the railroad tracks and the pump station, indicating that FIB increases in this reach were likely associated with human sources, although the latter remain unidentified. In the drains of upper Laguna Watershed, a pattern of HBM flowing from Salsipuedes to Gutierrez to Laguna drain could be identified. HBM also originated from a lateral to Laguna drain (loc. 10), but the contribution of this source to the HBM concentrations more downstream is low compared to the contribution from the Gutierrez drain.

A new DNA-based method, the Mnif assay, was able to confirm the general trends in the presence of human fecal waste, as determined by the HBM analysis. Based on several human and non-human fecal sources tested in this study, the Mnif assay was specific for human waste. The value of this new method is significant, as it confirms the presence of human fecal pollution by targeting a different DNA fragment associated with a different taxonomic group of bacteria as compared to the HBM assay. Moreover, the assay is likely less expensive and faster than the HBM assay. However, it is not quantitative and is less sensitive, and is therefore mainly useful in combination with the HBM assay, as an initial screening or confirmatory tool.

## 6. Acknowledgements

The research described in this report was funded by the City of Santa Barbara. The research was substantially performed, including the writing of this report, by Dr. Bram Sercu in the Holden Lab Group at UCSB. Others in the Holden Lab who contributed to field and laboratory research included Laurie Van De Werfhorst, Kaitlyn Lee (UCSB Summer Session Research Mentorship Program), and Line Darmedru (summer internship from ISARA, Lyon, France).

This research was performed cooperatively with the City through the assistance of Cameron Benson, Dr. Jill Murray, Tim Burgess, and additional staff of the Creeks Division.

We thank Dr. R. Ellender and Chris Flood (University of Southern Mississippi) for providing *Methanobrevibacter smithii* DNA for use as a positive control in the Mnif assay.

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## **APPENDIX C**

Fecal indicator bacteria and human fecal pollution in the Laguna Watershed-Phase II

## **Fecal indicator bacteria and human fecal pollution in the Laguna Watershed – Phase II**

Final report to City of Santa Barbara  
09/02/2009

Performed in the Laboratory of Patricia Holden, Donald Bren School of Environmental Science & Management, UC Santa Barbara ([holden@bren.ucsb.edu](mailto:holden@bren.ucsb.edu), 805-893-3195) by Bram Sercu, Associate Specialist I and Laurie Van De Werfhorst, Staff Research Associate II.

### 1. Summary

The Laguna Watershed Phase I study was performed in the summer of 2008, and a final report of the Phase I work was presented to the City of Santa Barbara on November 18, 2008. This report presents the data and conclusions of the Laguna Watershed Phase II study, performed during the spring and summer of 2009. The Laguna Watershed Phase II study was initiated to obtain a better understanding of the results obtained in Phase I, and involved additional sampling at locations sampled during Phase I and at new locations in Laguna Watershed.

The first goal was to better localize the origin of human fecal contamination flowing into the Gutierrez drain, as observed in Phase I, by collecting additional samples upstream in the storm drains.

The second goal was to obtain additional data for a lateral into Laguna drain, which exhibited a low degree of human fecal contamination but very low fecal indicator bacteria concentrations.

The third goal was to investigate the degree of human fecal pollution in the Quarantina drain, flowing onto East Beach, and in the upstream drain sections. This sub-watershed had not been sampled during Phase I.

The fourth goal was to determine the suitability of three new ELISA-based assays, quantifying chemical sewage markers, for source tracking human fecal pollution.

### 2. Description of field sites and timing

Eleven field sites were chosen, in coordination with the City of Santa Barbara. The selection of the sampling locations (Fig. 1, Table 1) was based on preliminary knowledge from Phase I and field visits. Samples were taken on June 3<sup>rd</sup>, June 10<sup>th</sup> and June 17<sup>th</sup> of 2009, for all 11 locations.



Fig. 1. Sampling locations and IDs for Laguna Watershed Phase II. The blue line represents the Laguna Channel.

Table 1. Sample location ID, coordinates and description of Laguna Watershed Phase II sampling locations

ID	Latitude	Longitude	Location
3	N 34° 24' 930"	W 119° 41' 151"	Laguna Channel, upstream of railroad tracks
10	N 34° 25' 398"	W 119° 41' 637"	Laguna drain lateral
15	N 34° 25' 386"	W 119° 41' 329"	Drain Salsipuedes St. & Haley St.
19	N 34° 25' 460"	W 119° 41' 394"	Drain E. Cota St. & Salsipuedes St. (sidewalk)
20	N 34° 25' 684"	W 119° 41' 423"	Drain De La Guerra St. & Nopal St. (sidewalk)
21	N 34° 25' 696"	W 119° 41' 276"	Drain Milpas St. & Ortega St.
22	N 34° 24' 900"	W 119° 40' 703"	Beach drain discharge (across Fess Parker's resort)
23	N 34° 25' 071"	W 119° 40' 734"	Drain Quarantina St. & Cacique St.
24	N 34° 25' 340"	W 119° 40' 960"	Drain Yanonali St. & Nopal St.
25	N 34° 25' 472"	W 119° 40' 822"	Drain Yanonali St. & Alisos St.
26	N 34° 25' 564"	W 119° 41' 259"	Drain Nopal St. & E. Cota St.

### 3. Materials and Methods

#### 3.1 Microbiological methods used in Phase I

Water sampling, quantification of fecal indicator bacteria by IDEXX, DNA extraction, SybrGreen qPCR for human-specific *Bacteroidales* (HBM qPCR) and PCR for *Methanobrevibacter smithii nifH* (Mnif PCR) was performed as in Laguna Watershed Phase I, and detailed in the November 18, 2008 final report. *Enterococcus* spp. qPCR was also performed.

In brief, 2L of water was sampled at each location and filtered in the laboratory for collection of bacterial cells. Fecal indicator bacteria (total coliforms, *E. coli* and *Enterococcus* spp.) were quantified using the Quanti-Tray/2000 assay (IDEXX Laboratories). DNA was extracted using the UltraClean Water DNA Kit (MoBio Laboratories, Carlsbad, CA). Mnif PCR was performed using a 2 step protocol using the Taq PCR Core Kit (Qiagen, Valencia, CA). PCR products were visualized using a 2 % agarose gel. HBM qPCR was performed on an iQ5 thermocycler (BioRad), using the SybrGreen Core Kit (Eurogentec). To test for inhibition during HBM qPCR, all DNA extracts were analyzed at 10 and 2 ng/reaction. Reaction inhibition occurred if the more diluted template produced higher final concentrations. In that case, the highest concentration was used as the final value. Samples were classified as not detected (ND), detected but non-quantifiable (DNQ), or quantifiable, in which case the concentration and standard errors are presented.

#### 3.2 qPCR for salmon testes DNA and *Enterococcus* spp.

The TaqMan qPCR assay for salmon testes DNA was performed prior to the *Enterococcus* spp. assay in order to determine the lowest template dilution without inhibition.

Both the salmon testes and *Enterococcus* spp. qPCR analyses were performed with TaqMan chemistry in a CFX96 BioRad thermocycler, using dual-labeled (BHQ-FAM) probes (Eurofins MWG Operon, Huntsville, AL). The qPCR MasterMix Plus (no ROX) (Eurogentec, San Diego, CA) was used in final reaction volumes of 25 µl, including 2.5 µl of diluted DNA template. A final concentration of 0.2 mg/ml bovine serum albumin was added to all reactions. The thermocycling program for all analyses was: 2 min at 50 °C, 10 min at 95 °C, 45 cycles of 15 sec at 94 °C and 60 sec at 60 °C. Concentrations were reported as not quantifiable (NQ), in case < 2 replicate wells were within the linear range of quantification.

The salmon testes DNA qPCR was based on the protocols by (Haugland, Siefring et al. 2005) and (Morrison, Bachoon et al. 2008), and uses the same primer (300 nM) and probe (100 nM) concentrations. The qPCR master mix is spiked with salmon testes DNA, to a final concentration of 0.25 ng/reaction. Four no sample DNA reactions (= no inhibition control) are run on each plate, in which only salmon testes DNA, PCR reagents and PCR-grade water are added. In addition, a 3 log salmon testes DNA standard curve is

run to determine amplification efficiency. Diluted template DNA (2.5  $\mu$ l) is added to all remaining reactions (in duplicate). Using the no inhibition controls, we calculated the average + 3  $\times$  standard deviation cycle threshold value ( $C_{t_{ni}}$ ). This value was used as the upper  $C_t$  value for no inhibition. All reactions with sample DNA that produced an average  $C_t > C_{t_{ni}}$  were considered to be inhibited. The salmon testes assay was run first, using 1:10 diluted DNA template, to determine the occurrence of reaction inhibition. If inhibition occurs, twofold dilutions are analyzed until no inhibition occurs. The lowest template dilution without inhibition is used for *Enterococcus* spp. qPCR.

The *Enterococcus* spp. qPCR assay is based on the protocol of (Haugland, Siefring et al. 2005). The primer and probe concentrations used in this study were 900 nM (forward primer), 300 nM (reverse primer) and 100 nM (probe). *Enterococcus* spp. concentrations were expressed as cell equivalents (c.eq.) per 100 ml, by assuming an *rrm* operon copy number of 6 for *Enterococcus*. Not-quantifiable concentrations were assigned a value of 100 c.eq./100 ml for graphing and statistical analyses.

### 3.3 ELISA

The ELISA assays (enzyme linked immunosorbent assays; Abraxis, [www.abraxiskits.com/product\\_markers.htm](http://www.abraxiskits.com/product_markers.htm)) are performed in 96-well plates, and are direct competitive ELISA tests, based on the recognition of the target analyte by specific antibodies. Three assays were tested, for caffeine (Microtiter Plate, #515575), for cotinine (Microtiter Plate, #515565), and triclosan (Microtiter Plate, #530114). When caffeine is present in a sample, it competes with the caffeine-HRP analogue for the binding sites of mouse anti-caffeine antibodies in the test solution. Then the caffeine antibodies are bound by a second antibody (goat anti-mouse) which is immobilized in the plate. Cotinine and triclosan assays work similarly, except they utilize rabbit and anti-rabbit antibodies in the solutions and goat anti-rabbit in the plates. After multiple washing steps, a substrate solution is added which allows color signal generation. The intensity of the blue color is inversely proportional to the concentration of the target present. After a specified period of time, the color reaction is stopped and the absorbance of each well is read at 450 nm. Target concentrations in the samples are determined by standard curve interpolation. The required equipment includes pipettes for aspirating and dispensing small volumes, and a 96-well plate reader capable of absorbance measurements at the required wavelengths.

For caffeine and cotinine assays, approximately 10 ml of water sample was filtered via 10 ml sterile disposable syringes through sterile 0.2  $\mu$ m Anotop filters (Whatman) into a 40 ml amber glass vial. For the triclosan assay, the procedure was the same as above, except 7.5 ml of sample was filtered, and the glass storage vials contained 2.5 ml of HPLC grade methanol to prevent loss of the analyte. All sample vials were stored on ice until return to the laboratory where they were frozen and kept at -20°C until analysis.

The assay procedure was followed strictly in accordance with the instructions provided in each kit, and all standards and samples were performed in duplicate. Most assays started with no sample dilution. If the first run revealed a sample higher than the highest standard

(> 5 ppb for caffeine and cotinine, > 2.5 ppb for triclosan), the sample was diluted using solutions provided in each kit and run on a second plate. Absorbance was read using a BioTek Synergy 2 plate reader. Standard curves and sample concentrations were calculated using BioTek's Gen5™ Reader Control and Data Analysis Software. The software was programmed to calculate the %B/Bo for each standard by dividing the absorbance for each duplicate by the mean absorbance value of the Zero Standard. The %B/Bo vs. target concentration was plotted for each plate, and a 4-parameter logistic function was used to generate the standard curve (= dose response curve) (Baud 1993). The formula for the 4-parameter function is:

$$Y = \frac{A - D}{1 + \left(\frac{X}{C}\right)^B} + D$$

where 'A' is the estimated response at zero concentration, 'B' is the slope function, 'C' is the mid-range concentration (C<sub>50</sub>), and 'D' is the estimated response at infinite concentration. The quality of standard curve fit was assessed via backcalculation of standards (= standards recovery): Observed concentration/expected concentration x 100. A standard recovery between 70-130% was considered to indicate the standard curve has good fit (<http://www.biocompare.com/technicalarticle/1160/Principles-Of-Curve-Fitting-For-Multiplex-Sandwich-Immunoassays-Rev-B-from-Bio-Rad.html>). If more than one standard set was included on a plate, each standard set was first evaluated separately to determine within assay variability, then all standard replicates were used together to generate one comprehensive standard curve to quantify samples.

Sample %B/Bo values were then used to interpolate the concentration of the target. Any samples with values less than the lowest standard replicate (that was still higher than the zero standard replicates) were considered not detected. If a sample had one duplicate in range and one below detection limit, that sample was also considered to be not detected. For all sample replicates, the mean, standard deviation, standard error, and %CV (coefficient of variation) were calculated. Plate to plate variability was assessed by calculating an overall %CV of all sample replicates from both plates, and performing an F-Test Two-Sample for Variances (two-tail) followed by either a t-Test (two-tail) Assuming Equal Variation or a t-Test (two-tail) Assuming Unequal Variation (Microsoft Excel) as indicated by the F-Test results.

## 4. Results

### 4.1 Physical-chemical data

Sampling dates and times, physical-chemical parameters, and flow for the Phase II samples are shown in Table 2. Table 2 also includes the sample ID that was internally used by UCSB for sample collection and storage, however, this ID will not be further used in this report and the site location ID will be used instead. Dissolved oxygen was not determined on 06/03/09 for most locations, because of malfunctioning of the UCSB probe. Flow was only collected at location 3, for comparison with the Phase I data.

Water temperatures were all between 16.9 °C and 21.8 °C, with lowest values usually observed for locations 3 and 25, and the highest for location 26. Dissolved oxygen was more variable and ranged between 0.96 mg/l and 7.13 mg/l, and was always lowest for location 26 and 3, and highest for locations 15 and 21. Conductivity was 1 – 2 mS/cm for most locations, and very constant in time. Only location 22 showed a high temporal variability in conductivity, due to the influence of tides on the beach (high conductivity on 06/10/09). Also location 19 had a slightly higher conductivity (> 4.9 mS/cm) compared to most other storm drain samples. The flow in Laguna Channel (location 3) decreased somewhat over the sampling time frame.

Table 2. Laguna Watershed Phase II samples: site location IDs, internal UCSB ID, sampling dates and times, temperature (Temp, °C), dissolved oxygen (DO, mg/l), conductivity corrected to 25 °C (cond, mS/cm), flow (m<sup>3</sup>/s). na: not available

ID	ID_UCSB	Time	Temp	DO	Cond.	Flow
06/03/09						
3	L0603-1	8:43	17.7	na	1.3	0.0064
10	L0603-2	11:13	20.2	na	1.5	na
15	L0603-3	10:22	20.6	na	1.5	na
19	L0603-11	10:35	20.6	na	5.0	na
20	L0603-4	10:48	20.0	na	2.2	na
21	L0603-5	10:59	19.6	na	1.4	na
22	L0603-6	9:12	20.3	na	4.3	na
23	L0603-7	9:26	21.5	na	2.0	na
24	L0603-8	9:36	19.5	na	1.7	na
25	L0603-9	9:51	18.5	na	1.6	na
26	L0603-10	10:07	21.0	na	1.8	na
06/10/09						
3	L0610-1	8:47	17.8	1.41	1.3	0.0050
10	L0610-2	11:10	20.9	4.19	1.5	na
15	L0610-3	10:21	21.2	7.13	1.5	na
19	L0610-11	10:32	20.8	2.5	4.9	na
20	L0610-4	10:46	20.8	3.15	2.3	na
21	L0610-5	10:58	20.1	6.03	1.4	na
22	L0610-6	9:15	16.9	4.97	32.3	na
23	L0610-7	9:31	20.6	2.61	1.7	na
24	L0610-8	9:43	19.9	4.56	1.6	na
25	L0610-9	9:57	18.9	6.49	1.6	na
26	L0610-10	10:10	21.5	0.96	0.8	na
06/17/09						
3	L0617-1	8:49	19.3	1.96	1.3	0.0047
10	L0617-2	11:20	21.2	3.07	1.5	na
15	L0617-3	10:26	21.3	7.12	1.5	na
19	L0617-11	10:40	21.1	2.85	5.3	na
20	L0617-4	10:51	21.1	2.55	2.1	na
21	L0617-5	11:03	20.6	6.57	1.4	na
22	L0617-6	9:16	20.2	2.07	1.9	na
23	L0617-7	9:31	21.6	2.72	1.9	na
24	L0617-8	9:41	20.0	5.21	1.5	na
25	L0617-9	9:52	19.3	4.31	1.6	na
26	L0617-10	10:10	21.8	1.01	1.2	na

## 4.2 Fecal indicator bacteria by IDEXX

All fecal indicator bacteria (FIB) concentrations and 95% confidence intervals are shown in Fig. 2 and Table 3.

Total coliforms (TC) are often out of range, especially in the Quarantina Drain network (locations 22 – 26). The lowest TC concentrations are found at loc. 10. The TC results are not discussed in detail because of the large portion of out of range concentrations.

The *E. coli* (EC) concentrations were also consistently lowest at location 10, with all of the samples below the limit of detection (< 10 MPN/100 ml). The EC concentrations in Laguna drain were similar within and between sites, except at location 19, showing a concentration spike of about 2 orders of magnitude occurred on 06/10, and location 10, having EC concentrations below the limit of detection. Spikes of high EC concentrations were observed (> 24,196 MPN/100 ml) at Quarantina drain locations 23, 24 and 26 on one of the three sampling days. All Quarantina drain locations, except the discharge at the beach, exhibited high temporal variability of the EC concentrations. ANOVA on log-transformed EC concentrations did not reveal any statistically significant differences between locations, when analyzed for Laguna (excluding location 10) and Quarantina drain separately.

In the Laguna drain network, location 19 appears to have a low influence on the EC concentrations downstream (location 15), as the EC spike on 06/10 did not influence the EC concentration at location 15. EC concentrations at locations 20 and 21 were similar, and their influence on concentrations downstream would be determined by the relative flow at both locations. In the Quarantina drain network, higher EC concentrations were found at location 26 compared to location 25, so the drain along Nopal St. may contribute more to EC concentrations downstream, although flow data are needed to calculate relative EC loads. EC concentrations increased slightly from location 22 to 23, probably related to pooling of drain discharge on the beach. The spike in EC concentrations at locations 22 and 23 on 06/10 coincided with breaching of the sand barrier to the ocean and high conductivity at location 22.

The *Enterococcus* spp. (ENT) concentrations were lowest (< 10 MPN/100 ml) at location 10. As with EC, more uniform ENT concentrations were observed for the Laguna drain locations (except location 19), and more variable ENT concentration were observed for the Quarantina drain locations. The highest concentrations (> 24,196 MPN/100 ml) were observed at locations 25 and 26. ANOVA on log-transformed ENT concentrations did not reveal any statistically significant differences between locations, when analyzed for Laguna (excluding location 10) and Quarantina drain separately.

The longitudinal patterns in ENT concentrations in the Laguna drain network were similar as for EC, with a low influence of the location 19 ENT concentration spike on the downstream concentrations, and only slightly higher concentrations at location 20 than at location 21. In the Quarantina drain network, patterns were somewhat different than for EC. For ENT, both drain sections at locations 25 and 26 had similar ENT concentrations, and concentrations decreased downstream at location 24. At location 22 ENT

concentrations increased, more than observed for EC, probably related to pooling of the drain discharge on the beach. Again, the spike in ENT concentrations at locations 22 and 23 on 06/10 coincided with breaching of the sand barrier to the ocean and high conductivity at location 22.

Table 3. Fecal indicator bacteria concentrations (MPN/100 ml) for Laguna Watershed Phase II samples, including lower (LCI) and upper (UCI) 95% confidence intervals.

	Time	Conc.	TC LCI	UCI	Conc.	EC LCI	UCI	Conc.	ENT LCI	UCI	
06/03/09											
	3	8:43	14136	9249	21016	246	151	376	145	77.9	236
	10	11:13	389.3	262	559	< 10	0	36.7	< 10.0	0	36.7
	15	10:22	5172	3384	7636	1187	847	1627	183	105	288
	19	10:35	2143	1402	3209	9.9	0.3	36.7	10	0.5	54.9
	20	10:48	2987	2071	4232	448	302	634	350	236	503
	21	10:59	19863	12220	33002	328	221	472	120	59.7	203
	22	9:12	> 24196	14395	∞	691	479	956	2909	1904	4461
	23	9:26	1860	1253	2687.6	20.2	2.6	71.3	20.1	2.5	59.2
	24	9:36	> 24196	14395	∞	> 24196	14395	∞	565	392	786
	25	9:51	> 24196	14395	∞	40.9	11.6	90.7	1314	936	1804
	26	10:07	> 24196	14395	∞	> 24196	14395	∞	> 24196	14395	∞
06/10/09											
	3	8:47	24196	16304	47161	529	367	737	173	103	282
	10	11:10	1017	725	1382	< 10.0	0	36.7	< 10.0	0	36.7
	15	10:21	12997	8504	18966	487	328	690	166	95.6	268
	19	10:32	> 24196	14395	∞	1782	1305	2431	4611	2927	6879
	20	10:46	> 24196	14395	∞	211	126	326	1187	847	1627
	21	10:58	7701	5490	10940	73.1	29.3	139	109	56.3	195
	22	9:15	> 24196	14395	∞	6488	4245	9415	9208	6205	12820
	23	9:31	> 24196	14395	∞	> 24196	14395	∞	4884	3100	7215
	24	9:43	19863	12220	33002	480	333	672	97.9	46.8	184
	25	9:57	7270	4757	10489	10	0.5	54.9	813	579	1114
	26	10:10	77010	54900	109403	6437	4463	8861	969	445	1716
06/17/09											
	3	8:49	12033	8108	17507	175	101	286	216	129	337
	10	11:20	4352	2762	6500	< 10.0	0	36.7	< 10.0	0	36.7
	15	10:26	8664	5838	12454	187	108	300	960	684	1317
	19	10:40	> 24196	14395	∞	30.6	6.9	89.4	86	44.5	169
	20	10:51	15531	10162	23531	370	249	537	569	417	764
	21	11:03	5475	3582	8045	30.6	6.9	89.4	85.2	39	156
	22	9:16	24196	16304	47161	327	214	477	591	421	812
	23	9:31	7270	4757	10489	63.2	29	137	30.6	6.9	89.4
	24	9:41	4352	2762	6500	52.1	22.9	119	97.9	46.8	184
	25	9:52	> 24196	14395	∞	855	610	1180	> 24196	14395	∞
	26	10:10	3013	1971	4420	745	356	1487	202	26	713

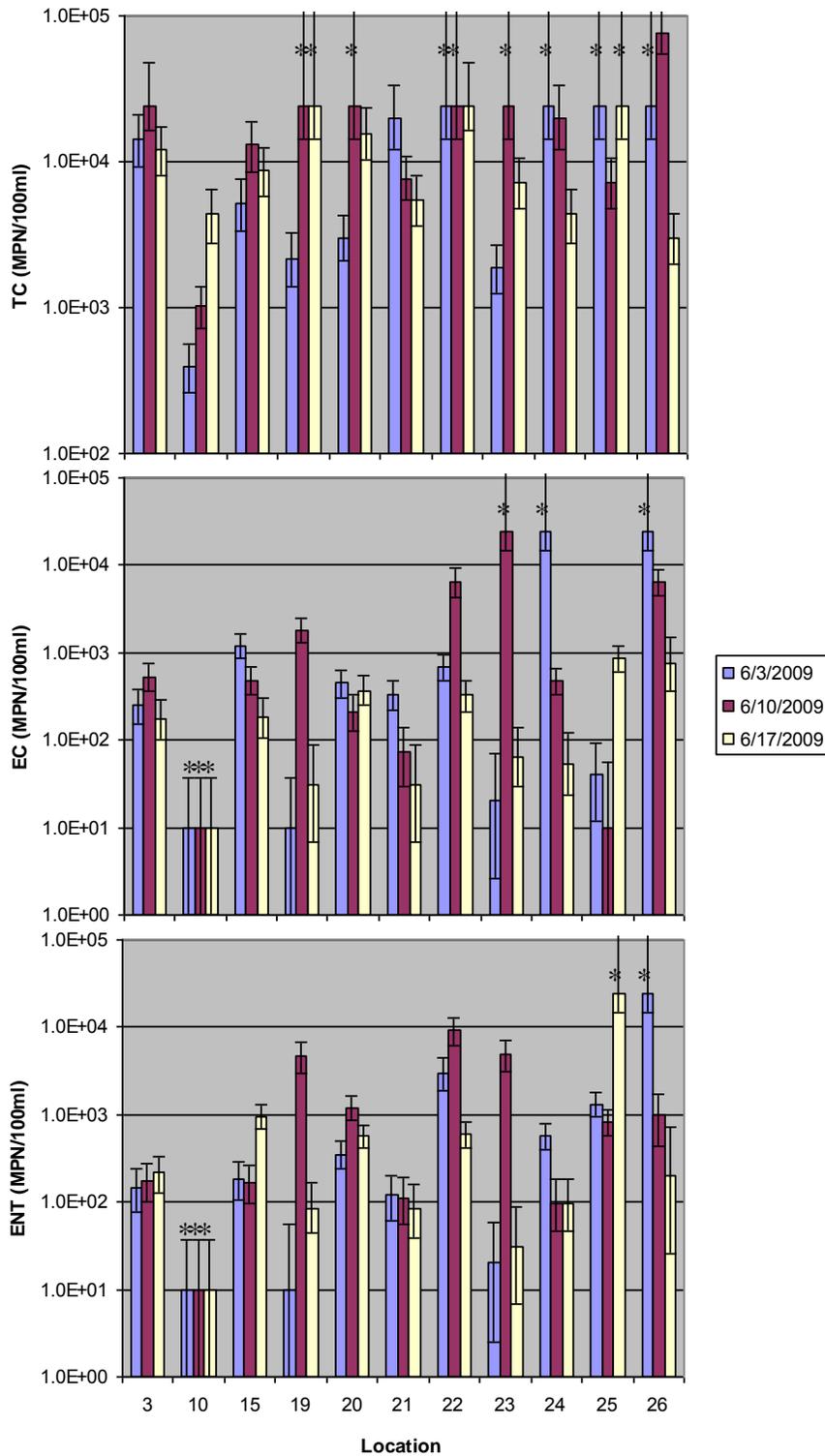


Fig. 2. Concentrations of fecal indicator bacteria in Laguna Watershed Phase II, as Most Probable Number (MPN) per 100 ml. Error bars indicate 95% confidence intervals. Samples outside the range of quantification are assigned the range limit value, and are indicated by \*. A. Total coliforms. B. *E. coli*. C. *Enterococcus* spp.

### 4.3 *Enterococcus* spp. by qPCR

The *Enterococcus* spp. concentrations by qPCR (qPCR-ENT) for Laguna watershed phase II are compared with ENT concentrations in Fig. 3 and listed in Table 4, including template dilution determined by salmon testes DNA qPCR.

Overall, the qPCR-ENT concentration patterns in the Laguna and Quarantina drain sections are very similar to those of ENT concentrations (Fig. 3). A scatter plot with regression line (Fig. 4) indicates that qPCR-ENT values are mostly higher than ENT values, but with a trendline slope of 0.7 there is a tendency for the two metrics for *Enterococcus* spp. to converge at higher concentrations. The qPCR-ENT/ENT ratios (Fig. 5) are in the low range of those reported in other studies, likely because freshwater generally induces little stress on bacteria (Haugland, Siefring et al. 2005; He and Jiang 2005; Morrison, Bachoon et al. 2008).

Three samples showed excellent consistency between qPCR-ENT and ENT as the ratios were nearly 1 (falling on the 1:1 line): locations 15, 19 and 22, all sampled on 06/10/09. Based on Fig. 5, especially locations 19 and 22 have good qPCR-ENT:ENT correspondence on 06/10/09 compared to the other sampling days. A 1:1 correspondence between qPCR-ENT and ENT would suggest that culturability is excellent in these samples, as compared to other samples. Culturability could be increased due to the absence of stressors; it also would tend to be improved when fresh waste is entering the system at those locations.

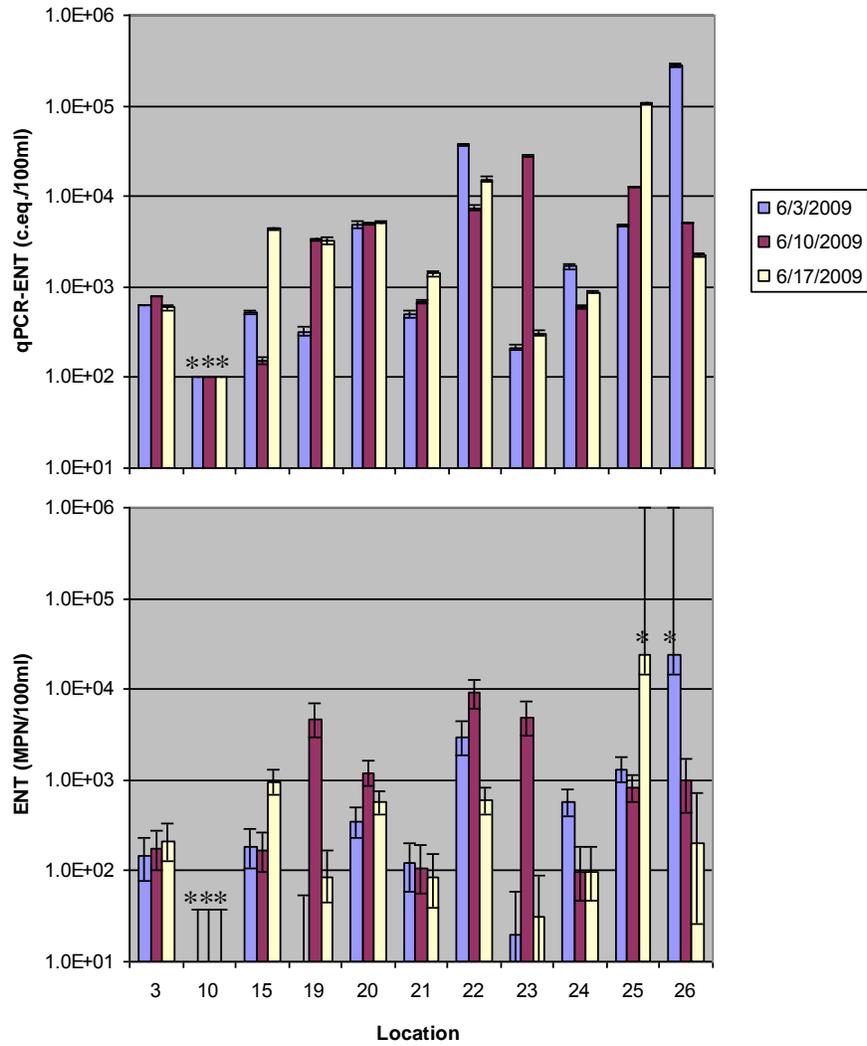


Fig. 3. Concentrations qPCR-ENT (A) and ENT (B) in Laguna Watershed Phase II. Error bars indicate standard error for qPCR-ENT and 95% confidence intervals for ENT. Samples outside the range of quantification are assigned the range limit value, and are indicated by \*.

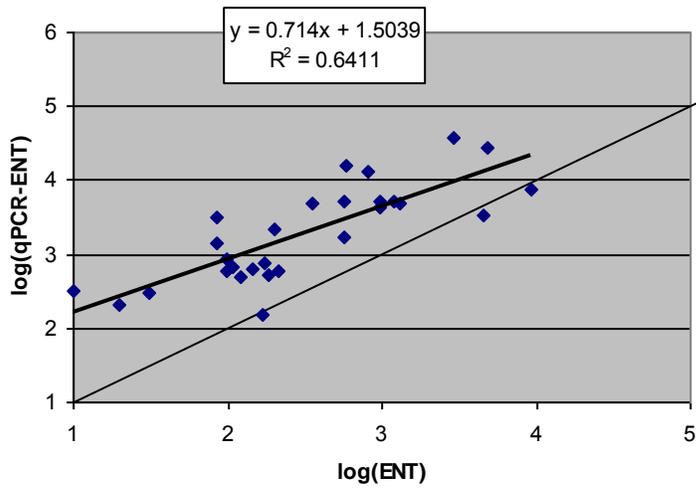


Fig. 4. Correlation between log-transformed ENT and qPCR-ENT concentrations. Only data within the range of quantification are shown.

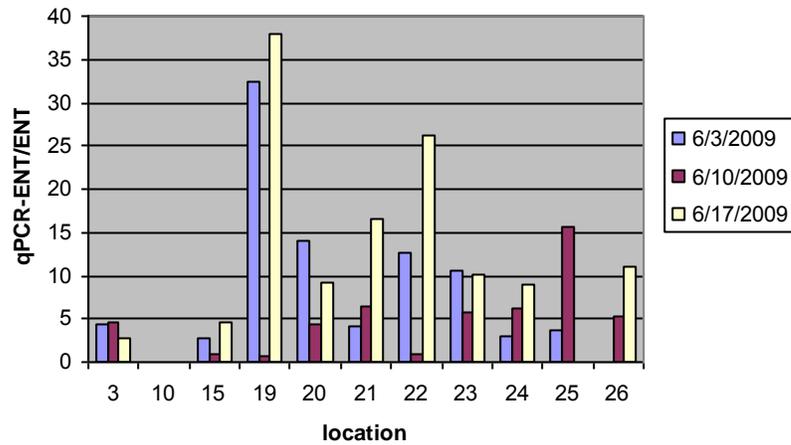


Fig. 5. qPCR-ENT/ENT concentration ratio for all Laguna Watershed Phase II samples, grouped per location.

Table 4. *Enterococcus* spp. concentrations by qPCR (c.eq./100 ml) for Laguna Watershed Phase II samples, including standard error (SE), and optimal template dilution determined by salmon testes DNA qPCR.

	Time	dilution	Conc.	SE
06/03				
3	8:43	10	620	4
10	11:13	10	DNQ	0
15	10:22	10	524	16
19	10:35	10	325	35
20	10:48	10	4898	357
21	10:59	10	506	45
22	9:12	10	37050	858
23	9:26	10	213	15
24	9:36	10	1679	91
25	9:51	10	4788	134
26	10:07	10	279324	13770
06/10				
3	8:47	10	785	5
10	11:10	10	DNQ	0
15	10:21	10	156	14
19	10:32	10	3335	39
20	10:46	10	5069	110
21	10:58	10	689	44
22	9:15	10	7417	487
23	9:31	20	27515	605
24	9:43	10	602	29
25	9:57	10	12769	143
26	10:10	10	5110	76
06/17				
3	8:49	10	588	51
10	11:20	10	DNQ	0
15	10:26	10	4438	101
19	10:40	10	3258	246
20	10:51	10	5205	159
21	11:03	10	1419	91
22	9:16	10	15453	842
23	9:31	10	310	15
24	9:41	10	878	13
25	9:52	10	106201	3402
26	10:10	10	2231	106

#### 4.4 Detection and quantification of human-specific DNA markers

The concentrations of human-specific *Bacteroidales* markers and presence/absence of *Methanobrevibacter smithii nifH* (Mnif) markers for the Phase II samples are shown in Figs. 6, 7 and Table 5.

HBM were consistently detected at locations 20, 22, 24 and 26, with location 26 exhibiting the highest concentrations. Especially on 06/03/09, the HBM concentration at location 26 was extremely high ( $10^9$  copies/L), and similar to concentrations found in El

Estero WWTP sewage influent samples (Sercu, Van De Werfhorst et al. 2009). The HBM concentration at location 26 was at least two orders of magnitude lower on the two other sampling days. HBM were detected once or twice at locations 3, 15, 19, 21, and 23. Mnif markers were detected once at locations 21 and 23, and twice (with higher intensity) at locations 24 and 26. HBM and Mnif were never detected at locations 10 and 25.

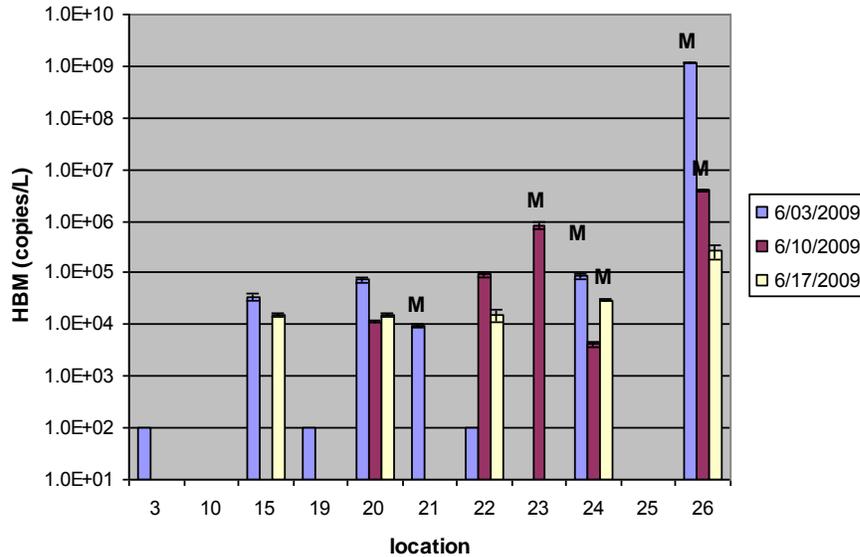


Fig. 6. HBM concentrations for Laguna Watershed Phase II samples. Error bars indicate standard error. Detectable but non-quantifiable HBM are assigned a value of  $10^2$  copies/L, HBM below the limit of quantification are considered to be zero. Samples positive for *Methanobrevibacter smithii nifH* PCR are identified by an “M” above the bar.

Based on the combined results of HBM qPCR and Mnif PCR, very strong evidence is present for significant human fecal contamination in the Quarantine drain section at location 26, flowing downstream to locations 24, 23 and 22. A high day-to-day variability was observed. Although HBM concentrations are similar for locations 22 and 24 on two days, Mnif markers were only detected at location 22. This could be due to different survival characteristics of the human-specific *Bacteroidales* and *Methanobrevibacter smithii*, or due to varying proportions of these markers in original waste samples.

In the Laguna Drain, human fecal contamination was found at locations 20 and 21, and potentially transported downstream to location 15, although the concentrations of HBM are fairly similar at location 15 which could suggest additional waste input at that location. Mnif was only detected once at location 21.

Part of this Phase II sampling was undertaken to investigate the origin of human fecal pollution observed at location 15 during Phase I. The results of the Phase I study were described in detail in the report to the City of Santa Barbara (November 18, 2008). HBM concentrations at location 15 were similar during Phase I and Phase II, about  $10^4 - 10^5$  copies/L. At location 3, HBM were detected 3 out of 7 days during phase I, compared to once out of 3 days during Phase II. However, the absence of Mnif markers at locations 3

and 15 during Phase II contrasts with its frequent detection during Phase I. Still, we can conclude that human fecal pollution was still present at location 15, and further downstream, during Phase II. In addition, we found that the drain sections associated with location 20 and 21 both contribute to the human fecal pollution at location 15. The short storm drain section associated with location 19 is not a significant source of human fecal pollution to location 15.

The specificity and sensitivity of the Mnif versus the HBM assay were very similar to those found in Phase I (Table 6). For Phase II, the specificity was 100 % (versus 87 % in Phase I), and the sensitivity 39 % (versus 43 % in Phase I).

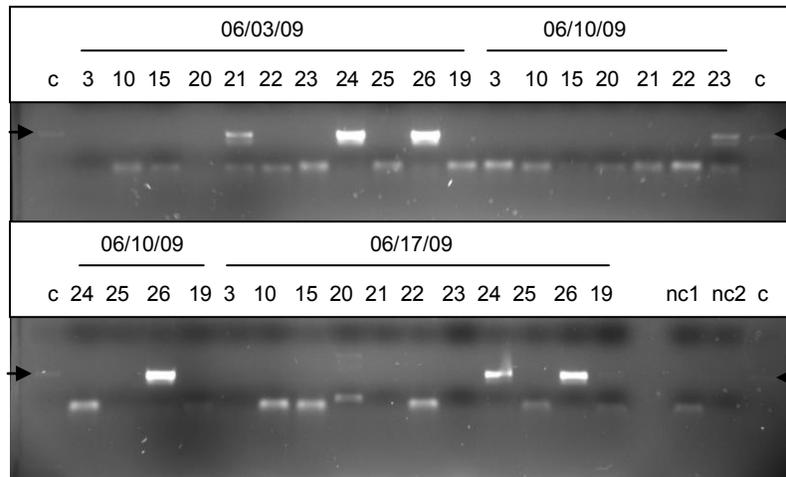


Fig. 7. Agarose gel (2%) showing presence/absence of Mnif markers after 2 rounds of PCR for Laguna Watershed Phase II samples. c: positive control (indicated by arrow), nc1: negative control round 1, nc2: negative control round 2. The positive control is used for determining the location of the positive samples.

Table 5. Human-specific markers in Laguna Watershed, Phase II. Human-specific *Bacteroidales* (HBM) concentrations and standard error (SE) are shown. ND: not detected, DNQ: detected but not quantifiable. *Methanobrevibacter smithii nifH* (Mnif) PCR results are indicated as strongly positive (++) , weakly positive (+) or negative (-).

Location	06/03/09			06/10/09			06/17/09		
	HBM Copies/L	HBM SE	Mnif	HBM Copies/L	HBM SE	Mnif	HBM Copies/L	HBM SE	Mnif
3	DNQ		-	ND		-	ND		-
10	ND		-	ND		-	ND		-
15	3.4E+04	5.3E+03	-	ND		-	1.6E+04	8.6E+02	-
19	DNQ		-	ND		-	ND		-
20	7.3E+04	6.3E+03	-	1.1E+04	2.6E+02	-	1.5E+04	1.3E+03	-
21	9.6E+03	7.4E+02	+	ND		-	ND		-
22	DNQ		-	9.6E+04	1.2E+04	-	1.5E+04	4.1E+03	-
23	ND		-	8.5E+05	1.3E+05	+	ND		-
24	8.7E+04	1.2E+04	++	4.2E+03	5.3E+02	-	3.0E+04	1.7E+03	++
25	ND		-	ND		-	ND		-
26	1.2E+09	1.5E+07	++	3.9E+06	2.2E+05	++	2.6E+05	8.6E+04	++

Table 6. Total number of positive (+) and negative (-) samples for the HBM and Mnif assays.

		HBM		sum
		+	-	
Mnif	+	7	0	7
	-	11	15	26
sum		18	15	33

## 4.5 Quantification of chemical sewage markers by ELISA

### 4.5.1 Standard curves

An initial assessment of the performance (detection limits, reproducibility) of all three ELISA assays was done by analyzing the standard curves. A standard curve for cotinine is shown as a representative example (Fig. 8). The  $R^2$  values of all standard curves, for all 3 assays, were high, ranging from 0.994 – 1. The standards recovery was 88 – 113 % for cotinine (n = 4), 86 – 130 % for caffeine (n = 4 to 6) and 64 – 147% for triclosan (n = 2 to 4).

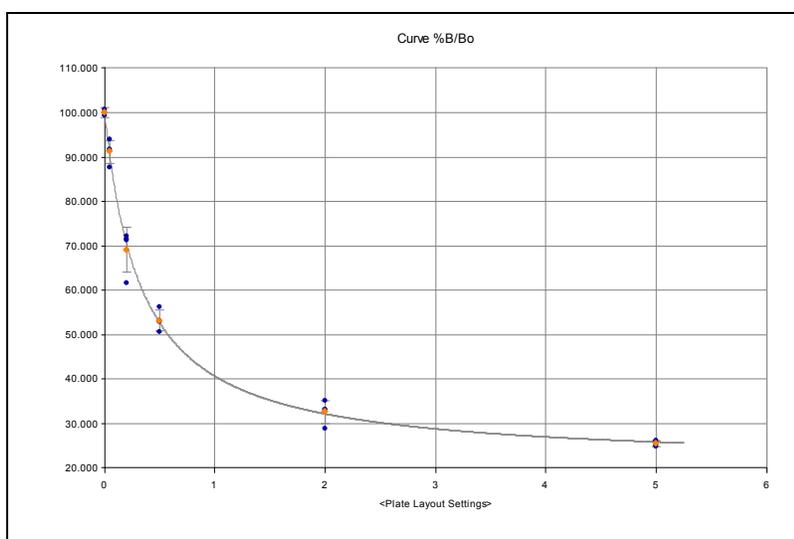


Fig. 8. Cotinine standard curve from all standard replicates run on the first plate ( $R^2 = 0.999$ ; standards recovery = 88-112%). X-axis is standard concentration (ppb).

For cotinine, 4 standard replicates were run on two plates (Table 7). The coefficient of variation (%CV) of individual standard replicates varied from 11.6 - 31.7 % for the first run, and 12.1 - 75.4 % for the second. Therefore, some plate-to-plate variability is seen for standards reproducibility, especially for the lowest standard concentration. F-Tests and t-Tests revealed no significant difference between the regression standard concentrations between runs.

The detection limit for cotinine stated in the manual was 0.045 ppb. In this study, the detection limit was calculated based on the lowest standard that could be distinguished from the zero standard. For cotinine, this standard was 0.05 ppb.

Table 7. Reproducibility of cotinine standards for 2 plates (run 1 and 2). All concentrations are given as parts per billion (ppb), as regression mean and standard error (SE).

Stand. conc.	Run 1 (n = 4)			Run 2 (n = 4)		
	Mean Regr.	SE Regr.	%CV	Mean Regr.	SE Regr.	%CV
0.05	0.044	0.007	31.3	0.053	0.020	75.4
0.2	0.220	0.032	29.3	0.204	0.024	23.4
0.5	0.486	0.028	11.6	0.518	0.048	18.7
2	2.027	0.321	31.7	1.905	0.176	18.4
5	5.596	0.421	15.1	5.662	0.342	12.1

For caffeine, 4 and 6 standard replicates were run on two plates (Table 8). The coefficient of variation (%CV) of individual standard replicates varied from 3.8 - 64.5% for the first run, and 9.9 - 43.3% for the second. Again, some plate-to-plate variability is seen for standards reproducibility, especially for the lowest standard concentration. F-Tests and t-Tests revealed no significant difference between the regression standard concentrations between runs.

The detection limit for caffeine stated in the manual was 0.175 ppb, which was also the lowest standard that could be distinguished from the zero standard.

Table 8. Reproducibility of caffeine standards for 2 plates (run 1 and 2). All concentrations are given as parts per billion (ppb), as regression mean and standard error (SE).

Stand. conc.	Run 1 (n = 4)			Run 2 (n = 6)		
	Mean Regr.	SE Regr.	%CV	Mean Regr.	SE Regr.	%CV
0.175	0.227	0.073	64.5	0.183*	0.035*	43.3*
0.5	0.428	0.050	23.2	0.517	0.046	21.6
1	1.048	0.090	17.1	1.000	0.063	15.4
2.5	2.573	0.087	6.8	2.575	0.263	25.0
5	4.916	0.093	3.8	5.055	0.205	9.9

\*n = 5

For triclosan, due to failure of one run for unknown reasons, only one plate was run. In addition, only half of the standard concentrations were run with 4 instead of 2 replicates (Table 9). The coefficient of variation (%CV) of individual sample replicates varied from 5.8 – 70.3 %. The triclosan kit comes with an assay control standard that the manual states should be 0.75 +/- 0.15 ppb. Despite this value, the manual also mentions that it is up to each individual lab to determine what the acceptable limits are. The control sample was run as three separate duplicate sets, and all were within the stated concentration range.

The detection limit for triclosan stated in the manual was 0.02 ppb. However, analysis of standard curves revealed that the 0.05 ppb standard curve was not always distinguishable from the zero standard, yielding effective detection limits between 0.05 and 0.1 ppb.

Table 9. Reproducibility of triclosan standards within one plate. All concentrations are given as parts per billion (ppb), as regression mean and standard error (SE).

Stand. conc.	n	Mean Regr.	Run 1	
			SE Regr.	%CV
0.05	3	0.032	0.013	70.3
0.1	4	0.106	0.009	17.0
0.25	4	0.272	0.014	9.9
0.5	2	0.476	0.044	13.1
1	2	0.907	0.037	5.8
2.5	2	3.676	0.570	21.9

The Abraxis manuals state test standard reproducibility at < 15% CV (cotinine) and < 13% CV (caffeine). For both assays, these values were usually exceeded, using 4 standard replicates per plate. For triclosan, no %CV limit was stated. Triclosan %CVs were of the same order of magnitude as for the other assays, up to 75 % for the lowest concentration.

In general, %CV values increase for the lower concentration ranges. For quality control purposes, we recommend not using the %CV values stated by Abraxis, but using experimental data to calculate what values can be attained in practice.

The use of 2 instead of 4 replicates was also assessed, by comparing reproducibility of standards. In general, higher %CV values and slightly lower  $R^2$  values were observed using 2 replicates, especially for the lowest standards. Therefore, we recommend running more than 2 replicates.

#### 4.5.2 Samples

As performed with the standards, the reproducibility of sample quantification was evaluated using coefficients of variation. For cotinine, the Abraxis manual states a CV < 20% for samples. Five samples fell outside of this range, with within-plate CVs up to 32%. For overall reproducibility (including between-plate), also five samples were outside this range, with CVs up to 38%. For caffeine, the Abraxis manual also states a test reproducibility of < 20% CV for samples. Seven samples fell outside this range, with within-plate CVs up to 52%. For overall reproducibility, four samples fell outside the range, with CVs up to 53%. The sample reproducibility for triclosan was very good (CV < 5%), probably because the only 2 samples detected were in the higher concentration range. Not enough positive samples were present to make a reliable assessment. For quality control purposes, we recommend not using the %CV values stated by Abraxis, but using experimental data to calculate what values can be attained in practice.

The concentrations of all three chemical markers in the Laguna Phase II samples are shown in Fig. 9. For cotinine, 14 samples were positive in one or more runs. Locations 22 and 26 consistently had detectable cotinine, and location 24 in all but one run (= 2 replicates) from 06/10. Cotinine concentrations were highest at location 26 (0.16 – 2.1 ppb). Cotinine was detected at locations 23 and 25 as well, although in lower concentrations (0.037 – 0.15 ppb). Interestingly, cotinine was only detected once in the Laguna drain section, at location 10, although at a concentration close to the limit of detection (0.048 ppb).

For caffeine, 12 samples were positive in one or more runs. Caffeine was consistently detected at locations 22, 24 and 26. Caffeine concentrations were usually highest at location 26 (3.1 – 57 ppb), although on 06/17/09 concentrations were similar at locations 22 and 26 (0.7 – 1.6 ppb). Caffeine was detected on two days at location 23, at relatively low concentrations (0.23 – 2.4 ppb).

For triclosan, only 2 samples were positive, both at location 26 (0.18 - 1.6 ppb).

All three chemical markers clearly indicate human fecal pollution at location 26, flowing downstream to locations 24, 23 and 22. In addition, there appears to be a second source of chemical markers between locations 23 and 22. Also, cotinine concentrations indicated some contribution of location 25 to human fecal pollution in the Quarantina drain, although much less than location 26.

Log-transformed caffeine and cotinine concentrations generally showed a good correlation, when values below the detection limit were included (Fig. 10). Without including the values below the detection limit, the  $R^2$  was 0.57.

The log-transformed caffeine and cotinine concentrations generally showed good correlation with log-transformed HBM concentrations (Figs. 11, 12). For caffeine,  $R^2$  was 0.83 when only including quantifiable samples, for cotinine  $R^2$  was 0.79.

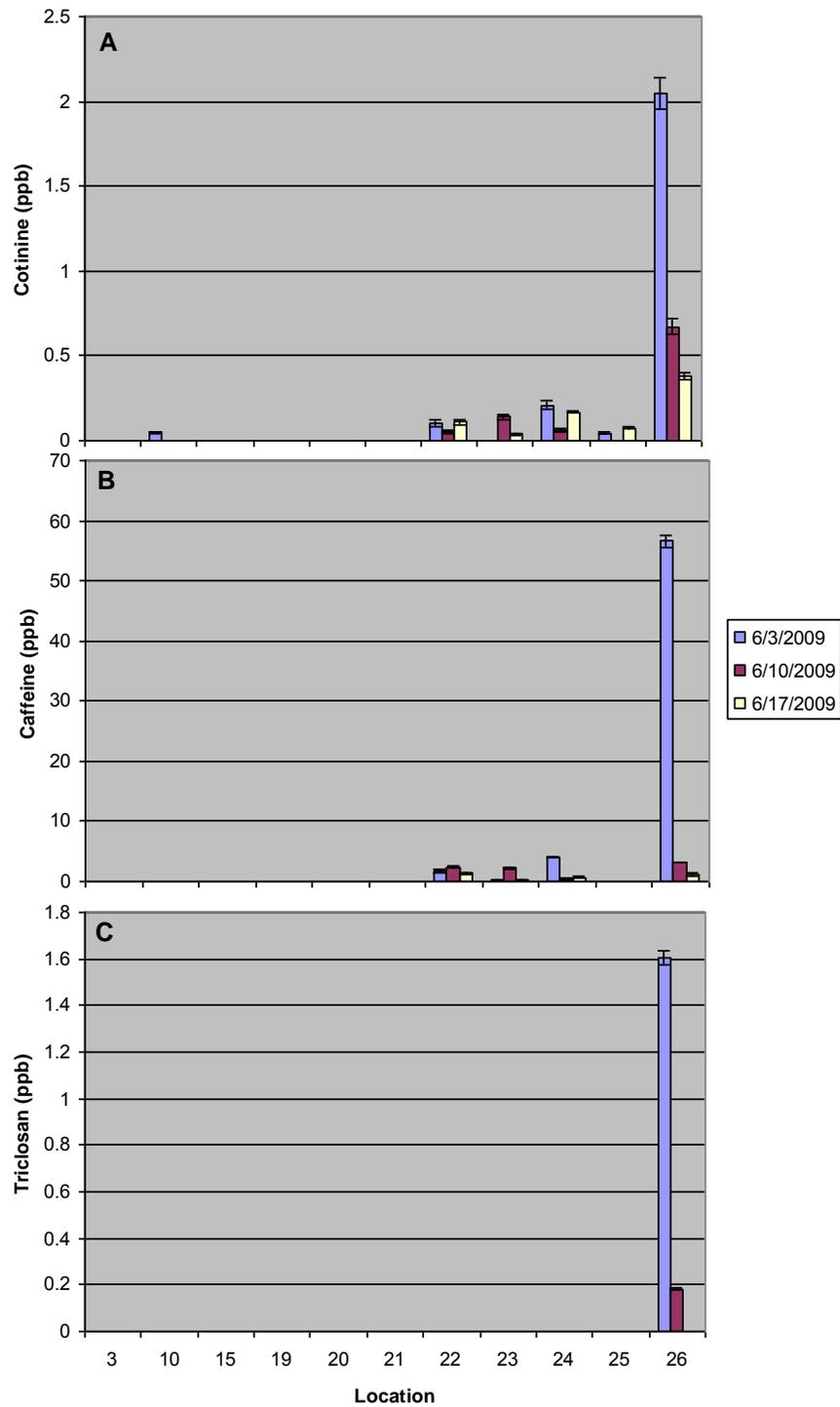


Fig. 9. Concentrations of cotinine (A), caffeine (B) and triclosan (C) in Laguna Watershed, Phase II. Error bars indicate standard error.

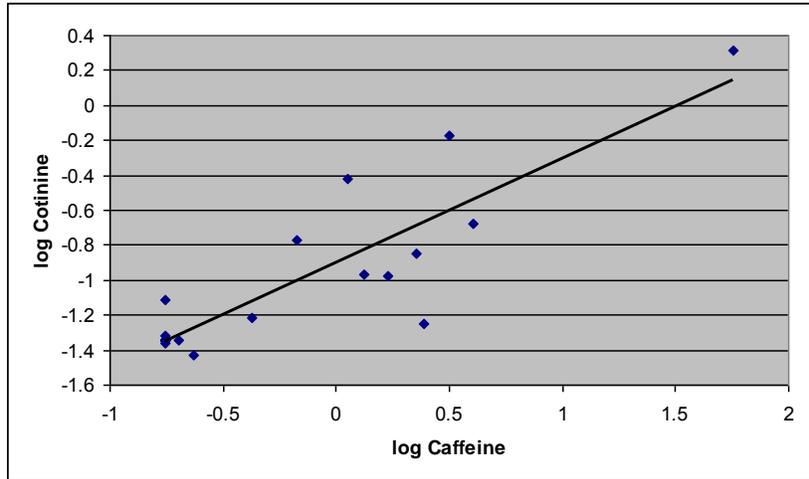


Figure 10. Scatter plot of log-transformed caffeine and cotinine concentrations. Values below detection limit were altered for visualization purposes (caffeine ND into -0.76; cotinine ND into -1.35).

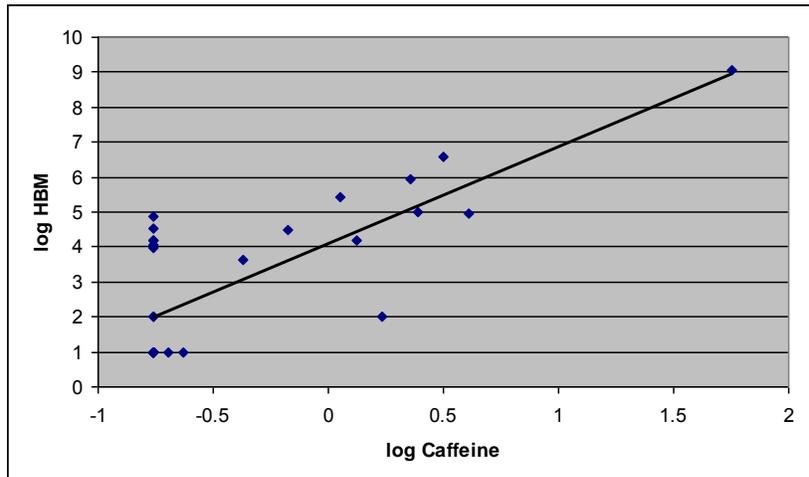


Figure 11. Scatter plot of log-transformed caffeine and HBM concentrations. Values below detection limit were altered for visualization purposes (caffeine ND into -0.76; HBM ND into 1, DNQ into 2).

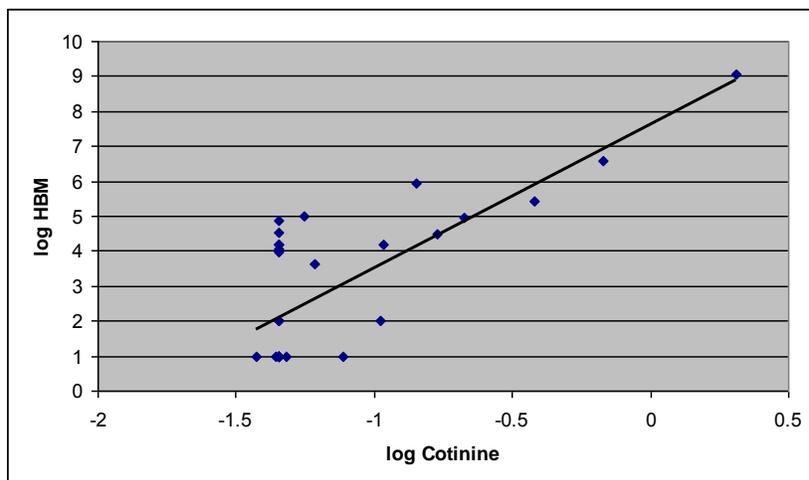


Figure 12. Scatter plot of log-transformed cotinine and HBM concentrations. Values below detection limit were altered for visualization purposes (caffeine ND into -1.35; HBM ND into 1, DNQ into 2).

In order to reference the chemical marker concentrations in Laguna Watershed storm drain samples, sewage influent samples of El Estero WWTP were analyzed as well (Table 10). Cotinine concentrations in this study were similar to those reported before in WWTP influent samples, by solid phase extraction (SPE) followed by LC-MS/MS (0.8 – 2.9 ppb) (Buerge, Kahle et al. 2008; Zhao and Metcalfe 2008). However, caffeine concentrations were higher than reported before in WWTP influent samples by SPE followed by HPLC, GC-MS or LC-MS/MS (0.2 – 73 ppb) (Buerge, Poiger et al. 2006; Zhao and Metcalfe 2008; Lin, Yu et al. 2009; Santos, Aparicio et al. 2009; Ying, Kookana et al. 2009; Yu and Chu 2009). This could be due to sample variability, and more samples should be analyzed using the ELISA assay to allow a more comprehensive comparison with published concentrations. Finally, the triclosan concentrations were in the low range of those found before in WWTP influents (0.20 – 12.3 ppb), by ELISA and by SPE-GC-MS (Kantiani, Farre et al. 2008; Ying, Kookana et al. 2009; Yu and Chu 2009).

Based on the listed analyte detection limits from the kit instructions for each assay, and the concentrations of each analyte in the raw sewage sample, the detection limits relative to raw sewage influent improved in the order triclosan (5 %) > cotinine (1 %) > caffeine (0.1 %). When chemical marker concentrations are expressed as % sewage (Table 11), triclosan indicates > 100 % sewage on 06/03/09 at location 26. This can be due to assay variability and hydrophobicity of the analyte, or to spatial heterogeneity of triclosan concentrations in sewage from different sources (Jackson and Sutton 2008). When only cotinine and caffeine are considered, sewage concentrations between 42 and 49% were detected on 06/03/09 at location 26, decreasing on 06/10/09 (~ 2 – 16 %) and 06/17/09 (~1 – 9 %). For the other locations < 5 % sewage was found.

Table 10. Chemical marker concentrations (ppb) in El Estero WWTP raw sewage influent and confluent. Two runs were performed for cotinine.

ID	Cotinine_1		Cotinine_2		Caffeine_1		Triclosan	
	mean	SE	mean	SE	mean	SE	mean	SE
CON	2.9	0.0	3.3	0.3			0.35	0.02
CON (1:10)	4.0	0.2	3.8	0.0				
CON (1:100)					183	25		
RAW	3.6	0.2	4.0	0.5			0.39	0.02
RAW (1:10)	4.8	0.3	4.3	0.1				
RAW (1:100)					134	14		

Table 11. ELISA assay results expressed as %raw sewage (%SEW), based on the results of the single raw sewage sample taken on 07/08/09. \* indicates samples that were quantifiable in only one of two runs.

ID	Caffeine % SEW	Cotinine % SEW	Triclosan % SEW			
06/03/09		3				
		10	1.1*			
		15				
		19				
		20				
		21				
		22	1.3	2.5		
		23	0.1*			
		24	3.0	5.0		
		25		1.1*		
06/10/09		26	42.2	49.1	407.4	
	06/17/09		3			
			10			
			15			
			19			
			20			
			21			
			22	1.8	1.3	
			23	1.7	3.4	
			24	0.3	1.5*	
		25				
	26	2.4	16.1	46.4		
06/17/09		3				
		10				
		15				
		19				
		20				
		21				
		22	1.0	2.6		
		23	0.2	0.9		
		24	0.5	4.0		
		25		1.8		
	26	0.8	9.1			

Based on the number of positives and negatives in Table 12, sensitivity and specificity of the ELISA assays vs. HBM was calculated. Sensitivity was 56 % (cotinine), 56 % (caffeine) and 11 % (triclosan). Specificity was 73 % (cotinine), 87 % (caffeine) and 100 % (triclosan). Although specificity was high for triclosan, sensitivity was very low. This is probably related to the high detection limit for sewage, based on the concentrations presented in Table 10. Based on the lower sensitivity, and the problems observed while running the assay, the triclosan assay appears least promising for detecting human fecal waste in storm drains. Both cotinine and caffeine showed similar specificity and sensitivity compared to HBM, but detection limits for sewage were tenfold lower for caffeine.

Table 12. Total number of positive (+) and negative (-) samples for the ELISA and HBM assays.

		HBM		sum
		+	-	
Cotinine	+	10	4	14
	-	8	11	19
	sum	18	15	33
Caffeine	+	10	2	12
	-	8	13	21
	sum	18	15	33
Triclosan	+	2	0	2
	-	16	15	31
	sum	18	15	

#### 4.6 Comparison of DNA-based and chemical sewage markers

Based on the numbers of positive and negative samples for all assays relative to HBM, the sensitivity decreased from caffeine = cotinine (56 %) > Mnif (39 %) > triclosan (11 %). The specificities decreased from triclosan = Mnif (100 %) > caffeine (87 %) > cotinine (73 %). Based on overall sensitivity and specificity, caffeine, cotinine and Mnif appeared good supplemental sewage markers for confirmation of HBM positives. Due to its very low sensitivity, triclosan was considered less useful for this purpose. In addition, one triclosan run failed for unknown reasons.

An overall comparison of the chemical sewage markers (caffeine, cotinine and triclosan) and the DNA-based markers (HBM and Mnif) is presented in Table 13.

In the Laguna drain section, none of the samples were positive for all markers. The highest HBM concentrations were consistently found at location 20, although none of the other markers were positive. Lower HBM concentrations were found once at location 21, backed up by positive Mnif. Downstream, HBM were quantified at location 15 and detected at location 3, none confirmed by other markers. Cotinine was detected once at location 10, at a concentration close to the detection limit, and not confirmed by any other marker.

Table 13. Chemical and microbial human-specific markers in Laguna Watershed, Phase II. Chemical markers are shown as mean (in ppb) and standard error (SE). Concentrations marked with “\*” are from samples that were quantifiable in only one out of two runs. HBM are shown as mean (copies/L) and standard error (SE). Mnif is shown as strongly positive (++), slightly positive (+), or not detected (-). ND: below the limit of detection, DNQ: detectable but non-quantifiable.

ID	Cotinine		Caffeine		Triclosan		HBM		Mnif
	mean	SE	mean	SE	mean	SE	mean	SE	
06/03/09									
3	ND		ND		ND		DNQ		-
10	0.048*	0.007	ND		ND		ND		-
15	ND		ND		ND		3.4E+04	5.3E+03	-
19	ND		ND		ND		DNQ		-
20	ND		ND		ND		7.3E+04	6.3E+03	-
21	ND		ND		ND		9.6E+03	7.4E+02	+
22	0.11	0.02	1.7	0.4	ND		DNQ		-
23	ND		0.20*	0.06	ND		ND		-
24	0.21	0.03	4.0	0.1	ND		8.7E+04	1.2E+04	++
25	0.044*	0.004	ND		ND		ND		-
26	2.1	0.1	57	1	1.6	0.0	1.2E+09	1.5E+07	++
06/10/09									
3	ND		ND		ND		ND		-
10	ND		ND		ND		ND		-
15	ND		ND		ND		ND		-
19	ND		ND		ND		ND		-
20	ND		ND		ND		1.1E+04	2.6E+02	-
21	ND		ND		ND		ND		-
22	0.056	0.011	2.4	0.1	ND		9.6E+04	1.2E+04	-
23	0.14	0.02	2.3	0.1	ND		8.5E+05	1.3E+05	+
24	0.061*	0.014	0.42	0.07	ND		4.2E+03	5.3E+02	-
25	ND		ND		ND		ND		-
26	0.67	0.05	3.2	0.1	0.18	0.01	3.9E+06	2.2E+05	++
06/17/09									
3	ND		ND		ND		ND		-
10	ND		ND		ND		ND		-
15	ND		ND		ND		1.6E+04	8.6E+02	-
19	ND		ND		ND		ND		-
20	ND		ND		ND		1.5E+04	1.3E+03	-
21	ND		ND		ND		ND		-
22	0.11	0.01	1.3	0.1	ND		1.5E+04	4.1E+03	-
23	0.037	0.002	0.24	0.02	ND		ND		-
24	0.17	0.01	0.67	0.10	ND		3.0E+04	1.7E+03	++
25	0.077	0.007	ND		ND		ND		-
26	0.38	0.02	1.1	0.3	ND		2.6E+05	8.6E+04	++

In the Quarantina drain section, all markers indicate human fecal pollution at location 26: HBM concentrations were highest, Mnif PCR signal was strong, triclosan was only detected at this location, and cotinine and caffeine concentrations were highest on two days (06/03/09 and 06/10/09). Disregarding the less sensitive triclosan, all markers also

indicated the presence of human fecal pollution at location 24 on two days, and 23 on one day. Only cotinine indicated the presence of human fecal pollution at location 25 on two days. At location 22, human fecal pollution was evidenced by HBM, cotinine and caffeine, but not Mnif.

In both drain sections, Mnif was only detected at the highest HBM concentrations, and in the upper drain sections, suggesting marker decay is faster than for HBM. Cotinine was detected three times when no caffeine was found, and caffeine was detected once with no cotinine detected. In all cases, the ELISA markers were present in concentrations close to the limit of detection, and no HBM or Mnif were found. Therefore, both caffeine and cotinine appear useful as alternative markers compared with DNA-based markers to detect low quantities of human fecal pollution.

#### 4.7 Correlation between FIB and HBM

Scatter plots of log-transformed concentrations indicate a weak correlation between FIB and HBM (Fig. 13). When including non-detectable HBM, relatively high EC (1700 MPN/100 ml) and ENT (> 24196 MPN/100 ml) were still found. Only for EC did the highest EC concentrations correspond to high HBM concentrations. Correlation coefficients were 0.44 (EC) and 0.19 (ENT), when only within range values were included (box in Fig. 13). Overall, the correlation with HBM was slightly better for EC.

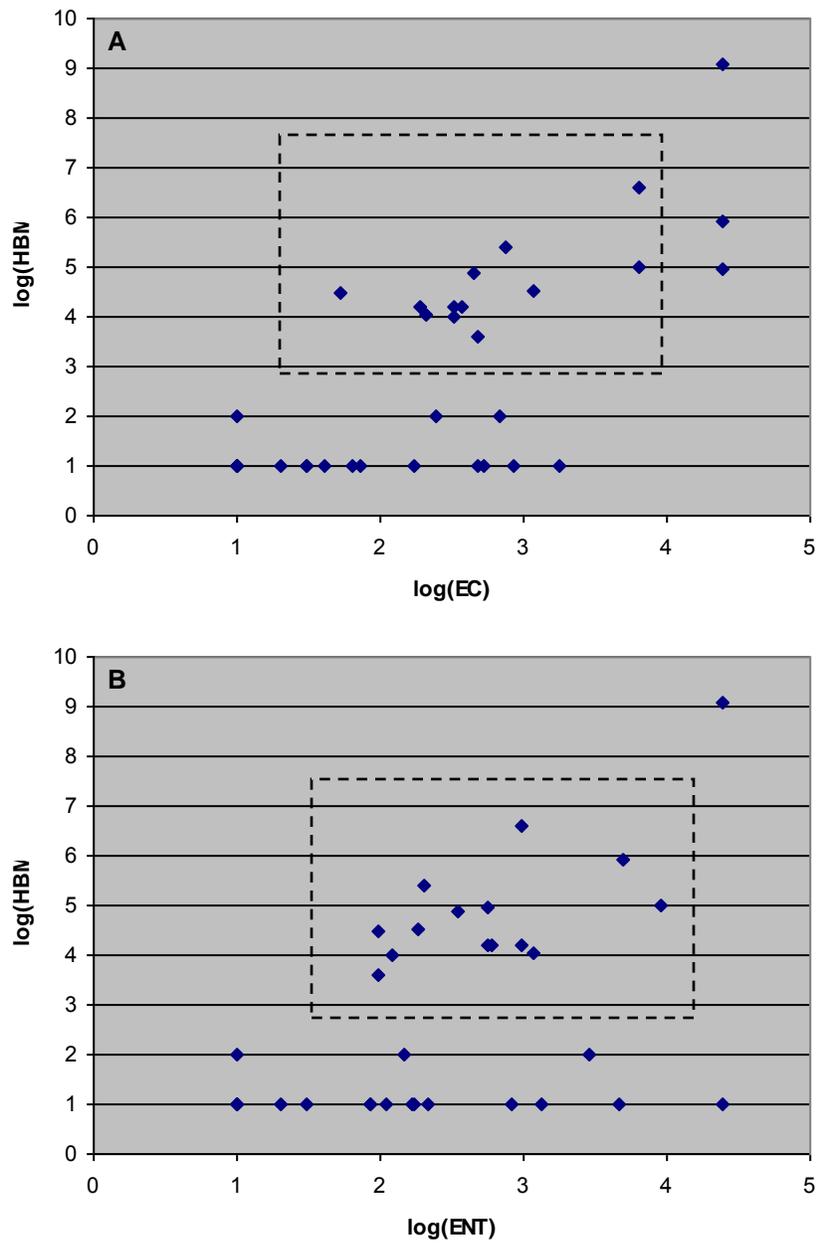


Fig. 13. Correlation between log-transformed FIB and HBM. A. EC, B. ENT. Data within the range of quantification are shown in box.

## 5. Conclusions

Fecal indicator bacteria, DNA-based markers and chemical markers for human fecal (sewage) pollution were used for localizing and quantifying the extent of human fecal pollution in two main storm drain sections of the Laguna watershed: the Laguna drain section and the Quarantina drain section. In the Laguna drain section, samples were taken at several locations upstream in the drain network, in order to better localize the source of human fecal pollution found in the Laguna watershed Phase I study (report of November 18, 2008 submitted by the Holden lab to the City of Santa Barbara). The quantification of chemical markers was done by ELISA test kits, and was newly implemented in the Holden lab for the purposes of this study.

Based on a combination of all sewage markers used, very significant human fecal pollution was found at location 26 in the Quarantina drain. On one day, pollution attained the equivalent of roughly 50 - 100 % raw sewage influent, based on DNA and chemical markers. The human fecal pollution could be detected on several occasions at the more downstream locations in the Quarantina drain section, even at the discharge on the beach (location 22). Possibly, an additional source of human fecal pollution is present close to the beach drain discharge. The drain section associated with location 25 may also add human fecal contamination to the drains, but this was only based on low concentrations of cotinine detected. In the Laguna drain section, overall levels of human fecal pollution were lower than in the Quarantina drain. Evidence for human fecal pollution was still present at location 15, and could be traced upstream to both locations 20 and 21. Both drain sections contributed to the contamination with human fecal pollution, and flow measurements are required to calculate the importance of each with respect to pollutant loads.

As observed during the Phase I research, FIB concentrations did not correlate well with HBM concentrations. Therefore FIB concentrations are not reliable as a first tier in a multi-tiered approach to source tracking human fecal waste in urban storm drains, which all contain relatively high FIB concentrations.

Of the three ELISA assays tested, the triclosan assay appears the least useful for source tracking human fecal waste in storm drains, because of problems with the assay reproducibility and low sensitivity. The assays for cotinine and caffeine appear suitable for confirming the presence of human fecal waste based on HBM analyses. The correlation with HBM is very good, and both assays have a good specificity and sensitivity relative to HBM. Mnif PCR still appears to be a good confirmation of HBM, as was observed before in Phase I. Overall, HBM qPCR is the most sensitive method based on the number of positive samples in the watershed.

The cotinine and caffeine ELISA assays may be very useful for source tracking human fecal waste for labs without molecular microbiology capacity, because they require less expensive equipment, are easier to perform, and potentially yield faster results. However, the cost of the assay should be considered, as kits and reagents are expensive. Based on

our experiences with the ELISA assays for sewage markers, the following recommendations are made:

- Experimental coefficients of variation of standards and samples appear higher than stated in the Abraxis manual. Therefore, it is recommended to determine the acceptable coefficient of variation levels in the lab. We observed coefficients of variation as high as 75%. In order to minimize coefficients of variation, it is recommended to use triplicates for all standards and samples, to use a multi-channel pipettor and perform all steps with a set order and timing.
- Detection limits should be determined experimentally, by using the standards provided with the kits. For each assay, we calculated the detection limit as the lowest standard replicate that is higher than any zero standard replicate. In general, higher coefficients of variation were observed with the lowest standards.
- The ELISA assays are designed for fresh water samples. Both the caffeine and cotinine kits mention salt water as a possible test interference substance when in excess of 50 %.

## 6. Acknowledgements

The research described in this report was funded by the City of Santa Barbara. The research was substantially performed, including the writing of this report, by Dr. Bram Sercu and Laurie Van De Werfhorst in the Holden Lab Group at UCSB.

This research was performed cooperatively with the City through the assistance of Cameron Benson, Dr. Jill Murray, James Rumbley, and additional staff of the City of Santa Barbara Creeks and Streets Divisions.

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## **APPENDIX D**

Grant Summary Form

# GRANT SUMMARY

Completed Grant Summaries are made available to the public on the State Water Resources Control Board's (SWRCB) website at <http://www.waterboards.ca.gov/funding/grantinfo.html>

Use the tab and arrow keys to move through the form. If field is not applicable, please put N/A in field.

Date filled out: 3/13/13

<b>Grant Information:</b> Please use complete phrases/sentences. Fields will expand as you type.
1. <b>Grant Agreement Number:</b> 07-585-550-2
2. <b>Project Title:</b> Laguna Watershed Study and Water Quality Improvement Feasibility Analysis
3. <b>Project Purpose - Problem Being Addressed:</b> This project addresses water quality problems at East Beach, in Santa Barbara, CA. From 1998 to 2006, East Beach at Mission Creek was posted with warnings due to indicator bacteria levels an average of 19% of the beach days. These postings are due in part to contributions from Laguna Channel, which also discharges into the ocean at East Beach. The City of Santa Barbara had previously collected an extensive amount of bacterial data for the Laguna Channel at several locations since 2001. While the lagoon at East Beach is also shared by Mission Creek, on most dates since 2001 Laguna Channel had higher concentrations of bacterial contamination than Mission Creek. Laguna Channel has also been found to contain human fecal markers through microbial DNA testing.
4. <b>Project Goals</b> a. <b>Short-term Goals:</b> The project included a watershed assessment to quantify sources of flow to Laguna Channel, microbial source tracking to confirm the presence of human waste in the channel, a feasibility analysis of potential water quality improvement projects, and preliminary design/CEQA permitting of the most cost-effective implementation project, the relining of two miles of sanitary sewer pipes. The feasibility analysis included a review of ozone disinfection at the outlet of Laguna Channel (near the existing pump station), which was identified as high priority in a Bacteria Reduction treatment study completed for the City with Prop 13 Clean Beaches Initiative funds. b. <b>Long-term Goals:</b> The long term goal of this project is to install water quality projects that lead to a decrease in the number of AB 411 beach warnings for fecal indicator bacteria at East Beach.
5. <b>Project Location:</b> (lat/longs, watershed, etc.) Laguna Channel Watershed a. <b>Physical Size of Project:</b> (miles, acres, sq. ft., etc.) 2020 acres b. <b>Counties Included in the Project:</b> Santa Barbara c. <b>Legislative Districts:</b> (Assembly and Senate) Assembly District 35, Senate District 19
6. <b>Which SWRCB program is funding this grant?</b> Please "X" box that applies. <input type="checkbox"/> Prop 13 <input type="checkbox"/> Prop 40 <input checked="" type="checkbox"/> Prop 50 <input type="checkbox"/> EPA 319(h) <input type="checkbox"/> Other
<b>Grant Contact:</b> Refers to Grant Project Director. <b>Name:</b> Cameron Benson <b>Job Title:</b> Creeks Manager <b>Organization:</b> City of Santa Barbara <b>Webpage Address:</b> www.sbcreeks.com <b>Address:</b> 620 Laguna St., PO Box 1990, Santa Barbara, CA 93102 <b>Phone:</b> (805)897-2508 <b>Fax:</b> (805)897-2626 <b>E-mail:</b> cbenson@santabarbaraca.gov

**Grant Time Frame:** Refers to the implementation period of the grant.

**From:** February 11, 2008

**To:** January 1, 2010

**Project Partner Information:** Name all agencies/groups involved with project. City of Santa Barbara, UCSB (subcontract), Geosyntec Consulting (subcontract)

**Nutrient and Sediment Load Reduction Projection:** (If applicable) n/a

Please provide a hard copy to your Grant Manager and an electronic copy to your Program Analyst for SWRCB website posting. All applicable fields are mandatory. Incomplete forms will be returned.