BASINS MODELING OF THE LOADING OF FECAL COLIFORMS AND PATHOGENS FROM LAND-BASED SOURCES INTO THE BIGHT OF THE CALIFORNIAS

PROJECT NUMBER: W-01-2

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NARRATIVE SUMMARY

Land-based sources of pollution, such as dry and wet weather urban runoff, are responsible for beach contamination and closures throughout the region of Bight of the Californias. Indeed, data presented by the Southern California Coastal Water Research Project (SCCWRP) (2000) shows that after a rain event of one inch or more, nearly 60% of the beaches in the 692 miles from Point Conception in Santa Barbara County to Punta Banda, near Ensenada, Mexico, exceeded water quality health standards. Geographic information system (GIS) technology coupled with hydrologic simulation models have become a valuable tool for watershed management and non-point source pollution modeling. Currently, there is a lack of suitable geospatial information and calibrated models that may be used to predict the spatial and quantitative nature of coliform (and pathogen) loading to the southern California Bight region.

The overall goal of this project was to establish the methodology and develop suitable input parameters for modeling (using the U.S. Environmental Protection Agency's Better Assessment Science Integrating Point and Nonpoint Sources [BASINS] model) of the mass loading of fecal coliforms from the watersheds encompassing the U.S.-Mexican border region of the Bight of the Californias.

Specifically, this project:

- Used the Non Point Source Model (NPSM) of BASINS, coupled with GIS, for the Tijuana River Watershed to estimate the mass loading of fecal coliform bacteria to the California bight
- Calibrated the model using historic data on fecal coliform densities in the Tijuana River
- Linked BASINS to GIS for the region of the bight from Encinitas to Ensenada to model the mass loading of fecal coliforms into this whole region of the bight
- Determined the ratio of coliforms to enteric viruses using polymerase chain reaction (PCR) methods to enumerate indicator and hepatitis A virus (HAV) densities in wet weather runoff samples in the region of the U.S.-Mexican border

Simulations using the BASINS model illustrated that, when considering the current and improved levels of wastewater treatment, the Tecate Wastewater Treatment Plant (WWTP) had the largest impact on fecal coliform levels in the Tijuana River because its loading is constant. Resources should initially be allocated to conduct more comprehensive studies on the quantitative effect of WWTP modification and improvement. The fecal contamination caused by residents without sewage collection system coverage, which is dependent on precipitation events, was nearly negligible when compared to the loadings from the WWTP.

Additionally, a real-time reverse transcriptase-PCR (RT-PCR) method for HAV detection in ocean water was developed as a result of this project. This was successfully accomplished by cloning Hepatitis Virus A (HAV) into a plasmid vector and designing/optimizing primers based on its sequence. HAV cDNA amplified by RT-PCR using RNA from an ocean water sample contaminated with Mexican sewage was sequenced, its identity confirmed, and it was cloned into a plasmid vector.

Ocean water samples were taken at the Tijuana River mouth (near the San Diego, California-Tijuana, Mexico border) and the Imperial Beach pier (0.85 mile north of the Tijuana River mouth in Imperial Beach, California) following four separate rain events Of the eight ocean water samples tested, six were HAV positive by conventional RT-PCR and eight were positive by real-time RT-PCR. This novel real-time RT-PCR method has greater utility in determining more accurately the health risk associated with recreational waters such as in Imperial Beach.

Subsequently, model calibration performed for 1995-1996 reduced these values and the calibrated model ultimately under-predicted the maximum observed level and over-predicted the mean observed concentration by 14%. Based on the tolerance ranges for model fit, the calibrated concentrations were “very good” estimates of the natural system (less than 15% difference between modeled and observed values) (EPA 2001).

The scenario that considered major improvements to the future wastewater collection and treatment infrastructure, full secondary treatment at the Tecate WWTP, and assumed that the population grew as expected until 2020 (but only 10% of the residents in Tecate and Tijuana remained without sewage collection systems), predicted a maximum fecal coliform concentration of 2.4×10^5 MPN per 100 mL and a mean concentration of 2.4×10^3 (Table 12). This simulation suggested that such improvements may result in a decrease by nearly one order of magnitude in bacteria levels.

Therefore, these results indicate that six out of six samples exceeded the fecal coliform indicator threshold (as measured by the Graduate School of Public Health, San Diego State University). Total coliforms exceeded the threshold in

two out of three samples, fecal coliforms exceeded the threshold in two out of three samples, and the enterococcus threshold was exceeded in three out of three samples (as measured by County of San Diego, Department of Environmental Health). In summary, two indicators in the second rain event pier sample were the only bacterial counts (out of the fifteen measured), which did not exceed a standard. At least one indicator exceeded the threshold in every sample measured.

This method, which is sensitive, relatively rapid six hours) and highly specific for selected viral pathogens, will allow in the future a much more accurate human health risk assessment for bathing in contaminated ocean waters such as at Imperial Beach.

Publications that have been published or submitted as a result of this project include:

Gersberg, R.M., Pitt, J., King, A., Johnson, H. and R. Wright. 2000. “Use of the BASINS Model to estimate the loading of heavy metals from the binational Tijuana River watershed.” Watershed 2000 Specialty Conference, Water Environment Federation, 9-12 July, Vancouver, British Columbia.

Brooks, H.A., Gersberg, R.M. and A.K. Dhar. Quantification of Hepatitis A virus in seawater using real-time RT-PCR. Submitted to *Applied Environmental Microbiology*.

This project contributed significantly to the education and training of a number of graduate students including J. Pitt and H. A. Brooks, who are referenced above.

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INTRODUCTION

Land-based sources of pollution, such as dry and wet weather urban runoff, are responsible for beach contamination and closures throughout the region of Bight of the Californias. Indeed, data presented by the Southern California Coastal Water Research Project (SCCWRP) (2000) shows that after a rain event of one inch or more, nearly 60% of the beaches in the 692 miles from Point Conception in Santa Barbara County to Punta Banda, near Ensenada in Mexico, were found to exceed water quality health standards.

Geographic information system (GIS) technology coupled with hydrologic simulation models have become a valuable tool for watershed management and nonpoint source pollution modeling. Currently, there is a lack of suitable calibrated models that may be used to predict the spatial and quantitative nature of coliform (and pathogen) loading from coastal watersheds of Mexico and the United States, into the southern California bight region. The aim of this study was to use the Nonpoint Source Model (NPSM) imbedded in the Better Assessment Science Integrating Point and Nonpoint Sources (BASINS) environmental analysis system to estimate annual loadings of fecal coliform bacteria in runoff from the coastal watershed in the border region of the Bight of the Californias. That model was then used in a predictive fashion to estimate pollutant loading from the watershed under a variety of coastal development scenarios.

RESEARCH OBJECTIVES

The overall goal of this project was to establish the spatial information and modeling framework (using the NPSM within BASINS) for protecting the nearshore marine environment in the bight from land-based sources of pathogen pollution.

The specific objectives of the project were as follows:
1. Use the NPSM of BASINS together with GIS for the Tijuana River Watershed (TRW) to calculate the mass loading of fecal coliform bacteria to the southern California bight. This modeling would permit the quantitation of the mass loading of coliforms to the U.S.-Mexican border section of the bight.

2. Calibrate the model using historic data on fecal coliform densities in the Tijuana River. All watershed models, such as BASINS, need to be validated by calibration of the model's predicted values against directly measured values. For the Tijuana River watershed, coliform densities have been measured in past years by both the International Boundary Water Commission (IBWC) and City of San Diego (1996-2000) at Dairy Mart Road, and by the IBWC and County of San Diego (1992-1995) at Dairy Mart Road or the international boundary. These historic datasets were used as the calibrating datasets for the model.

3. Link BASINS to GIS for the border region of the bight (from Encinitas to Ensenada) to model the mass loading of fecal coliforms into this entire region of the bight. This would allow the extension of the BASINS modeling to other similar, semi-arid watersheds in the border region of the bight. The first such use of BASINS for modeling of a semi-arid watershed was demonstrated by Gersberg, et al. (2000), who used BASINS in a predictive fashion to estimate both annual discharge volume and metal loading from the Tijuana River watershed to the coastal ocean for 1998-1999.

4. Determine the ratio of coliforms to enteroviruses (using Polymerase Chain Reaction [PCR] methods) in both wet and dry weather runoff samples from the United States and Mexico. This data would enable the direct modeling of pathogens and coliforms so that human health risk in the coastal region of the bight may be predicted. This effort allowed the output of the BASINS model to be linked to a quantitative risk assessment model. It should be noted here that fecal coliform bacteria are simply indicators of fecal contamination, and as such, do not actually cause human disease. Despite that fact that beach posting and closures are based on fecal indicator densities, it is mostly the human enteric viruses that are associated with many of the adverse health effects (e.g. hepatitis A, acute gastroenteritis) of swimming in contaminated coastal waters of the bight. Therefore, this effort will allow us to convert the BASINS output (for fecal coliform bacteria) to mass loading (and densities) of human viruses discharged to the bight.

This Final Report will be divided into two main parts. The BASINS modeling part of this project (representing Research Objectives 1-3 above) will be presented first. Following this, the PCR methodology research for virus detection in ocean waters and runoff (Objective 4 above) will be described.

RESEARCH METHODOLOGY/APPROACHES

Basins Modeling

This research incorporated the available data into the Hydrologic Simulation Program—Fortran (HSPF) model interfaced with ArcView® GIS in the BASINS environmental analysis system. The data required for HSPF and BASINS are described in detail in the following sections. Additionally, the process for performing simulations with HSPF, including model calibration and validation, is described.

Geographic Data

To facilitate watershed-based analysis and modeling, four types of geographic and environmental data are delivered with the BASINS system and are used to initiate a BASINS project, which is the file format used to store information in BASINS. These data, further described in Table 1, include base cartographic data, environmental background data, environmental monitoring data, and point sources/loading data (EPA 2001). The U.S. Environmental Protection Agency (EPA) includes these data sets to provide a starting point for regions that have limited local data.

These data sets proved valuable in this research to ensure that the BASINS and HSPF projects contained the information necessary to successfully perform analyses; however, the data only covered the U.S. portion of the TRW. Therefore, collaboration with faculty in the Department of Geography at San Diego State University (SDSU) to obtain geographic data for the entire watershed was a vital part of this research. In addition to the following information on the TRW geographic data coverages, further detail on the origin and creation of the local geographic data sets is provided in the "Metadata Dictionary for the Tijuana River Watershed Project" (Tijuana River Watershed Project 1997).

Watershed Boundaries

The initial step in this watershed-based analysis was to clearly define the watershed boundary. A watershed is defined as a drainage basin or an area of land in which all waters drain to a single river system. Since the dataset bundled with the BASINS software included only the U.S. portion of the TRW, a coverage for the entire boundary the TRW was developed using a digital elevation model (DEM), further described with the topography data below.

Topography Data

Surface topography data for the United States and Mexico was developed from the U.S. Geological Survey (USGS) topographic quadrangle maps (1:24,000 scale) and Instituto Nacional de Estadística Geografía e Informática (INEGI) topographic maps (1:50,000 scale). These original USGS maps were scanned and the 20-foot and 40-foot contours extracted, and the INEGI maps were digitized to determine the contours. The resultant contour maps were merged in ARC/INFO® to generate a 30-meter DEM for the entire watershed. The DEM represents the elevation values for cells within the watershed.

Reach Files

To perform simulations on streams, BASINS requires the inclusion of a Reach File. Reach File Version 1 (RF1) provides a stream network that includes major rivers, while Reach File Version 3 (RF3) builds off the RF1 coverage and identifies a more detailed stream network. The Reach Files available with the BASINS software were modified to include the Mexican portion of the TRW.

To create a hydrographic representation of the TRW, stream channels within the TRW were identified on a DEM using the hydrologic techniques within ARC/INFO®. The resultant stream network, RF3, included many small, intermittent streams that only contained flow during very wet years, which can cause model errors while performing simulations. To maintain accuracy and reduce the potential for errors, only stream segments identified in the RF1 were included. Additionally, the stream files were attributed with the fields necessary for BASINS simulations. Figure 1 represents the stream network in the TRW.

In-stream HSPF simulations require the model user to input geometric and hydraulic properties of stream reaches into the HSPF hydraulic function tables (F-Tables) (Bicknell, et al. 2001; EPA 1999). These data were obtained via field measurements, observations, and flow gage rating curves. Pitt completed base flow stream measurements and channel characteristics for three stream reaches in the TRW. To determine the hydraulic properties for Campo Creek and Rio Alamar, Pitt analyzed the USGS rating curves at the Campo Creek and Dulzura (Rio Alamar) gage sites. Pitt performed similar analyses on the IBWC rating curves for the Tijuana River near the international border. The F-Table geometric channel characteristics for Campo Creek, Rio Alamar, and the Tijuana River were obtained through field observations.

Subwatershed Boundaries

Using a DEM, the TRW is naturally divided into 12 subwatersheds based on its topography and hydrologic characteristics. Due to the presence of reservoirs and dams in both the United States and Mexico, only four of the 12 original subwatersheds are simulated in this research. Specifically, Barrett Dam in the United States and Presa Rodriguez in Mexico terminate the surface flow from the northern and southern portions of the watershed, respectively. The subwatersheds impounded by these reservoirs are only released under extremely high flow conditions (Gersberg, et al. 2000). Therefore, only the central subwatersheds are hydrologically active and will be included in model simulations. These subwatersheds will be referred to as the interior subwatersheds throughout the remainder of this report. Figure 2 is a map of the original subwatersheds and the interior subwatersheds in the TRW. This figure also depicts the stream reach network and associated dams and reservoirs.

HSPF, a lumped parameter model, can be modified to function as a pseudo-distributed parameter model in which subwatersheds are further delineated to create smaller homogenous parcels. In this case, the parameter values assigned to hydrologic characteristics are constant throughout each corresponding delineated subwatershed, and have no spatial variability.

In order to execute HSPF through BASINS, each subwatershed can only contain one reach segment. Therefore, the interior subwatersheds were further delineated into a system of hydrologically connected subwatersheds using the BASINS 3.0 Manual Watershed Delineation tool (EPA 2001). Delineations of the

interior subwatersheds maintained consistency with the related topographic features. Figure 3 illustrates the delineated interior watersheds of the TRW and Table 2 identifies the delineated subwatershed name, stream reach identification number, and corresponding area for each delineated subwatershed. During HSPF simulations, each delineated subwatershed will be parameterized separately; thus, creating a patchwork of different parameter values that represent the interior subwatersheds.

BASINS Stream Network

After performing successful watershed delineations, BASINS users must use the stream network tool to create three data layers that are used for modeling purposes. Upon executing HSPF from BASINS, the model uses three coverages—Streams, Outlets, and Subbasins—to identify the characteristics of each subwatershed and to eliminate extraneous data included in the ArcView® attribute tables. Specifically, the Subbasins coverage represents the geographic features of the associated subwatershed, including slope, latitude of the subbasin centroid, and elevation. The Streams coverage includes specific attributes of the RF1 theme, including the number of inlet subbasins, the stream reach length, slope, width, and depth. Finally, the Outlets coverage includes the latitude and longitude and an identification number for the point on a stream reach where the adjacent delineated subbasins connect. HSPF uses all of this information during simulations to determine the subbasin area that runs off into a particular stream and then determines the resultant pollutant load at a particular outlet.

Land Use Data

Land use data for the U.S. side of the TRW was initially compiled by the San Diego Association of Governments (SANDAG). The polygon boundaries for this portion of the watershed were created using 1995 SPOT satellite imagery. Land use polygons for the Mexican portion of the TRW were generated from the interpretations of 1:12,500 and 1:50,000 scale aerial photographs. These polygon boundaries were then digitized using a SPOT satellite image as a reference. After completing the boundaries, topology was generated and appropriate land use codes and descriptions were assigned. To achieve an overall land use coverage for the TRW, the Mexican portion was then merged with the U.S. portion from SANDAG. This coverage had 18 unique land uses.

HSPF requires parameter values to be inputted for every land use in each subwatershed (Bicknell, et al. 2001). Therefore, to simplify model parameterization, the land uses from the original TRW coverage were grouped using the BASINS Land Use Reclassification utility. To maintain consistency, land uses were reclassified into groups with similar levels of pervious cover. The resulting land use coverage contained six unique land uses. Table 3 depicts the original and grouped land uses in the interior subwatersheds of the TRW.

Meteorological Data

Considering that nonpoint source pollution is a weather-driven process and that hydrologic processes vary over time, meteorological data comprise a very important part of hydrologic simulations. Time-series data for such simulations are often stored and manipulated in weather data management (*.wdm) files. Times-series data management includes data collection, formatting, generation, aggregation, disaggregation, display, and analysis. The BASINS 3.0 software contains WDMUtil, a *.wdm file management program used to format and manipulate *.wdm files for use in HSPF. Figure 4 illustrates how meteorological data are processed in HSPF.

To perform time-series analyses, hourly meteorological data, including air temperature, precipitation, evaporation, wind speed, solar radiation, potential evapotranspiration, dewpoint temperature, and cloud cover, were required. These data were available through the EPA for Lindburgh Field (San Diego's airport) from 1970 through 1995. In order to simulate more recent years, hourly precipitation, temperature, wind speed, dewpoint, and cloud cover data at Lindbergh Field (COOP ID #047740) were obtained for 1996 through June 2001 from the National Climatic Data Center (NCDC), a branch of the National Oceanic and Atmospheric Administration (NOAA) (National Climatic Data Center 2002). These data were then used to compute the hourly evaporation, solar radiation, and potential evapotranspiration required for HSPF simulations using WDMUtil.

The meteorological data from Lindbergh Field are representative of the western interior subwatersheds. However, due to the climatic variability in Southern California, and the size of the entire watershed, Lindbergh Field was not considered representative of the eastern interior subwatersheds, which tend to have more precipitation and lower humidity. In order to predict the surface runoff from the eastern interior subwatersheds more accurately, weather data from Campo, California, were used. Figure 5 illustrates the weather stations used for simulations in the interior subwatersheds. The SDSU Geography Department staff assisted in the initial preparation of the meteorological data from the Campo weather station. Daily temperature and precipitation data (1970 through September 1999) from the Campo Weather Station (COOP ID # 041424) were obtained from the NCDC (NCDC 2001). These daily data were disaggregated using WDMUtil to compute hourly rainfall and temperature data based on the observed hourly data from the Morena Dam and Lindbergh Field. WDMUtil was also used to compute hourly potential evapotranspiration. Due to the lack of hourly meteorological data at this smaller weather station, evaporation, wind speed, solar radiation, dewpoint temperature, and cloud cover data from Lindbergh Field were used in the Campo *.wdm file, including a two-hour time lag to account for the temporal differences between Campo and San Diego.

Considerable effort was required to transform and manipulate this meteorological data into the HSPF-required format. It is generally accepted among model users

that weather data manipulation and formatting can take up to 30% of the total modeling effort (Donigian, et al. 1984). The completion of this meteorological data allowed simulations to be performed from 1970 through September 1999 in the entire watershed and through June 2001 for the western interior subwatersheds.

Stream Flow Data

After importing all necessary data into HSPF and upon successful model runs, it was necessary to obtain observed flow data for various stream reaches in the TRW. These data were used for comparison with the modeled flow data during the calibration process. Flow data were obtained from USGS for Campo Creek (USGS site number 11012500) for October 1936 through September 2000 (USGS 2000) and Rio Alamar near Dulzura (USGS site number 11013000) for October 1936 through September 1990 (USGS 1990). Additionally, daily flow data for the Tijuana River near the international border were obtained from the IBWC for 1962 through 2001 (IBWC 2001). These stream flow datasets were appropriately formatted and imported into GenScn for analysis. Figure 5 represents the locations of the stream flow gages in the TRW.

Water Quality Data

Performing water quality simulations requires a variety of water quality data. These data include pollutant accumulation rates, point source contributions, and observed constituent concentrations. Bacterial loading levels from the population of Tijuana and Tecate, Baja California that did not have access to sewage collection and treatment were also obtained for this research.

Accumulation Rates

Land use-specific accumulation rates (parameter ACQOP) are required by HSPF to simulate nonpoint source pollution. Fecal coliform accumulation rates were determined for the Santa Monica Bay region by SCCWRP (Ackerman and Schiff 2002). Specifically, SCCWRP collected water samples from open, agricultural, commercial, industrial, transportation, high-density residential, and low-density residential land uses. After performing laboratory analyses to determine the level of fecal coliform concentrations, accumulation rates (fecal coliforms per acre per day) were calculated for each land use (Ackerman and Schiff 2002). These accumulation rates were slightly modified or regrouped to correspond with the land uses being simulated in the TRW (Table 4).

Point Source Loadings

To account for the point source pollution in HSPF models, daily loadings of pollutants (quantity per day) are required. On average, the Tecate Wastewater treatment plant (WWTP) discharges 125 liters per second (Brown 1998), or 4.4 cubic feet per second (cfs). Its effluent contains approximately 1.6×10^7 fecal coliforms per 100 milliliters (mL) (Regional Environmental Consultants 1991). These values correspond to a daily loading of 1.728×10^{15} most probable

number (MPN) of fecal coliforms into the Tecate Creek, which eventually connects to the Rio Alamar and flows into the Tijuana River.

Unsewered Residential Areas
Between 41% (Wakida and Riveles 1997) and 29% (Brown 1998) of the 1.3 million residents of Tijuana live in areas without municipal sewer systems. Each resident in an urban developing country produces an average of 250 grams of feces per day and each gram of feces contains approximately 10^8 fecal coliforms (Feachem, et al. 1983). Therefore, the unsewered population of Tijuana, Mexico contributes between 9.43×10^{15} and 1.33×10^{16} fecal coliforms per day to the residential areas of the city.

These pollutants generated by the population of Tijuana eventually wash off into the Tijuana River during precipitation events. HSPF distributes the total pollutant load over the residential land use in the Tijuana River-border interior subwatershed using the accumulation rate parameter ACQOP. In addition to the typical fecal coliform accumulation rates for residential land uses (see Table 4), the unsewered population of Tijuana contributes between 1.32×10^{12} and 1.86×10^{12} fecal coliforms per acre per day to these areas of Tijuana. The calculated accumulation rates for the estimated proportion of Tijuana residents without sewer systems were similar. Therefore, these values were averaged and an additional 1.59×10^{12} fecal coliforms per acre per day were incorporated into the parameter ACQOP for the residential areas of Tijuana.

Similarly, about 19% of the 100,000 residents of Tecate are not connected to municipal sewer systems (Brown 1998). This population contributes 2.66×10^{11} fecal coliforms per acre per day to the residential areas of Tecate. During precipitation events, some of these pollutants wash off of the landscape and enter Tecate Creek, which eventually connects to Rio Alamar and the Tijuana River.

Observed Water Quality Data
Actual fecal coliform levels at Dairy Mart Road, just north of the international border in Imperial Beach, California, were available from various monitoring programs. Actual data, which included weekly observations, at best, were obtained for 1995-1996 and 1998-2000 (IBWC 2000). The first time period was used for model calibration, while the first two years of the second was used for model validation. The final year of fecal coliform data were not used to maintain consistency with the flow simulation period, which terminated in September 1999 due to the limited weather data. These bacteriological water quality data were reformatted and imported into the GenScn post-processor for analysis. Additionally, actual fecal coliform data were available for Campo Creek. These data included an arithmetic mean of fecal coliforms levels from samples collected over the 1995-1996 and 1996-1997 storm seasons.

RESEARCH FINDINGS

Campo Creek Flow Results

Model calibration was performed to achieve an overall annual water balance between modeled and observed streamflow data. The calibrated model over-predicted the streamflow for some storm seasons and under-predicted for others. Parameter adjustments resulted in a mean difference between observed and modeled stream flow of 25% for October 1990 through September 1996 at Campo Creek (Table 5). Figure 6 illustrates the time-series comparison of modeled and observed flow for the calibrated model at Campo Creek.

Model validation for stream flow at Campo Creek was performed for October 1996 though September 1999 using the set of parameter values determined during the model calibration process. During this time period, a comparison of the modeled and observed stream flow rates resulted in a mean difference of 33% (Table 6). These results are further illustrated in Figure 7, which graphically depicts the validated time-series analysis.

Campo Creek Fecal Coliform Results

Simulations to predict the fate and transport of fecal coliforms in Campo Creek were only performed for two storm seasons (1995-1996 and 1996-1997) due to limited empirical data. Specifically, the only available observed data was an arithmetic average of fecal coliform concentrations from samples collected over these two storm seasons. The first storm season, 1995-1996, was used for model calibration while the 1996-1997 storm season was used for model validation. Initial fecal coliform simulations were based on land use-specific accumulation rates in the Santa Monica Bay region and typical maximum storage capacities, which equal 1.8 times the corresponding accumulation rate (Cocca 2001). For October 1995 to March 1996, the model predicted an average fecal coliform concentration of 34 MPN per 100 mL. This was 94% below the observed average of 6.5×10^2 MPN per 100 mL. After performing model calibration on this same time period, the model predicted an average of 7.8×10^2 MPN per 100 mL, just 19% above the average observed value.

Model validation, performed using the same parameters as the calibrated model, resulted in 4.8×10^2 MPN per 100 mL of fecal coliforms in Campo Creek, which corresponded to 25% below the average observed concentration for October 1996 to March 1997 (6.5×10^2 MPN per 100 mL). Although the observed data for model comparison were limited, the Campo Creek fecal coliform simulations identified accurate parameter values for the eastern subwatersheds.

Rio Alamar Flow Results

All streamflow simulations for Rio Alamar were performed after calibration of Campo Creek. Due to limited observed stream flow data, simulations were performed for the 1980s at Rio Alamar, rather than for the 1990s. To achieve an annual water balance between modeled and actual flow rates, parameters were adjusted for all reaches upstream of Rio Alamar besides Campo Creek. Figure 8

provides a graphical representation of the calibrated modeled and observed daily time-series for 1980-1985 at Rio Alamar. Model calibration resulted in an average 43% difference between modeled and observed stream flows for this same time period (Table7).

The model consistently under-predicted the observed flow at Rio Alamar for 1980-1985 during the model calibration process; however, model validation for 1986-1989 resulted in consistent over-prediction. Specifically, the validated model, predicted an average 72% above the observed stream flow data from the USGS flow gage (Table 8). This is primarily due to the large percent difference for 1987, in which the modeled stream flow was 165% higher than the observed flow.

Figure 9 graphically represents the daily values for the validated stream flow model at Rio Alamar. This figure illustrates the over-prediction of storm peaks throughout the validated time period, especially for 1986 and 1987. Additionally, this figure shows storm peaks predicted by the model that, based on observed data, did not occur.

Tijuana River Flow Results

After completing model calibration and validation through the Rio Alamar, simulations were performed on the western interior subwatersheds of the TRW. These simulations included stream flow calibration for October 1990 through September 1996 and validation for October 1996 through September 1999

To reflect the stream flow in the Tijuana River accurately, the Tecate WWTP and the Tecate Brewery were included as point sources of flow. The point source of flow for the WWTP (4.4 cfs) began in late 1995, corresponding to the plant opening (Placchi 1998), while the flow of the Tecate Brewery (0.7 cfs) applied to the entire simulation period.

Model calibration included altering the parameter values for the reaches downstream of the Rio Alamar. The calibrated model over-predicted the stream flow for some storm seasons and under-predicted for others. These simulations resulted in a mean difference between the modeled and observed stream flow of 59% at the Tijuana River for October 1990 to September 1996 (Table 9). The 1995-1996 storm season, which was the first to include the flow of the Tecate WWTP, was especially problematic during model calibration (+122% difference). Figure 10 illustrates the calibrated daily modeled and observed stream flow at the Tijuana River for October 1990 through September 1996.

Model validation for the Tijuana River was performed for October 1996 through September 1999. Similar to the calibration results, the model over-predicted the stream flow for some storm seasons and under-predicted for others. The validated model resulted in a mean difference of 65% between modeled and observed stream flow for the Tijuana River during the 1996-1999 storm seasons

(Table 10). The model grossly over-predicts the stream flow during the period from October 1996 through September 1997 (+125% difference), which is the second year of the operation for the Tecate WWTP. Figure 11 is a graphical representation of the validated modeled and observed daily stream flow rates for the Tijuana River near the international border.

Tijuana River Fecal Coliform Results

Simulations to predict fecal coliform loadings in the Tijuana River were performed for four years. Initial simulations and model calibration were performed for 1995-1996, and January 1998 through September 1999 were used for model validation. Subsequent wastewater collection and treatment scenarios were conducted for the calibration time period.

Initial fecal coliform simulations included the calibrated parameter values for Campo Creek, while the other stream reaches in the interior subwatersheds were assigned the land use specific accumulation rates calculated for the Santa Monica Bay region (Table 4) and typical corresponding maximum storage capacities equal to 1.8 times the accumulation rate (Cocca 2001). Table 11 presents the results for the initial fecal coliform simulations at the Tijuana River for 1995-1996, which only slightly under-predicted both the maximum and the mean fecal coliform loadings, when compared to the actual values obtained from a sampling site at Dairy Mart Road in Imperial Beach. To achieve better concordance with the actual data, model calibration was performed, which included increasing the storage capacity (SQOLIM) and decreasing the wash-off rate (WSQOP).

To depict the bacterial water quality in the Tijuana River more accurately, the residential populations of Tijuana and Tecate, which are without sewage collection systems, were incorporated into the model as nonpoint sources of pollution. This contribution resulted in maximum and average fecal coliform concentrations two to three orders of magnitude below the observed values. However, to further characterize the bacterial pollutants in the TRW, the Tecate WWTP was also included in the model. When considered independently, this loading resulted in model predictions within the same order of magnitude as the observed fecal coliform levels (Table 11). Similarly, the incorporation of both the residential population without sewer collection systems and the Tecate WWTP resulted in fecal coliform concentrations within the same order of magnitude as the observed values for the same time period (Table 11). Specifically, the model under-predicted the mean concentration by 14% for the entire simulation period. The model also under-predicted the actual dry season flows by 88% and over-predicted the wet season flows by 462%. However, the observed data for both the wet and dry seasons had values in the same order of magnitude (10^4 MPN per 100 mL) and the simulated fecal coliform concentrations followed a more traditional pattern for wet and dry season values. Namely, the simulated mean dry season level was 1.4×10^3 MPN of fecal coliforms per 100 mL and the mean wet season concentration was 1.0×10^5 MPN per 100 mL, which are in the same

order of magnitude as the fecal coliform levels in the TRW presented by Gersberg, et al. (1994). Figure 12 illustrates the observed and modeled calibrated daily time series of fecal coliform concentrations for 1995-1996 in the Tijuana River.

The geometric mean of the model validation results for January 1998 through September 1999 was higher than that for the calibration results. Specifically, the model predicted a mean fecal coliform concentration of 6.4×10^5 MPN per 100 mL, which was 141% above the observed value of 2.7×10^5 MPN per 100 mL. Alternatively, the model under-predicted the observed maximum concentration of 1.30×10^7 MPN of fecal coliforms per 100 mL by 93%, with a predicted fecal coliform level of 1.0×10^6 MPN per 100 mL. When compared to observed values from the Dairy Mart Road sampling sites, the model predicted fecal coliform levels within the same order of magnitude for both the wet (10^5 MPN per 100 mL) and dry seasons (10^5 MPN per 100 mL). Figure 13 graphically depicts both the observed and modeled fecal coliform levels for the validated model.

Using the calibrated model parameters, various scenarios of wastewater treatment and collection were simulated for 1995-1996. Table 12 presents the results for these simulations. The first scenario depicts what the current fecal coliform levels in the TRW would be if all residents had sewage collection systems and the Tecate WWTP had effluent concentrations similar to typical secondary treatment (approximately 5.0×10^5 MPN of fecal coliforms per 100 mL) (Feachem, et al. 1983). This scenario resulted in a maximum fecal coliform concentration three orders of magnitude below the corresponding observed values at the Dairy Mart Road sampling site and a mean concentration two orders of magnitude below. Specifically, the geometric mean of the modeled values was in the hundreds, similar to the ambient water quality standard of 200 MPN per 100 mL (Table 12). The simulated dry season fecal coliform concentrations had a geometric mean of 1.8×10^2, which is below this standard, and the simulated wet season results were well above the standard, with a mean concentration of 4.8×10^3 MPN of fecal coliforms per 100 mL.

The next situation modeled calculated a “worst-case scenario” for 2020 in which the population increased as expected and the Tecate WWTP was running at maximum capacity (10.59 cfs), yet no improvements were made to sewage collection or treatment infrastructure. Independently, the expanded population resulted in a mean of 5.2 fecal coliforms per 100 mL and a maximum of 1.1×10^6 (Table 12) and the Tecate WWTP loading resulted in a mean of 1.4×10^4 fecal coliforms per 100 mL and a maximum of 2.5×10^6 (Table 12). The cumulative impact of these sources was similar to those presented for the Tecate WWTP loading. The geometric mean of these results were 79% above and within the same order of magnitude as the observed data for 1995-1996.

The final scenario simulated the effect of improvements to the infrastructure in Baja California in 2020, while considering the expected population growth.

Sewage collection infrastructure improvements included a reduction in the population of Tecate and Tijuana that were not covered by sewage collection systems to 10% for each city. When simulated independently, the fecal coliform contribution of the residents in Baja California was similar to that of the worst-case scenario, which considered no infrastructure improvement (3.0 MPN per 100 mL assuming infrastructure improvements and 5.2 MPN per 100 mL for the worst-case scenario). The wastewater treatment improvements simulated for this scenario assumed that the Tecate WWTP was running at maximum capacity due to the increased population demands, but the effluent contained only 5.0×10^5 fecal coliforms per 100 mL. Independent simulations of the Tecate WWTP resulted in mean loadings one order of magnitude below the 1995-1996 observed levels (Table 12). The results associated with a combination of the residential and Tecate WWTP loadings were similar to those that considered only the WWTP contribution. Specifically, the maximum level caused by the combination of factors was 2.4×10^5 MPN of fecal coliforms per 100 mL while the mean was 2.4×10^3 MPN per 100 mL (Table 12). These fecal coliform concentrations are one order of magnitude below the worst-case scenario results and 83% below the mean observed fecal coliform concentrations for 1995-1996.

CONCLUSIONS

Simulations to predict the loading and transport of fecal coliforms in the Tijuana River were performed for a total of four years. The residential population of Tijuana and Tecate, Baja California that did not have sewer main and lateral coverage and the Tecate WWTP were included in the model to reflect known sources of fecal coliform loading within the TRW. The residential populations were included as a nonpoint source of pollution and their impact was distributed over the residential land uses in Tijuana and Tecate. These fecal coliform loadings were subsequently washed off into nearby receiving waters during and after precipitation events. The Tecate WWTP was included as a continuous point source loading of fecal coliforms into the Tecate Creek.

After including these additional sources of fecal contamination, the model was within the same order of magnitude as the observed maximum and average values and the Tecate WWTP contributed the majority of the fecal coliform loading (Table 11). Subsequently, model calibration performed for 1995-1996 reduced these values and the calibrated model ultimately under-predicted the maximum observed level and over-predicted the mean observed concentration by 14%. Based on the tolerance ranges for model fit, the calibrated concentrations were “very good” estimates of the natural system (less than 15% difference between modeled and observed values) (EPA 2001).

During 1995-1996, the model accurately predicted an expected difference between wet and dry season fecal coliform concentrations. Specifically, the wet season mean concentrations were two orders of magnitude above the dry season levels. The observed data for this time period did not reflect such a difference, (10^4 MPN per 100 mL for both wet and dry season geometric means).

The lack of variation among the observed data during these storm seasons may be due to poorly designed monitoring programs, in which samples were collected only when the concentrations were expected to be high. Alternatively, there may be additional sources of fecal contamination not captured in the data input into the model.

The validated model for 1998-1999 predicted 141% above the observed mean value. This was well out of the tolerance ranges for good model fit. The simulated results for this time period did not display wet and dry season variation similar to the calibrated results (10^5 MPN per 100 mL for both). However, these values were consistent with the geometric means of the observed data, which were also in the fifth order of magnitude for wet and dry season concentrations. The constant loading from the Tecate WWTP may be responsible for the consistent results across seasons or other sources of fecal contamination not considered in the model may contribute to the TRW on a regular basis.

Since the model achieved very good agreement with the observed fecal coliform values for the calibrated time period, different scenarios were simulated to determine the relative impact of various levels of wastewater treatment and sewer main and lateral coverage. These scenarios were modeled for the same time period as the calibrated model, 1995-1996. Additionally, all model parameters remained the same, except for those altered to represent the different management scenarios.

The scenario that considered major improvements to the future wastewater collection and treatment infrastructure, full secondary treatment at the Tecate WWTP, and assumed that the population grew as expected until 2020 (but only 10% of the residents in Tecate and Tijuana remained without sewage collection systems), predicted a maximum fecal coliform concentration of 2.4×10^5 MPN per 100 mL and a mean concentration of 2.4×10^3 (Table 12). This simulation suggested that such improvements may result in a decrease by nearly one order of magnitude in bacteria levels.

These simulations illustrated that, when considering the current and improved levels of wastewater treatment, the Tecate WWTP had the largest impact on fecal coliform levels in the Tijuana River, since its loading is constant. Resources should initially be allocated to conduct more comprehensive studies on the quantitative effect of WWTP modification and improvement. The fecal contamination, which is dependent on precipitation events, caused by residents without sewage collection system coverage was nearly negligible when compared to the loadings from the WWTP.

The results of these simulations generally indicate that the fecal coliform loading associated with the residential populations not covered by sewage collection systems have a minor impact on the overall bacterial water quality in the TRW, when compared to the Tecate WWTP. Additionally, the model consistently under-

predicts the maximum concentration levels when compared to the observed fecal coliform levels for the same time period.

RESEARCH METHODOLOGY/APPROACHES

PCR Detection of Hepatitis A Virus (HAV)

Sampling Sites: Ocean water samples were collected from two beach locations—200 yards north of the Tijuana River mouth and on the south side of the Imperial Beach Pier. A map of these sampling sites is shown in Figure 14. All samples were collected following a rain event, which was defined as precipitation of 0.2 inches or more. This definition is based on the Department of Environmental Health general advisory, which is issued after 0.2 inches or more of rainfall, and warns the public of possible water contamination by urban runoff (County of San Diego 2003). Samples were collected only after it had rained sufficiently to cause the Tijuana River to increase its flow significantly. In the beginning of the wet season, the amount of rain to cause increased flow in the river was greater because the ground was very dry and soaked up much of the water before it could reach the river. Samples were collected within six hours following the peak flow of the Tijuana River so that the increased flow from the river theoretically had enough time to reach the Imperial Beach pier, 0.85 miles (1,500 yards) north of the river mouth. Peak flow was measured by an automatic sampler (ISCO automatic sampler with flow gauge) in the river. Some rain events had greater precipitation in the distant areas of the Tijuana River watershed and very little locally. This type of rain event would still cause the river to rise, although since the water was coming from far away, the peak flow would often occur up to 24 hours after the rain event. In this case, samples would not be collected because the rain water was passing through a relatively unpopulated area of the Tijuana River watershed and therefore were not representative of the desired sampling for this study. The sampling objective was to collect water that had passed through the local urban areas and had a greater risk of being contaminated by human waste.

Determining Fecal Coliform Bacterial Count in Water Samples

A sample measuring 100 mL was taken for analysis of fecal coliforms. Samples were held on ice and processed within three hours of collection. The MPN method was used to enumerate fecal coliforms. Multiple tube fermentation was employed with five replicates of three serial dilutions (American Public Health Association 1992), and was performed by the Graduate School of Public Health at San Diego State University. Total coliform, fecal coliform, and enterococci were also tested for by the County of San Diego Department of Environmental Health, and the results used when testing times corresponded with the sampling times of this study.

Processing of Water Samples for Hepatitis A Virus Detection

Four rain events were sampled during which a total of eight samples were collected. Each 4 liter (L) sample was collected in a two-gallon bucket and processed within one or two days of collection. Each sample was spiked with 250

microliters (µl) of the Taura syndrome virus (TSV) homogenate. TSV is a member of the picorna-like virus family, and therefore shares physicochemical characteristics with HAV, which is a picornavirus, that will allow both viruses to act similarly through the filtration process. This viral spike will allow for assessment of the viral recovery from the filter method used to concentrate the sample. Following addition of the spike, the sample was stirred to facilitate sediment-TSV binding. Viruses tend to absorb to sediments, and have been estimated to harbor between 10-times and 10,000-times the amount of virus found in water (Metcalf, Melnick, and Estes 1995). Therefore, by stirring the spike into the sample, both the HAV-sediment binding and the HAV filtration recovery were assessed. The sample was then filtered via a vacuum pump system through a type HA 0.45-micrometer (µm) pore size, negatively charged membrane filter (Millipore, Burlington, Mass.) where the virus was captured. The filter was washed with 200 mL of 0.5 millimoles (mM) H_2SO_4 to rinse out the cations. Subsequently, the virus was eluted from the filter with 10 mL of 1 mM NaOH, into a tube containing 0.1 mL of 50 mM H_2SO_4 and 0.1 mL of 100x TE buffer to neutralize the recovery, reducing the volume of the sample to 10 ml.

This eluate was added into a Centriprep Concentrator (Millipore) and filtered at 1,500 x g for 10 minutes, reducing it further to a volume of about 450 µl. This sample processing was based on a virus filter/concentration method developed at the University of Tokyo (Katayama, Shimasaki, and Ohgak 2001). In order to reduce filter clogging, water samples that were high in particulate matter were filtered through a series of Whatman filters (of 11µm and 2.5 µm pore size) before applying the method described above.

RNA Extraction
The total volume of filtrate (450 µl) for each sample was transferred to one tube and RNA was extracted using TRI Reagent™ and the manufacturer's protocol (Molecular Research Center Inc., Ohio). The RNA pellet from each sample was dissolved in 20 µl of RNAse, DNAse free water (Invitrogen Corporation, Carlsbad, Calif.).

Conventional Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Amplification of HAV
The cDNA was synthesized using random hexamer primers and the GeneAmp® Gold RNA PCR Core kit (Applied Biosystems, Foster City, Calif.). The reaction mix for the cDNA synthesis contained 20 µl of RNA, 1x GeneAmp RT-PCR Buffer, 2 mM of $MgCl_2$, 1.25 mM of deoxynucleoside triphosphates, 1.25 µM of random hexamers, 6.25 mM of dithiothreitol (DTT), 25 U of RNase inhibitor (RNasin), and 62.5 units of reverse transcriptase in a total reaction volume of 40 µl. All cDNA synthesis reactions were carried out at 42ºC for 1 hour.

Conventional RT-PCR
The HAV detection was carried out using conventional RT-PCR. The conventional RT-PCR reaction mixture contained 2 µl of cDNA, 1x PCR buffer II,

2 mM of $MgCl_2$, 0.8 mM of deoxynucleoside triphosphates, 0.8 mM of each of forward and reverse primer, and 5 U of AmpliTaq Gold® DNA Polymerase in a reaction volume of 25 µl. The thermal profile for conventional RT-PCR was: 10 minutes at 94ºC, following 40 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds, and 72ºC for 1 minute.

HAV primers have been designed to specifically target a region of the hepatitis A viral protein coding region, the VP3/VP1 junction. This region is a conserved sequence in the 5' end of the HAV genome. In comparing different strains of HAV to wild-type HAV, as well as to other picornaviruses, sequence conservation was found to be high between HAV strains and to be low between HAV and other picornaviruses (Cohen, et al. 1987; Deardorff 2001). These Hepa 1 and Hepa 2 primers have successfully amplified HAV (Deardorff 2001; Cohen, et al. 1987). The specificity of the primers were verified (Deardorff 2001).

Gel Electrophoresis
Amplified cDNA products were separated on a 2% agarose gel containing 2.5 µl of 10 mg/mL ethidium bromide. Each gel was electrophoresed for three hours at 80 volts (V) in a 1X TBE buffer. A ladder (Promega, Madison, Wisc.) was used to aid in determining product band size. The DNA products were visualized with exposure to UV light using a Fisher Biotech 312 nm Variable Intensity Transilluminator (Fisher Scientific, Pittsburgh, Penn.). Each gel was photographed using a Photo-Documentation Hood (Fisher Scientific).

Cloning and Sequencing of HAV cDNA
HAV was isolated from an ocean water sample taken in Punta Bandera, Mexico using the method described above. Total RNA was isolated from this sample and RT-PCR was performed as described above. The amplified cDNA was run on a 2% agarose gel and a cDNA band around the 250 base pair region was cut out. The cDNA was then purified from this band, sequenced, and the sequence analyzed in the National Center for Biotechnology Information (NCBI) BLAST search program (www.ncbi.nlm.nih.gov/BLAST). Sequence analysis confirmed the identity of the band to be Hepatitis A virus. Once confirmed, the HAV cDNA was cloned into a TOPO cloning vector (Invitrogen) following the manufacturers protocol. The recombinant plasmid was isolated using an alkali lysis method. The plasmid DNA was then used for generating a standard curve in real-time RT-PCR (see below).

SYBR Green Real-Time RT-PCR
The primers used for SYBR Green real-time RT-PCR are listed in Table 13. The primers for HAV were designed based on the sequence of the cloned HAV plasmids using the Primer Express Software version 1.0 (Applied Biosystems). Four primer sets were tested for HAV amplification efficiency, and the best primer set was chosen for real-time RT-PCR. The chosen HAV primers, HAV1FWD and HAV3RVS, amplify a region of 76 base pairs within the VP1 and VP3 region.

SYBR Green real-time RT-PCR was conducted using an iCycler iQ™ real-time PCR detection system (Bio-Rad). For real-time RT-PCR, cDNA was synthesized using random hexamers as described above. cDNA samples were diluted 1:10, 1:100, and 1:1000 with DNase, RNase free water containing herring sperm DNA (5 ng/mL) and 5µl were taken for each amplification reaction. Real-time RT-PCR was carried out in a 25 µl reaction volume that contained 7.1 µl of iQ SYBR® Green Supermix (Bio-Rad), 0.8 mM each of the forward and reverse primer, and 1 µl of stock cDNA/5 µl of diluted cDNA. Each sample had three replicates, and was repeated twice to ensure reproducibility of results. All reactions were carried out in 96 well plates. The thermal cycle profile for SYBR real-time PCR was 95ºC for 10 minutes, followed by 40 cycles of 95ºC for 10 seconds and 60ºC for 1 minute.

Plasmid Standard
For each SYBR Green real-time RT-PCR assay, a dilution series of HAV plasmid was run to generate a standard curve and serve as a positive control. Plasmid DNA serial dilutions were made in sonicated herring sperm DNA (5 ng/ml). This diluent captures small quantities of sample DNA for PCR more efficiently. The dilution of plasmid DNA ranged from 1.1×10^5 copies of HAV plasmid down to a single copy of HAV plasmid.

Data Analysis for SYBR Green Real-Time RT-PCR
SYBR Green real-time RT-PCR data analyses were performed by the iCylcer iQ™ real-time PCR detection system software (Version 3.0A). In the iCylcer iQ™ detection system, the individual wells are calibrated dynamically (against the fluorescence of SYBR Green) using an internal passive reference fluorophore, fluorescein which is in the iQ™ SYBR® Green Supermix (Bio-Rad). A sample is considered positive when ΔR_n exceeds the threshold value. The threshold value is the midpoint of ΔR_n and the cycle number plot. The threshold value of all amplifications was chosen to be 0.25. The threshold cycle (C_T) is the cycle at which a statistically significant increase is detected in R_n. This threshold cycle corresponds inversely to copy number. In other words, the higher the copy number in a sample, the lower the threshold cycle.

RESEARCH FINDINGS
Detection of HAV in Tijuana River Mouth and Imperial Beach Pier Samples by Conventional RT-PCR
HAV was detected in six out of the eight samples (6/8, 75%) by conventional RT-PCR. Four of these positive samples were from the Tijuana River mouth and two were from the Imperial Beach pier (Table 14). The TSV spike was detected in five out of the eight samples (5/8, 62.5%).

The third rain Tijuana River mouth sample was concentrated using two different procedures; once with Whatman filter pretreatment and once without. The application of Whatman filter pretreatment produced an HAV positive result with

conventional RT-PCR, while in the absence of this pretreatment HAV was not detected.

Detection of HAV in Tijuana River Mouth and Imperial Beach Pier Samples by Real-Time RT-PCR

Real-time RT-PCR detected HAV in all four Tijuana River mouth samples (4/4, 100%) and in all four Imperial Beach pier samples (4/4, 100%). Therefore, all samples that had tested positive with conventional RT-PCR also tested positive with real-time RT-PCR. In addition, two samples that had tested negative with conventional RT-PCR, tested positive with real-time RT-PCR (Table 14). It was observed that some stock cDNA samples did not successfully amplify until they were diluted between 1:10 and 1:1000. The TSV spike was detected in seven out of the eight samples run with real-time RT-PCR (7/8, 87.5).

Comparison of Sensitivity of Conventional RT-PCR verses SYBR Green Real-Rime RT-PCR

The sensitivity of conventional RT-PCR and SYBR Green real-time RT-PCR were compared by using a serial dilution of HAV plasmid DNA as a template for amplification. The limit of detection for conventional RT-PCR was determined to be 24 copies of HAV. For SYBR Green real-time RT-PCR the limit of detection was one copy of HAV. A linear relationship was observed in real-time RT-PCR between the input plasmid DNA and the C_T values with correlation coefficients (r^2) greater than 0.99. The mean C_T values of replicate assays ranged from 19.57 ± 0.28 (1.1×10^5 copies) to 38.31 (1.1 copies) for the HAV standard curve. Therefore, the data indicated that SYBR Green real-time RT-PCR is eight-fold more sensitive than conventional RT-PCR when plasmid DNA was used as template.

Bacterial Concentrations of Samples

The concentrations of total coliforms, fecal coliforms, and enterococcus were determined for ocean water samples (Table 14) as described in Research Methods/Approaches. Analysis across rain events showed considerable variation. In general, the Tijuana River mouth samples had higher concentrations of bacteria than the Imperial Beach pier samples. The fecal coliform count in the Tijuana River mouth ocean water samples varied from 220,000 MPN per 100 mL to 500,000 MPN per 100 mL whereas the bacterial count in the corresponding pier samples varied from 400 MPN per 100ml to 90,000 MPN per 100 ml. Within a single rain event, the reduction of fecal coliform counts between the river mouth and pier sample varied from 3.3% to 550%. The three standards used in California to indicate coastal recreational water quality, as stipulated in State Assembly Bill 411, are as follows: 10,000 MPN per 100 mL (total coliforms), 400 MPN per 100 mL (fecal coliforms), and 104 MPN per 100 mL (enterococcus). Therefore, these results indicate that six out of six samples exceeded the fecal coliform indicator threshold (as measured by the Graduate School of Public Health, San Diego State University). Total coliforms exceeded the threshold in two out of three samples, fecal coliforms exceeded the threshold in two out of

three samples, and the enterococcus threshold was exceeded in three out of three samples (as measured by County of San Diego, Department of Environmental Health). In summary, two indicators in the second rain event pier sample were the only bacterial counts (out of the fifteen measured), which did not exceed a standard. At least one indicator exceeded the threshold in every sample measured.

CONCLUSIONS

The research objective was to develop a real-time RT-PCR method for HAV detection in ocean water. This was successfully accomplished by cloning HAV into a plasmid vector and designing/optimizing primers based on its sequence. HAV cDNA amplified by RT-PCR using RNA from an ocean water sample contaminated with Mexican sewage was sequenced, identity confirmed, and cloned into a plasmid vector. Then four primer sets were designed based on this sequenced HAV plasmid DNA. The primer sets were tested for amplification efficiency using the plasmid DNA as template, and the best primer set was chosen for subsequent work. This HAV plasmid DNA was also used to generate standard curves and to serve as a positive control in sample assays.

The second objective was to compare the sensitivity of HAV detection by conventional RT-PCR, to the sensitivity of HAV detection by real-time RT-PCR. The limit of detection for conventional RT-PCR was determined to be 24 copies of HAV and for real-time RT-PCR the limit of detection was found to be one copy of HAV. Therefore, the data indicate that real-time RT-PCR is eight-fold more sensitive than conventional RT-PCR when plasmid DNA was used as template.

There appeared to be a sufficient HAV load in the ocean waters surrounding the Tijuana River mouth and Imperial Beach pier following rain events to fall within the sensitivity range of conventional RT-PCR in most of the samples. However, the two samples that were negative by conventional RT-PCR either had lower HAV loads or greater concentrations of inhibitors, both of which required the increased sensitivity of real-time RT-PCR to amplify HAV. Amplification of HAV by real-time RT-PCR proved to require dilutions to minimize the effect of inhibitors, and therefore required the increased sensitivity to detect HAV at very low concentrations. Therefore, this real-time RT-PCR method has greater utility in determining more accurately the health risk associated with recreational waters.

RECOMMENDATIONS FOR FURTHER RESEARCH

As discussed throughout this report, this research can be expanded and improved by a variety of data enhancements. For the modeling part of this research, site-specific meteorological data may reduce the potential for model over-prediction near the Rio Alamar stream reach. Additionally, the inclusion of local evapotranspiration data and other hourly, not daily, data at the Campo Creek weather station would likely improve simulation results. Although data disaggregation from daily to hourly time steps is a commonly accepted practice in

hydrologic modeling, individual storms may be misrepresented, resulting in inaccurate rainfall intensities and durations.

Stream velocity and geometry along with other stream-specific hydraulic properties to populate the HSPF F-Tables would also improve future hydrologic simulations. Such data should be collected at various points along the stream reach and during different times of the year to incorporate stream geometry variation and seasonal fluctuations in the TRW. Simulation modeling in the TRW would also benefit from seasonal data on stream behavior regarding the fraction of flow entering the interflow and lost to deep groundwater, as well as the vegetative demand on water quantity (i.e. for evapotranspiration).

Data improvements to these local data sets are likely to improve the overall agreement of the hydrologic simulation results with observed data; however, the most dramatic effects in the TRW are likely to result from updating and verifying the stream flow data from the IBWC gage near the international border. This can be completed by performing statistical analyses to compare the IBWC data with all other known flow data sources on the Tijuana River near the international border. These sources include the SDSU flow gage at the Hollister Street Bridge in Imperial Beach and the USGS flow gage near Nestor, Calif., which stopped collecting data in 1982. Comparative analyses of these data sources can provide a monthly average flow for the Tijuana River, which can be used to supplement the IBWC data when the flow gage is malfunctioning as a result of sedimentation during storm events.

The flow simulations in the Tijuana River can be further improved by obtaining accurate historical flow data for both the Tecate WWTP and the Tecate Brewery. Since HSPF can accommodate different daily point source loadings, these data can provide a more realistic representation of the point sources in the watershed, thus improving the overall water balance.

Enhancements to stream flow simulations in the TRW will likely cause some improvements to modeled fecal coliform results; however, with the current fecal coliform loadings into the TRW, the effects will be minimal. Therefore, to improve the simulation results, more frequent bacteriological monitoring data are necessary at a variety of locations along the stream reach network in the TRW. This monitoring should include a consistent and frequent sampling schedule, as well as samples collected continuously over particular storm events. This will allow for a time-series comparison with modeled output at various locations, thus improving model calibration. Additionally, this information can be used to predict the presence of a first flush and to simulate the fecal coliform concentrations over an entire storm event. Hourly streamflow data are also necessary to accurately predict flow rates and water quality over specific storm events.

For this research on the PCR detection of viruses, further investigation is necessary to extend real-time RT-PCR methods to detection and quantitation of

a whole range of viruses in ocean water. The exquisite sensitivity, quantitative ability, and high throughput utility offered by real-time RT-PCR to monitor recreational waters is unparalleled by current methods. Further improvements need to be made to increase viral recovery from water samples and reduce inhibitor recovery during the viral concentration process. Viral concentration methods should also aim to decrease the number of steps in order to decrease viral loss and increase time efficiency. Lastly, further epidemiological studies should be performed to address the human health risk associated with recreational coastal waters that receive urban runoff. Information gained from such epidemiological studies is necessary for an accurate assessment of the health risk associated with this type of recreational water contamination.

PROBLEMS/ISSUES ENCOUNTERED

There were no unforeseen problems or issues in this project that were beyond the scope of those normal in the course of doing applied research.

RESEARCH BENEFITS

The major benefit of this research on BASINS modeling of fecal coliforms in the TRW is the development of a predictive model of coliform sources and loading to the southern California bight, which can be of great value to policy- and decision-makers in the region for choosing between alternative sewage infrastructure investment scenarios. The PCR research on the detection of human-specific viruses, such as HAV, resulted in the development of a new real-time PCR method to detect HAV in ocean waters, which is a novel addition to the scientific field of environmental microbiology. This method, which is sensitive, relatively rapid six hours) and highly specific for selected viral pathogens, will allow in the future a much more accurate human health risk assessment for bathing in contaminated ocean waters such as at Imperial Beach.

Publications that have been published or submitted as a result of this project include:

Gersberg, R.M., Pitt, J., King, A., Johnson, H. and R. Wright. 2000. “Use of the BASINS Model to estimate the loading of heavy metals from the binational Tijuana River watershed.” Watershed 2000 Specialty Conference, Water Environment Federation, 9-12 July, Vancouver, British Columbia.

Brooks, H.A., Gersberg, R.M. and A.K. Dhar. Quantification of Hepatitis A virus in seawater using real-time RT-PCR. Submitted to *Applied Environmental Microbiology*.

This project contributed significantly to the education and training of a number of graduate students including J. Pitt and H. A. Brooks, who are referenced above.

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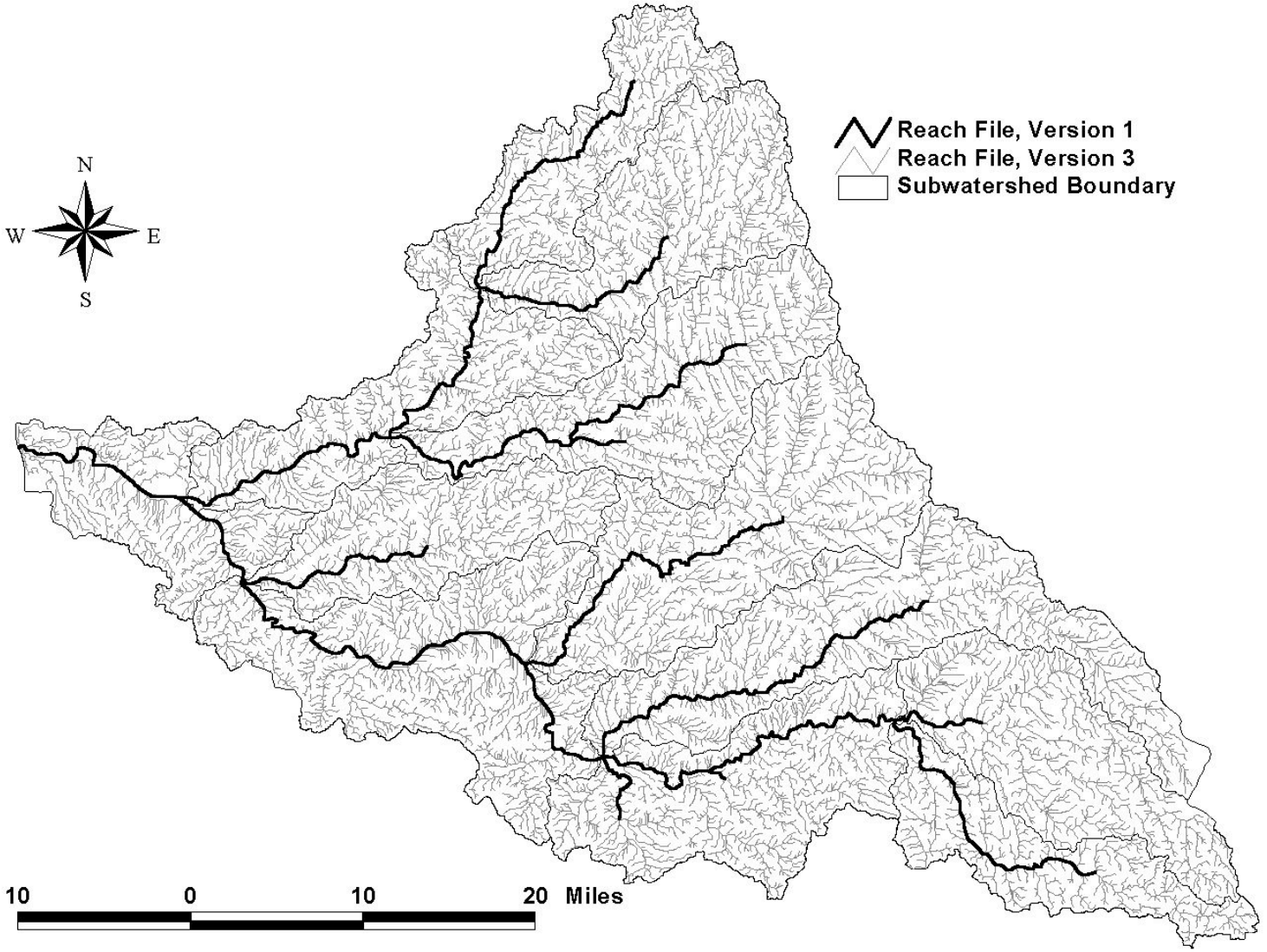

Figure 1. Stream Network in the Tijuana River Watershed

Figure 2. Original and Interior Subwatersheds in the Tijuana River Watershed

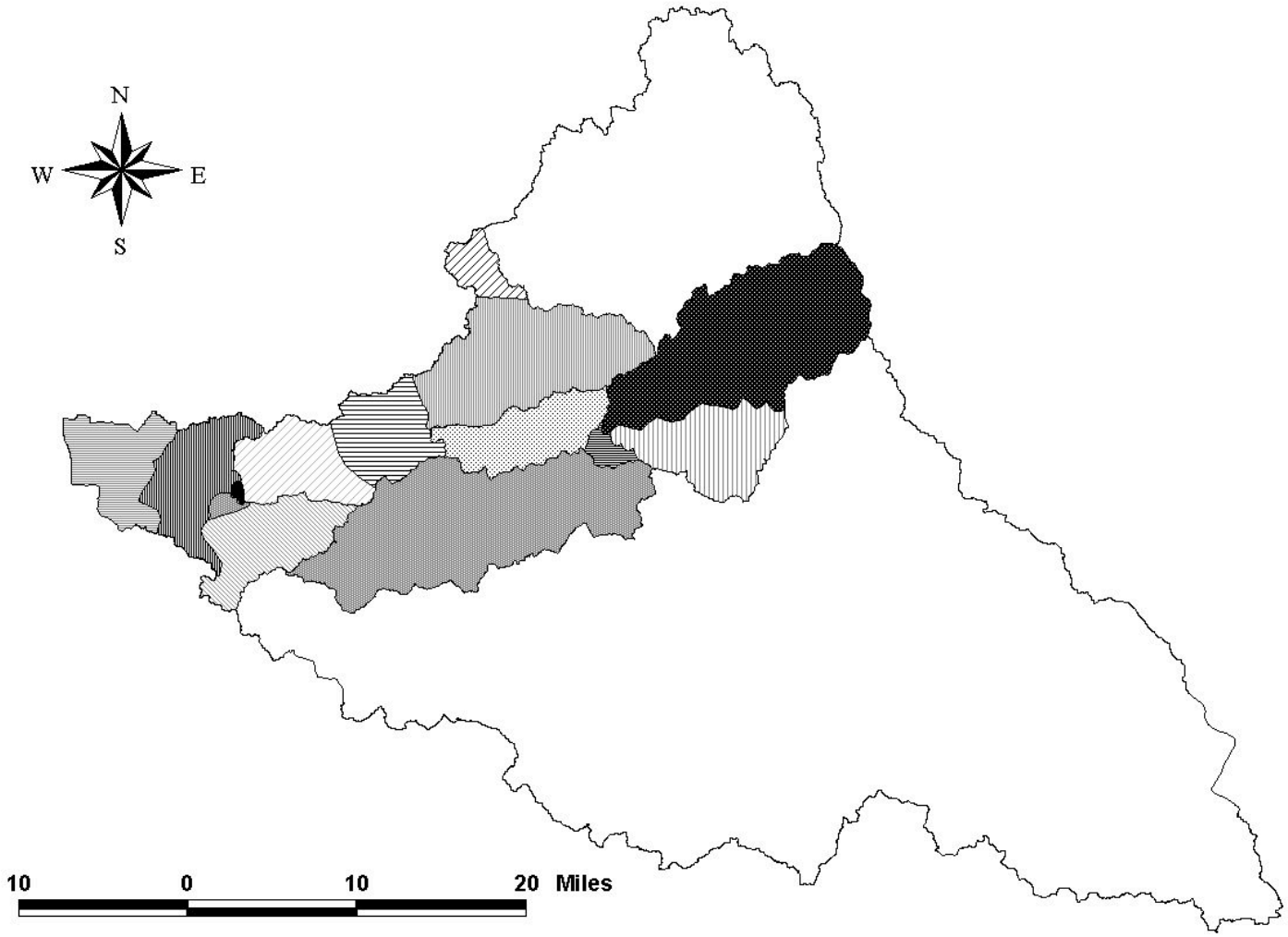

Figure 3. Delineated Interior Subwatersheds in the Tijuana River Watershed (shading represents a pseudo-distributed modeling approach in which each shade signifies different parameter values)