Identification and Application of Physical and Chemical Parameters to Predict Indicator Bacterial Concentration in a Small Californian Creek

Hun-Kyun Bae[1]*, Betty H. Olson[1]*, Kuo-Lin Hsu[1], Soroosh Sorooshian[1]

ABSTRACT: This study of Aliso Creek in California aimed to identify physical and chemical parameters that could be measured instantly to be used in a model to serve as surrogates for indicator bacterial concentrations during dry season flow. In this study, a new data smoothing technique and ranking/categorizing analysis was used to reduce variation to allow better delineation of the relationships between adopted variables and concentrations of indicator bacteria. The ranking/categorizing approach clarified overall trends between physico-chemical data and the indicators and suggested sources of the bacteria. This study also applied a principle component regression model to the data. Although the model was promising for predicting concentrations of total and fecal coliforms, it was somewhat weaker in predicting enteroccocci. *Water Environ. Res.,* **81,** 633 (2009).

KEYWORDS: indicator bacteria, principal component analysis, physical and chemical parameter.

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Introduction

The quality of water in coastal areas can affect human activities and natural ecosystems. Reports raising water quality concerns in coastal and beach areas coupled with large-scale studies of California's beaches have focused on indicator bacteria contamination of the surf zone. This contamination is the main public health concern to state and coastal water quality agencies and managers in charge of protecting beachgoers from exposure to disease (Ha and Stenstrom, 2003; Grant et al., 2001; Reeves et al., 2004; Schiff and Kinney, 2000; Steets and Holden, 2003; Noble et al., 2003). The state has invested substantial resources in monitoring programs to ensure beach water quality. The monitoring system, however, which relies on grab samples and culture methodologies for indicator bacteria concentrations, is labor intensive and does not provide real-time data because results take 18 to 24 hours. Because 70% of bacteria in waterbodies is naturally removed within 24 hours, beach closures are often issued after the fact (Christen, 2002; State Water Resources Control Board, 2001). Methods that provide bacterial concentrations in timely manner are needed to manage water quality effectively.

[1] Henry Samueli School of Engineering, Department of Civil and Environmental Engineering, University of California, Irvine, California.

* Henry Samueli School of Engineering, Department of Civil and Environmental Engineering, University of California, Irvine, CA 92697; e-mail baeh@uci.edu or bholson@uci.edu.

In addition to timely results, identification of pathogens and management and treatment of sources of pollution are also essential to maintaining water quality within federally mandated standards. Grant et al. (2001) recommended the management of coastal wetlands to protect beach water quality after finding high concentrations of enterococci (ENT) in urban runoff, bird feces, marsh vegetation, and sediments. Reeves et al. (2004) focused on source tracking of indicator bacteria pollution to urban runoff, particularly residential runoff, from inland areas to Talbert Marsh in Orange County, California. Schiff and Kinney (2000) found sources of indicator bacteria were diffuse and widespread throughout the entire upper watersheds in their study of Mission Bay in San Diego, California. Indicator bacteria exceeded California water quality objectives regardless of land use type within the watersheds. Managing bacterial contaminations at the source presents managers with several challenges. However, water quality monitoring systems focus on the surf zones of beaches. These studies and current monitoring systems suggest that the state needs to develop a rapid method to predict indicator bacteria concentration based on monitoring results from upland areas to beach areas.

This study investigated the relationships between bacterial concentrations and other water quality factors that could serve as surrogates for rapid detection of indicator bacteria concentrations based on the Aliso Creek Watershed in California. The study then estimated indicator bacteria concentrations upstream of a beach using those identified surrogates. Principal component regression (PCR) was applied to estimate indicator concentrations once the orthogonal variables were identified by principal component analysis (PCA). The application of PCA to PCR is an extension of this approach in environmental engineering that previously had been adopted only to discriminate pollution source apportionment (Masunaga et al., 2001; Ozeki et al., 1995; Ehrlich et al., 1994; Kennicutt et al., 1994; Buck et al., 2005; Papa et al., 2007; Pejcic et al., 2007). The PCR approach is particularly important in this research project because the physical and chemical factors produce additive, opposite, or synergistic effects on indicator bacteria concentrations. The results indicated that surrogates could be used to predict bacterial concentrations and could help improve water quality management.

Methodology

Site Description. The Aliso Creek Watershed covers 78.7 km^2 (30.4 mi^2) in southern Orange County, California. Its main

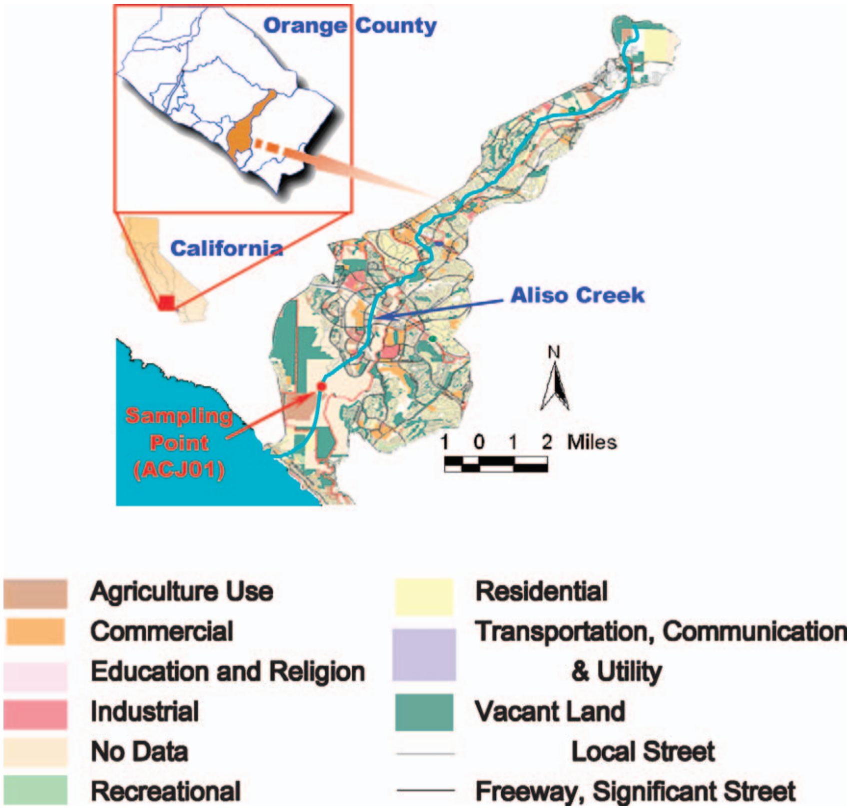

Figure 1—Study area, Aliso Creek Watershed, California (delineated using ArcView GIS 3.3 version and Automated Geospatial Watershed Assessment; red circle shows the water quality monitoring station (ACJ01); light blue line indicates the main tributary of watershed. The watershed consists of ten different land uses; the outlet of Aliso Creek is directly connected to Aliso Beach.).

tributary, Aliso Creek, originates in the Santa Ana Mountains and extends to Aliso Beach, the Regional Water Quality Control Board has identified as an ''impaired'' stream for water quality problems. The board's Reconnaissance Study of Aliso Creek also noted substantial issues with channel degradation, geotechnical instability, loss of natural habitat and recreation opportunities, and potential for flooding (Tetra Tech Inc., 2005). This watershed includes several highly urbanized cities comprising commercial, industrial, and residential areas and agricultural and undeveloped areas. Thus, natural and anthropogenic sources could be generating nonpoint source contaminants in this watershed. Water from inland areas could affect the water quality of Aliso Beach because the main tributary of the watershed is directly connected to the beach. Figure 1 shows the study area and its land use.

Selection of Physical and Chemical Data for Trend Analysis. A new technique—ranking/categorizing analysis—was developed for trend analysis because raw data showed complicated patterns. For the ranking/categorizing analysis, first the data was ranked in respect to one of the independent variables and all other variables were rearranged in ascending order to match. Second, the data were categorized in uniform interval increments of the ranked variable's unit. For example, every 2 mg/L for dissolved oxygen or every 5 nephelometric turbidity units (NTU) for turbidity. After grouping, geometric mean values for each group were calculated. Finally, the geometric means of the independent and the dependent variables were plotted to find the relationship between two variables. Using this technique, extremes in the data are moved toward mean values generating a new graphical expression in which relationships are more clearly delineated.

Principal Components Analysis and Principal Components Regression. In multivariate statistics, visualizing multidimensionality can be difficult. In multidimensional dataset, groups of variables sometimes move together because more than one variable may be measuring the same driving principle governing behavior of the system. When this happens, we can take advantage of this redundancy of information by replacing a group of variables with a single new variable. Principal components analysis is one method to achieve this simplification to find patterns in a high-dimensional dataset. The method can capture variance in a dataset in terms of principal components—which are a set of orthogonal variables—so that they are uncorrelated and define a projection that extracts the maximum amount of variation. The first principal component is a single axis that lies on the data with maximum variance and generates a new variable by projecting each observation on that axis. The second principal component is an axis that is perpendicular to the first axis and generates another new variable with the second largest data variance. The full set of principal components is the same size as the original set of variables. However, the data can be compressed without much loss of information by reducing the number of dimensions and summarizing the most important parts of the data. Overall, PCA is a powerful tool for analyzing and expressing multidimensional data while highlighting similarities and differences; patterns in multidimensional data sets, however, could be hard to find (Smith, 2002; Hsu et al., 2002).

Results and Discussion

Relationships between indicator bacteria and each physical and chemical parameter were investigated. Relationships between physical and chemical parameters and bacterial concentrations and growth have been reported for both laboratory and field studies. Lindqvist (2006) measured growth of bacteria using turbidity; Augustin et al. (1999) studied temperature, and Toit et al. (2000) studied pH. Watier et al. (1996) modeled effects of temperature, pH, and ethanol concentrations on growth kinetics of the microorganism *Pectinatus* sp. Field work by Byamukama et al. (2005) investigated the relationships between two different fecal indicators, *E. coli* and *Clostridium perfringens*, and five chemophysical parameters—dissolved oxygen, pH, temperature, electrical conductivity, and total suspended solids (TSS). Auer et al. (1993) and Steets et al. (2003) reported that particle size and sediments have strong relationships with indicator bacteria.

Because Orange County Watershed and Coastal Resources Division (OCWCRD) collects water quality data, it would be ideal if these data could be used as surrogates to predict periods of indicator bacteria violations. The physical and chemical parameters for this study included dissolved oxygen, turbidity, streamflow, pH, and temperature because they often are related bacterial growth or concentration in water. Water quality samples were taken during dry season from May to September from 2003 to 2005 at a sampling station (ACJ01). Truncated indicator bacteria data (greater than or less than detection limits) were removed along with the corresponding data points for ranking/categorizing analysis because their exact values were unknown. Cyclical patterns of varying periodicity and magnitude are shown for each of the parameters, but all of the temporal representations were somewhat obscured by variation within the data sets. Figure 2 shows the raw data.

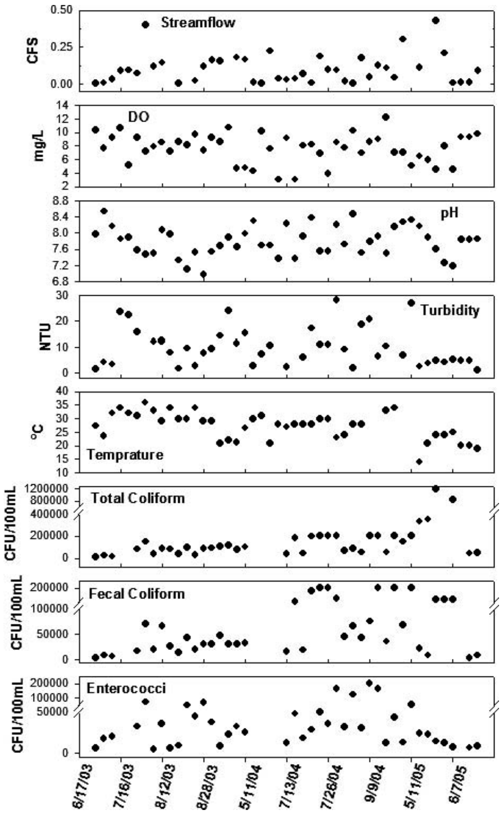

Figure 2—Physical, chemical, and biological data for Aliso Creek Watershed (total coliform during 2004 contained truncated data because the dilution series used did not allow for more than 100,000 CFU/100 mL; sampling in 2005 for total coliform was adjusted to cover higher concentrations of indicator bacteria).

Physical and Chemical Parameters Versus Bacterial Concentration. Removing the temporal aspect of the data emphasized the magnitude of variability for several parameters examined. Figure 3 shows compares indicator bacteria data and raw data of physical and chemical parameters. As shown, variation made it difficult to find relationships between indicator bacteria and physical and chemical parameters. The data sets required smoothing because of variability between instantaneous measures and culture-and-grab samples and weak, biphasic associations. As a result, the ranking/categorizing analysis was used. The ranked data sets were grouped by intervals based on physical or chemical parameters, and the mean value within the corresponding bacterial grouping was computed to reduce the effect of high variability. For example, to find the relationship between total coliform and dissolved oxygen, the dissolved oxygen data were ranked in ascending order and each total coliform value moved with the dissolved oxygen value. Then, the dissolved oxygen data were categorized in uniform interval increments of 2 mg/L, and geometric mean values were calculated for both dissolved oxygen and total coliform data falling within each interval. Finally, geometric means of the independent variable, dissolved oxygen, and the dependent variable, total coliform, were plotted. Raw total coliform and dissolved oxygen data showed a clear, but negative, relationship after the ranking/categorizing method was applied (Figure 4a). In some instances, such as ENT and dissolved oxygen, outliers in the raw data made interpretation of the trend difficult (Figure 3c). These relationships, however, also were clarified after the ranking/categorizing methodology was applied to pH and temperature for all indicator bacteria. The results of applying this smoothing technique to all physicochemical variables and indicator bacteria are shown in Figure 4.

Figure 4a, 4b, and 4c shows a negative relationship between dissolved oxygen and indicator bacteria concentration. As expected, total coliform and fecal coliform showed the same negative pattern with dissolved oxygen (fecal are a subset of total coliform). The relationship between ENT and dissolved oxygen was slightly different; the negative effect of oxygen was not observed until dissolved oxygen was greater than 7 mg/L (threshold effect). These results for dissolved oxygen reflect the role of oxygen in the metabolism of each group. Aerobic, anaerobic, facultative, microaerophilic, and aerotolerant anaerobic bacteria span a continuum from oxic to anoxic metabolism. Because total coliform, fecal coliform, and ENT are facultative anaerobes with a preference for a fermentative metabolism, the biology of the organisms correspond with the data showing negative relationships. As would be expected, total coliform and dissolved oxygen showed a weaker negative

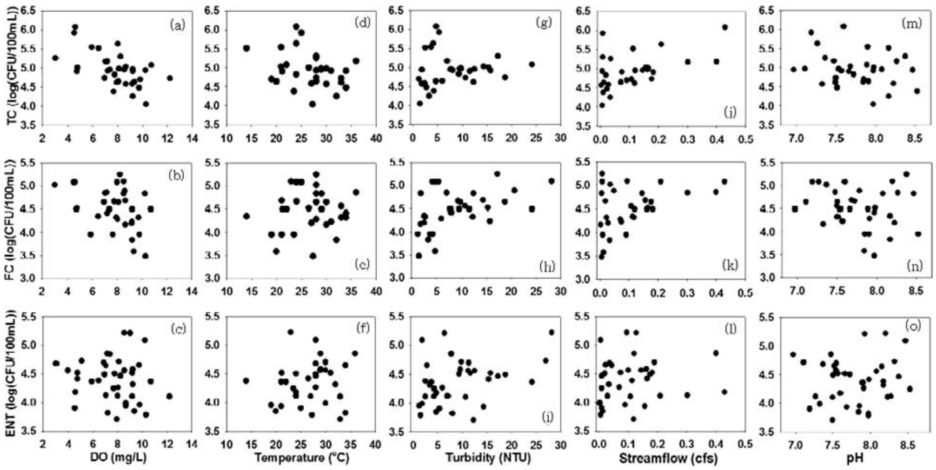

Figure 3—Raw data obtained for indicator bacteria and physical and chemical parameters at location ACJ01 in the Aliso Creek Watershed.

relationship compared to fecal coliform or ENT and dissolved oxygen. This difference is because total coliform grow in a broader range of environments, including plant matter, soil, and animal intestines. Low redox potential of the gut would suggest a negative association with oxygen; in fact, the two fecal bacteria (fecal coliform and ENT) have a stronger fit than the total coliform group.

Because organisms associated with soil and plant materials are adapted to lower temperatures than those of warm blooded organisms (fecal coliform and ENT), the effect of increasing temperature were inversely related to concentration (see Figures 4d, 4e, and 4f). Optimum temperature for environmental strains of bacteria in temperate zones is approximately 20°C. In Figures 4d, 4e, and 4f, the difference in characteristics between bacteria of fecal origin (coliform and ENT) and those of mixed origin (total coliform) is reflected in temperature results. Fecal coliform and ENT were proportionally related to temperature; whereas, total coliform were inversely related. Faust et al. (1975) reported that temperature was a significant parameter that could affect the rate of decline of *E. coli*. At low temperatures (approximately 9°C), multiplication of bacteria did not occur and the rate of decline in cell number was slow; at

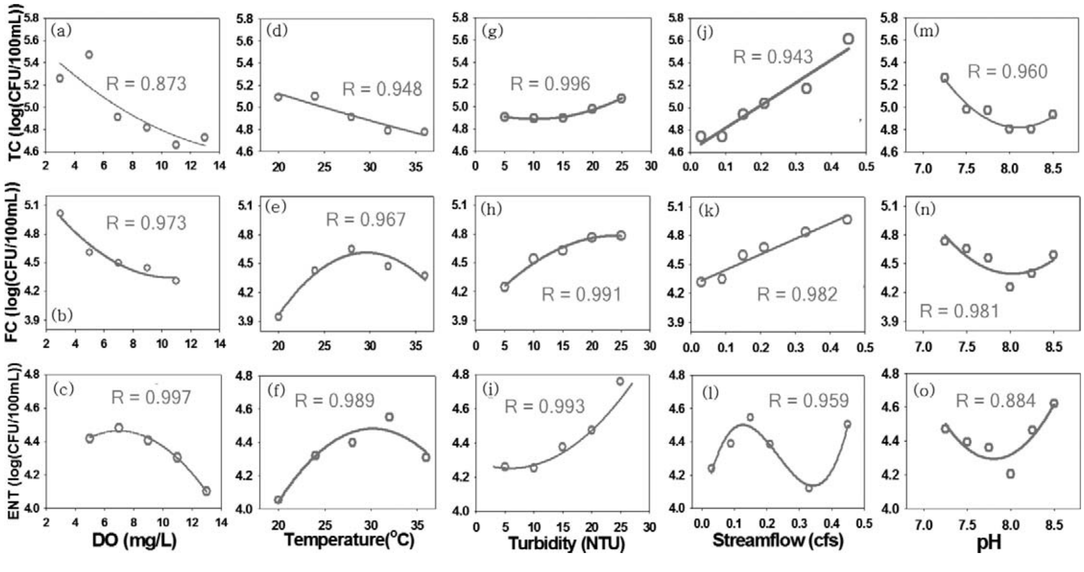

Figure 4—The results of ranking/categorizing analysis (relationships of indicator bacterial concentrations at ACJ01 in Aliso Creek Watershed after application of ranking/categorized means technique; coefficient of correlation values indicate the relationships among indicator bacterial mean concentrations linked with ranked and categorized mean values of physical/chemical parameters).

medium temperatures (approximately 17°C), the number of bacteria increased during the first 24 hours; and at the high temperatures (approximately 28°C), the decline in cell numbers started immediately, and the rate of decline was rapid. Jiang et al. (2007) reported that fecal bacteria grew in dry weather runoff water from Aliso Creek at approximately 21°C. Results of these works support the outcome of this study, especially for fecal coliform and ENT versus temperature. Total coliform didn't follow fecal coliform results because soil and plants survive well at ambient temperatures. Fecal coliform and ENT showed similar patterns relative to temperature, which is consistent with the work of Faust (1975). Total coliform contains bacteria of environmental and animal origin and will vary depending upon which is the dominant portion. Total coliform in this study probably came from the storm drain from irrigation of residential areas, whereas fecal coliform and ENT came from animal feces.

At higher temperatures, which are associated with increased sunlight, die off would be expected because of increased UV radiation. Increased metabolic activity linked to temperature may also result in more rapid die-off because of starvation related to metabolic rates. Fujioka et al. (1981) reported that fecal coliform and fecal streptococci in seawater were inactivated within 1 to 3 hours in the presence of sunlight (solar radiation measurements were between 415 and 703 $cal/cm^2 \cdot d$), whereas these organisms survived for days in the absence of sunlight. This study data agreed with this finding as fecal coliform and ENT decreased at higher temperatures. Much of the creek receives direct sunlight because the creek is not lined with trees.

Concentrations of all indicator bacteria increased as turbidity increased (see Figures 4g, 4h, and 4i). Fecal coliform and ENT showed better relationships with turbidity than total coliform, although an approximately log 1.0 (CFU/100 mL) increase in total coliform is observed as turbidity increases from 5 NTU to 25 NTU. The initial increase in slope for ENT was low compared to that of fecal coliform, which had a more rapid rate of increase. On the other hand, at the highest turbidity levels, fecal coliform reached an asymptote, whereas ENT appeared to increase steadily. These differences may relate to different types of source inputs. The fact that bacteria had a positive relationships with turbidity has been reported in the literature (Steets and Holden, 2003; Byamukama et al., 2005; Auer et al., 1993; Faust et al., 1975).

The association with particles and sediments has been shown to relate to survival rate. The strong positive relationships between turbidity and bacterial concentrations in this study are reasonable results and may precipitate after entering sea water. Fecal indicators associated with particles that settled during low-flow periods could be released to the waterbody by resuspension of sediments from activities such as swimming or boating. More data would be required to determine if there is actually a trend between these two variables. However, relationships between total coliform concentrations and turbidity have been demonstrated during storm events, although concentration of both are much greater (Noble et al., 2003).

Total coliform and fecal coliform showed a strong positive relationship to streamflow (Figures 4j and 4k), but ENT had a different pattern for which there is no biological explanation (Figure 4l). Hence, no interpretation should be made of the relationship between stream flow and ENT concentrations. However, positive relationships between contaminants and streamflow can be predicted easily if runoff from inland sources contains contaminants from dry deposition and irrigation practices. Increasing streamflow also may cause the resuspension of sediment in streambeds to which contaminants can be adsorbed or absorbed. Based on this, bacteria should have a positive relationship with streamflow. The ENT, however, showed a sinusoidal pattern with streamflow, which might reflect dual inputs: directly into the stream and via runoff. These results might explain spikes in ENT concentrations, which were unrelated to those of total coliform and fecal coliform in this study. Further support comes from densities of fecal streptococci in bird feces, which have been found to be 40 to 1000 times that of fecal coliforms (Gray, 2004). Jiang et al. (2007) found that bird biomarkers were a significant source of fecal pollution in Aliso Creek. Grant et al. (2001) also has reported high concentrations of bird waste entering coastal beaches from wetland drainage.

Although variations in pH are shown in the data, the literature suggests that survival for this group of organisms should not be affected at pH greater than 7 to 8.5. The pH values for this study remained within this range. Polynomial relationships were displayed for all indicator bacteria. Toit et al. (2000) reported that *Enterococci* species grew from 3 to 10 pH and maximized growth between 8 and 9 pH. The relationships derived from the ranking/categorizing analyses suggest the following hypotheses: total coliform in Aliso Creek came from natural systems such as soil and plants, whereas fecal coliform and ENT came from warm-blooded animals. Total coliform and fecal coliform most likely came from dry-flow runoff, whereas ENT appears to come from both direct deposition and dry flow runoff.

Multi-Variables of Water Quality Versus Bacterial Concentration. In this section, the relationships between each indicator bacteria group and multiple physical and chemical parameters were investigated. Single variables of physical and chemical parameters showed good relationships with bacterial concentrations using their grouped mean values and the relationships could be used for tracking origins of each indicator bacteria. However, the relationships between single variables and indicator bacteria concentration obtained from the ranking/categorizing analysis were not sufficient to use for prediction purposes because no single variable appeared dominant. Considering multiple parameters simultaneously was, therefore, another option to augment the difficulties of single parameter analysis. Some physical and chemical parameters can be related to each other, and sometimes in inverse relationships such as dissolved oxygen and temperature. As a result, multiple variable relationships between indicator bacteria concentrations and physical and chemical parameters were investigated.

The PCA was adopted to investigate the effects of multiple physical and chemical parameters on bacterial concentrations and to determine whether the number of dimensions for input variables could be reduced. Additionally, PCA reduced any co-linearity that may exist between variables and the number of dimensions without losing important data. Both cases were used for principal component regression approaches. Although each principal component showed different variances, none had significantly high variance. Therefore this study tested two cases; one considered all the principal components that satisfied more than 95% of total variances; the other considered the first four principal components that satisfied more than 90% of total variances. The variances were: first principal component with 35.1%; second principal component with 25.3%; third principal component with 19.9%; four principal component with 10.8%; and fifth principal component with 8.9%.

The principal component analysis showed that pH and temperature were the most important variables of the five in the first principal component. Streamflow and dissolved oxygen were the most important in the second principal component. And turbidity

Table 1—Eigenvector and eigenvalue of each principal component.

		1st principal component	2nd principal component	3rd principal component	4th principal component	5th principal component
weight	Streamflow (cfs)	−0.3798	0.6818	−0.0400	0.0040	−0.6239
	Dissolved oxygen (mg/L)	0.2939	0.7102	0.0751	0.2258	0.5939
	pH	−0.5856	0.0529	0.4444	−0.5573	0.3823
	Turbidity (NTU)	0.3457	−0.0290	0.8797	0.1313	−0.2976
	Temperature (^{o}C)	−0.5540	−0.1647	0.1464	0.7881	0.1529
Eigenvalue		1.7581	1.2677	0.9940	0.5385	0.4418

and pH were the most important in the thrid principal component (Table 1). Hence, none of the variables was dominant in all the components. This result explains why all five components are necessary for the principal component regression analysis.

Figure 5 shows the results from both PCR tests for each indicator bacteria. Figure 5a, 5c, and 5e shows the results of PCR using all principal components (100% of total variances); Figure 5b, 5d, and 5f shows the results using the first four (91%). Root mean squared error (RMSE) of each case was measured as an objective function; each result indicated that estimations from PCR using all principal components were slightly better than those using the first four principal components. The only exception was ENT, in which there was no difference (RMSE = 0.32). The RMSEs for estimation from PCR using all principal components for 100% of total variances were 0.42 for total coliform and 0.39 for fecal coliform. Those using the first four principal components for 91% of total variances were 0.49 for total coliform and 0.46 for fecal coliform. Error values were within the range of 7 to 10% of the average value of actual indicator bacteria concentrations, 4.95 log (CFU/cell) for total coliform; 4.50 log (CFU/cell) for fecal coliform; and 4.39 log (CFU/cell) for ENT would be considered to be a good representation of the actual data. The RMSE did point out differences in the fit between the regression models and observations. Therefore, RMSE cannot adequately address whether the model predicts extreme values which, would be important in determining if PCR would be useful in instantaneously predicting violations in standards. In this respect, PCR using all principal components corresponded better with extreme changes in concentrations (high or low peaks) for total coliform and fecal coliform than

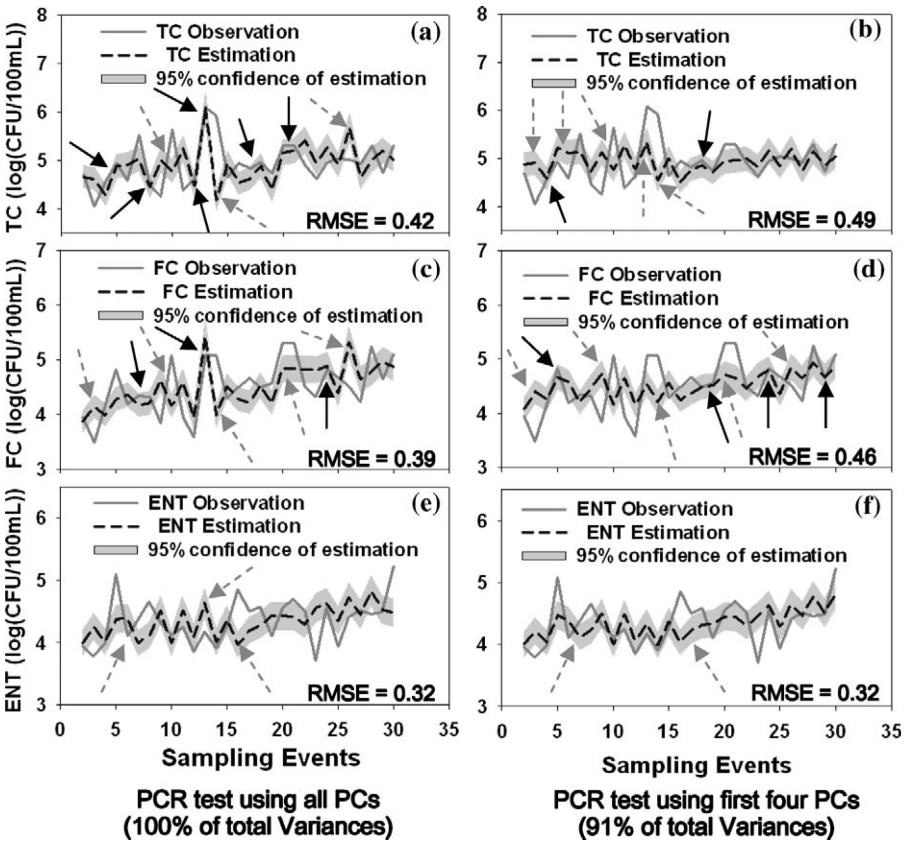

Figure 5—Results of principal component regression.

did the four principal components. The objective function (RMSE), however, showed no significant difference between estimations using all principal components and those using the first four principal components. This is significant, because standards for fecal indicators are based on an excess established concentration. As shown in Figure 5, black arrows indicated estimations that closely followed changes in actual bacterial concentrations, and red dotted arrows indicated over- or under-estimations of actual bacterial concentrations. Furthermore, PCR estimations using all five principal components for total coliforms indicated that 52% of actual concentrations fell within the 95% confidence interval of estimations (Figure 5a); whereas, using the first four principal components resulted in 28% of total coliform concentrations within 95% confidence intervals (Figure 5b). For fecal coliform estimations (Figure 5c and 5d), 45% of actual events fell into 95% confidence areas for both cases. For ENT estimations (Figure 5e and 5f), both cases of the PCR test showed similar results, which means the dimensions of the PCR could be reduced to four. Neither estimation for ENT, however, predicted extreme events. Overall, total coliform PCR estimations were the best among the three different indicator bacteria.

Conclusion

Clear relationships for physical and chemical parameters versus indicator bacteria concentrations from raw data were difficult to identify because biological variables respond differently as variables change. The new technique developed in this study, ranking/categorizing analysis, however, generated clear patterns that could be used to track the factors that most influenced indicator bacteria concentrations. The authors hypothesize that total coliform in Aliso Creek came from natural systems such as soil and plants, whereas fecal coliform and ENT came from warm-blooded animals. This suggests that multiple parameters that could be measured instantaneously also are useful in predicting indicator bacteria concentrations.

Using PCR with five physical and chemical parameters showed reliable estimations for indicator bacteria and could be used to rapidly identify the indicator bacteria concentration in a waterbody. The application of PCR would require measurement of all five physical and chemical variables, which easily could be obtained through online measurements and submitted to automated modeling programs. The PCR approach was promising for predicting concentrations of total coliform and fecal coliform, but was somewhat weaker in the prediction of ENT. The analysis also suggested that different sources were responsible for the occurrence of these two groups of indicator bacteria, which could explain the reduction in prediction reliability in the case of ENT. Additional studies with larger data sets are needed to validate these findings in physical and chemical parameters and bacterial concentrations in dry season flow. Data from wet seasons also should be examined.

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