

## **DRY WEATHER BACTERIA MONITORING AND VARIATION WITH LAND USE FOR KRANJI RESERVOIR CATCHMENT, SINGAPORE**

**Lloyd H. C. Chua<sup>(1)(3)</sup>, Peter Shanahan<sup>(2)</sup>, Edmond Y. M. Lo<sup>(1)</sup>,  
Eng Ban Shuy<sup>(1)</sup>, Janelle Thompson<sup>(2)</sup>, Cameron C. Dixon<sup>(2)</sup>,  
Kathleen B. Kerigan<sup>(2)</sup>, Jean Pierre Nshimiyimana<sup>(2)</sup>, Jessica M. Yeager<sup>(2)</sup>,  
Li-Jun Lee<sup>(1)</sup>, and Yu-Ling Por<sup>(1)</sup>**

1. Department of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798.
2. Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139.
3. Tel: +65 67905249. Fax: +65 67921650. Contact email: hcchua@ntu.edu.sg.

### **Abstract**

An initial assessment of bacteria concentrations under dry weather conditions was made for the Kranji catchment in Singapore. Under Phase 1 of the study, high counts of *E. coli* and Enterococci were observed at 5 sampling locations, which were monitored for a period in excess of 1 year. The USEPA (1986) guideline concentration for *E. coli* of 235 per 100ml was exceeded for almost all samples collected. Comparing between the 5 sampling locations, it was found that *E. coli* and Enterococci concentrations were highest at KC02, which had a sub-catchment with the highest percentage development, compared to the other 4 monitoring sites. Limited storm sampling showed evidence of elevated concentrations of *E. coli* and Enterococci contained in storm runoff. Phase 2 of the study saw an expansion of the monitoring program to obtain a wider spatial coverage of sites being monitored. The drainage system upstream of KC02 was progressively sampled which led to the identification of a possible source, where an *E. coli* concentration of  $2 \times 10^6$  per 100ml was measured about 1.4 km along the drainage network, upstream of KC02. An analysis of the data obtained from Phase 2 showed that *E. coli* varied according to land use, in increasing order according to the following sequence of land use categories: Undeveloped → Farm → Residential → Sewage Treatment Plant (STP). DNA analysis revealed that the locations where the human factor (HF) marker was detected generally corresponded with locations at which high coliform concentrations were found and which were known or suspected to be associated with sanitary wastewater. Nonetheless, numerous locations at which coliform counts were well above USEPA water-quality criteria often did not show human factor presence. Others have questioned whether coliform may grow naturally in tropical waters and more analyses are needed to investigate the possible contributors to observed coliform counts in Kranji catchment.

**Key Words:** Dry Weather, Bacteria Monitoring, *E. coli*, Enterococci, Land use.

### **1. INTRODUCTION**

A proposal for improvements to the Kranji Reservoir has recently been made under the Western Catchment Masterplan (PUB 2008). These improvements will result in the expansion of activities in the reservoir and the waterways within the catchment to include activities such as kayaking, fishing, barbecue, and picnic activities. There is thus a need to quantify the levels of bacterial contamination within the catchment and bacteria loading to the reservoir and waterways before activities that involve higher degrees of contact with the water can be introduced. An investigation was thus undertaken to study bacteria loadings from the Kranji catchment area. It is envisaged that the results of this study will guide the regulatory bodies in management efforts to reduce overall bacteria loading.

Many studies on bacteria loading into receiving waterbodies have focused on storm runoff, since runoff has been found to contain elevated amounts of pollutants. Indeed, bacteria loading to streams in urbanized areas have been identified as one of the most common pollution sources affecting aquatic

systems since urbanization results in increased surface runoff and pollutant accumulation on impervious surfaces (Schueler, 1994). However, other studies (Peterson et al., 2005; Stein and Tiefenthaler, 2005) have also shown that dry weather flow can be a significant contributor to total load as well. This is understandable since dry weather flow can contribute as much as or even more of the total flow to a receiving waterbody than storm flows and bacteria concentrations in dry weather flow can have an important impact on the overall loading. This consideration has led to the present study to monitor bacteria concentrations in the drainage system and effluent from several sewage treatment plants in the Kranji catchment during dry weather. Additional studies of bacteria loadings during wet weather are planned as a future study.

Study of pathogen loading impacts on water quality incorporating indicator bacteria such as *Escherichia Coli* (*E. coli*) or Enterococci is important when recreational water quality is a concern. Although fecal coliforms have been used as indicator bacteria for pathogens for recreational water quality monitoring, studies have shown that gastrointestinal disease correlates much more strongly with *E. coli* and Enterococci as compared to fecal coliform bacteria (Bartram and Rees, 2000). On the other hand, progress in molecular biology has allowed microbiologists to increase precision in identifying bacteria pollution sources using Deoxyribonucleic acid (DNA) Polymerase Chain Reaction (PCR) based analysis. DNA PCR based analysis is now increasingly used in identifying nonpoint sources of bacteria in polluted water (Bernhard and Field, 2000). Recently, Santoro and Boehm (2007) used DNA based methods to study the occurrence of human-specific *Bacteroides* fecal marker (HF marker) at an open-coast marine beach in California. The authors found molecular genetic markers from fecal *Bacteroides Prevotella* microorganisms to be useful in linking bacterial pollution to their sources. This bacterial group is found only in human and animal guts (Fogarty and Voytek, 2005) and in contrast to *E. coli* and Total Coliforms cannot grow in oxygenated sediments and surface waters at tropical temperatures. This traceable characteristic makes the *Bacteroides Prevotella* group suitable as an indicator of sources of bacteriological water pollution in tropical environments where environmental growth of *E. coli* and Total Coliforms may confound results (Rivera, 1988; Jensen, 2001; Winfield, 2003).

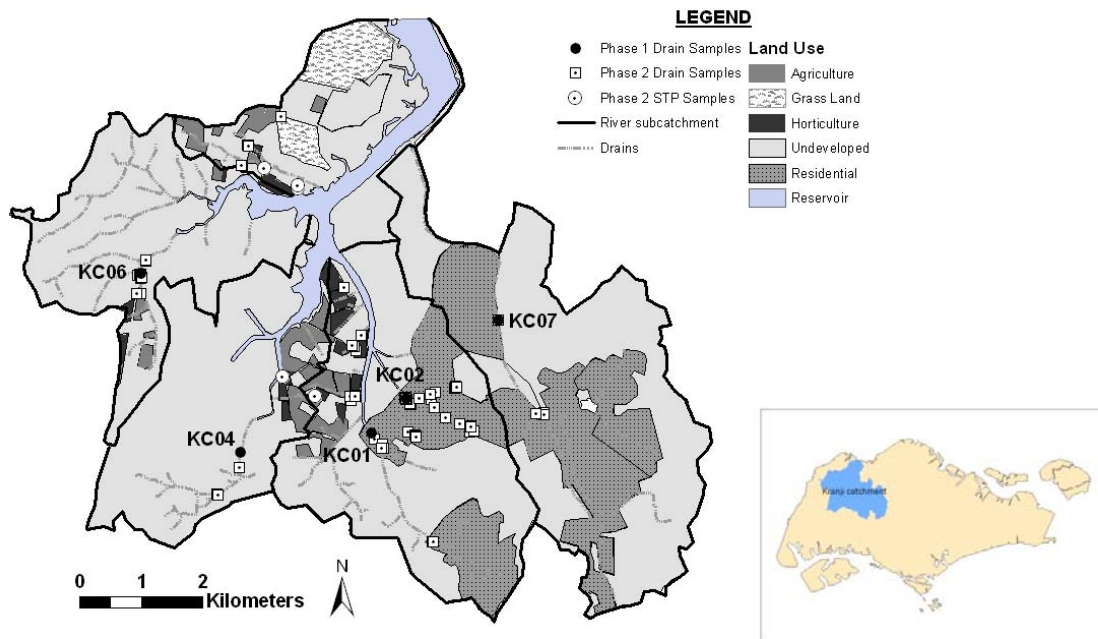
The objective of this study was to provide initial estimates of bacteria concentrations in the drainage system serving the Kranji catchment. This was achieved through a field monitoring program consisting of grab sampling and, in a limited number of cases, sampling with auto-samplers at specific locations within the Kranji catchment. In Phase 1 of the study, the sampling locations were chosen based on land use considerations. Subsequently, in Phase 2, the monitoring effort was expanded to target the sub-catchment that revealed the highest bacteria concentration under Phase 1, with the aim of identifying the likely source(s) of bacteria contribution. Sampling of the effluent from several sewage treatment plants was also undertaken under Phase 2.

## **2. STUDY SITE AND MONITORING PROGRAM**

Figure 1 shows a map of the Kranji catchment, which is located in the northwest of Singapore and is approximately 5,450 ha in size. It is mostly undeveloped with some rural land and manufacturing industry. Most of the land around the reservoir is designated as open space under current zoning regulations, with the exception of some agricultural land use, a small golf course to the west, and some light industry to the east (PUB 2008).

Phase 1 of the monitoring program consisted of a routine sampling program during which dry weather samples were collected from locations marked as KC01, KC02, KC04, KC06 and KC07 in Fig.1. This program was conducted during the period from November 2005 to December 2006. The overall goal of the Phase 1 monitoring was to obtain a gross indication of the bacteriological distribution in the catchment. KC01, KC02, KC04, KC06 and KC07 are existing flow monitoring station locations, and the samples collected at these stations represent contributions, in terms of water quality, from a combination of land use types. The land use at KC01 consists of high-density residential (36%) and forested (50%) areas, KC02 is the most developed consisting mainly of high-density residential (68%) and forested (17%) areas. KC04 and KC06 are predominantly undeveloped, with land use at KC04 consisting of grassed-over land cover (77%) and forest (15%) and land use at KC06 consisting of

grassed-over land cover (78%) and farms (15%). Land use at KC07 consists mainly of high density residential (32.5%), forest (35%), grassed-over land cover (19%) and low density residential (13.5%) areas. The numbers within parentheses indicate percentage of land area. Samples were collected routinely at a rate of more than once per month during dry weather. Phase 2 of the monitoring program consisted of dry-weather sampling from sampling points located predominantly within the KC02 sub-catchment, Sewage Treatment Plants (STP) and other locations within the Kranji catchment. The overall goal of the Phase 2 monitoring was to identify possible sources contributing to the high *E. coli* and Enterococci concentrations detected in KC02 during Phase 1 (results to be presented below) and to sample from targeted land use sites within the Kranji catchment in order to establish the dependence of dry-weather *E. coli* concentrations with land use. For this purpose, STPs were also included as a separate category. Phase 2 monitoring was conducted in January 2009 and the sampling locations under Phase 2 of the study are indicated in Fig.1.



**Figure 1. Kranji catchment map, sampling and STP locations.**

Samples were collected mainly by grab sampling using Nasco sterile Whirl-Pak<sup>®</sup> bags by hand. For each sample, approximately 400-500 milliliters of surface water were collected in the sterile plastic bags and chilled during transport to the laboratory. Appropriate collection and preservation of samples was observed to ensure proper quality control and assurance during sample collection and transport. For continuous monitoring, samples were collected by auto-samplers, and the procedures found in Eaton et al. (2005) were observed. All bottles being reused were either bleached and soaked in deionized water or baked at 450°C. Sample containers were filled without pre-rinsing with sample, leaving a space for aeration. Samples were then chilled in coolers during transport to the laboratory.

Global Positioning System (GPS) was used to determine sample locations, and in addition, a log for every sample collected was kept detailing the identification of each bottle/container, sample type (e.g., if DNA was to be analysed), date/time, water temperature and weather conditions. In addition to sampling from the drainage system, effluent from 5 STPs was collected and analysed. The sampling locations under Phase 2 of the study are included in Figure 1.

Millipore Sterivex<sup>™</sup>-GS 0.22µm Filter Units were used to collect DNA samples. The filter units accumulate sediments and bacteria on the membrane incorporated in the interior of the plastic filter case. The sediments and bacteria represent the environmental sample from which a DNA sample will be extracted. Although it is desirable to pump one liter of water sample through the filter in order to

have a robust DNA sample, the turbidity of the water in the drains did not always allow a full liter to pass through before clogging the filters. Once collected, the filter units containing the DNA samples were kept frozen at about -80°C.

### 3. WATER QUALITY ANALYSIS

During Phase 1, coliform bacteria were analysed using the IDEXX Colilert-18 media and Quanti-Tray/2000 MPN trays (IDEXX, 2007). These tests result in a most probable number (MPN) quantification of the bacteria count. During Phase 2, the Hach m-ColiBlue24<sup>®</sup> (Hach Company 1999) method was used to determine *E. coli* concentrations. This is a membrane filtration test that allows enumeration of total coliform and *E. coli* within 24 hours. After incubation, total coliform colonies appear red and *E. coli* appear blue and can be counted. Hach discontinued the sale of their m-ColiBlue24<sup>®</sup> broth due to lack of sensitivity in random testing of the product shortly after the end of our experiments. Communication with Hach revealed that the discontinued m-ColiBlue24<sup>®</sup> gave lower than expected concentrations of *E. coli* (Hach Customer Service 2009); therefore, we have assumed that even if this is a problem in our study, our values are still valid, but may be taken as conservatively low. The membrane filtration tests result in a count of colony forming units (CFU) per sample—the MPN and CFU quantifications are functionally equivalent. During both Phases 1 and 2, analysis of *Enterococci* concentrations was performed using IDEXX Enterolert media and Quanti-Tray/2000 MPN trays (IDEXX, 2008).

Blank samples containing zero *E. coli* and zero total coliform were included in the analyses to demonstrate a lack of outside contamination of the samples. No traces of sample contamination were detected. On certain sampling days, duplicate samples were taken and processed in the same way as the original sample (Eaton et al., 2005). On days when duplicate samples were taken, one duplicate sample was taken for every nine field samples. Following the procedures of Eaton et al. (2005), the duplicate sample was taken in the field and processed in the laboratory in the same way the original sample was taken and processed. Duplicate samples were evaluated based on quality assurance goals published by Oregon's Department of Environmental Quality (2001). To achieve these goals, the relative percent difference between the bacteria density in the original sample and that in the duplicate sample should be less than 25 per 100ml for samples with values greater than five times the detection limit, or 60 per 100ml. For samples with densities less than or equal to 60 per 100ml, the absolute difference between the same dilution of the two samples should be less than two times the detection level, or 24 per 100ml. Only a single sample with *E. coli* values between twelve and two hundred did not meet our quality assurance goals. This single exception can be attributed to errors in our sampling or analysis methods or, because duplicate sampling consists of taking two separate samples of the water, the second sample could have included more sediment due to stirring of the water body while taking the first sample, contributing additional *E. coli* to a sample.

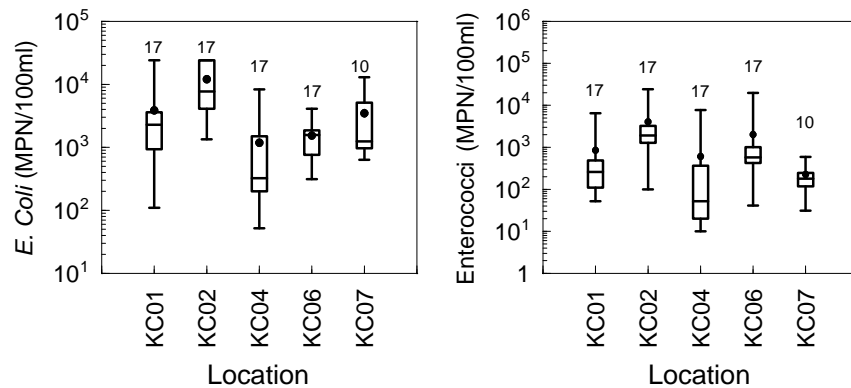
DNA analyses for 23 samples were carried out at the Parsons Laboratory, Massachusetts Institute of Technology. Sample extraction was performed using the MO BIO solid DNA extraction kit protocol (MO BIO Laboratories, Inc. 2008) and the DNA samples were then stored at -20°C before analysis. The samples were electrophoresed on 1% agarose, and then observed under UV-light to ensure the quality of the DNA samples following extraction, before amplification using the PCR technique. PCR amplification reports either the presence or absence of the human factor gene. Both positive and negative controls were incorporated in the PCR analysis. The positive control verifies if the DNA samples do not have any inhibiting substances that could affect the amplification and the negative control verifies that the PCR mixture was not contaminated during laboratory manipulations. Distilled water was used as a negative control and DNA containing the human factor (HF) marker was generously provided by Prof. A. Boehm, Stanford University, and used as a positive control.

### 4. RESULTS AND DISCUSSION

#### Phase 1

Figure 2 contains box plots of the sample analysis for *E. coli* and *Enterococci* respectively, obtained

under Phase 1 of the study. The box plots indicate the minimum, 1<sup>st</sup> quartile, median, 3<sup>rd</sup> quartile and maximum values, and mean values are indicated by filled circles. The numbers in the plots indicate the number of samples analysed. Relatively high bacteria counts are observed at all the 5 sampling locations. The US Environmental Protection Agency (USEPA, 1986) guideline concentrations for *E. coli* for surface waters recommends that the geometric mean of *E. coli* concentrations from 5 surface water samples over a thirty-day period should not exceed 126 per 100ml or the concentration in a single surface water sample should not exceed 235 per 100ml. Overall, the monitoring stations located in the catchment had bacteria counts that exceeded USEPA guidelines for single-sample maximums at each of the sampling locations, with only a few exceptions.



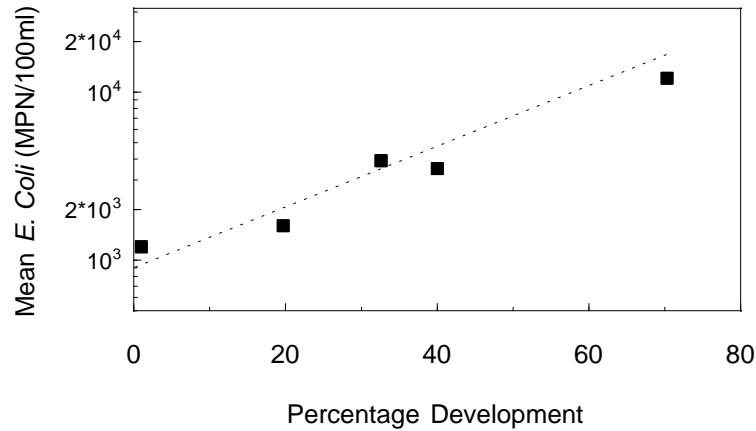
**Figure 2. Box plots of Phase 1 *E. coli* and Enterococci concentrations for dry weather samples**

There is a wide variability in the data indicated by the large differences between the maximum and minimum values. There are insufficient numbers of samples to elucidate temporal patterns in the bacteria density, however, the range and order of magnitude of the data are in general similar for all the sampling locations. It is noted, however, that three out of the seventeen samples exceeded the maximum detection limit for *E. coli*, and one out of the seventeen samples exceeded the maximum detection limit for Enterococci for the KC02 samples. Samples obtained from the other sampling locations were within the maximum detection limit for *E. coli* and Enterococci. Thus, *E. coli* and Enterococci concentrations for KC02 are underestimated.

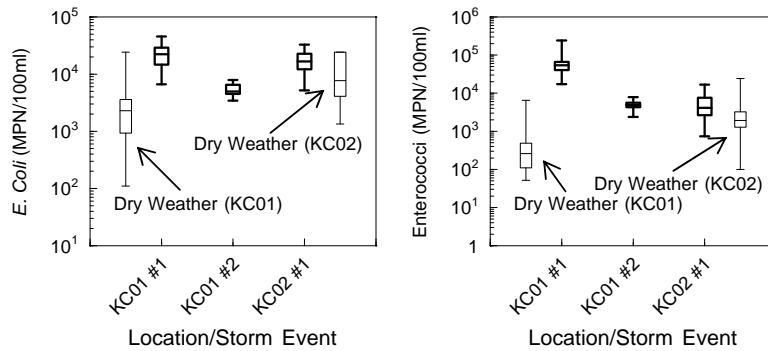
The results from the Phase 1 monitoring effort showed a possible dependence of *E. coli* concentrations with percentage development of catchment land use. The plot of mean *E. coli* concentration as a function of percentage development in Figure 3 indicates a positive correlation between mean dry weather *E. coli* concentrations with percentage development. Correlation between mean Enterococci with percentage development was not as significant and is not plotted. The correlation of *E. coli* highlights the impacts of urbanization on dry-weather bacteria loading. For urban areas, under dry weather conditions, bacterial contamination in the storm drains may be due to sewer leaks, cross-connections between sewer and drain lines, or the illegal discharge of wastes from mobile toilets at construction sites. Contamination may also be due to the discharge from the sewage treatment plants (STPs) scattered throughout the catchment.

Although this study was conducted primarily to study bacteria loading under dry weather conditions, storm runoff was sampled for three storm events in order to obtain an initial assessment of storm runoff versus dry weather flow bacteria concentrations. Storm runoff samples for two storm events at KC01 and one storm event at KC02 were collected for this purpose. Eight samples were collected and analyzed for each storm event. The storm runoff data for *E. coli* and Enterococci are shown in Fig. 4, which also includes the results from the dry weather analysis for KC01 and KC02, shown in Fig. 2. Elevated *E. coli* and Enterococci concentrations during storm events are linked to catchment impervious surface coverage consisting of roofs, roads, driveways, sidewalks and parking lots. These surfaces serve to concentrate and convey storm-water borne pollutants to downstream receiving waters (Schueler, 1994). Notwithstanding the limited number of storm events sampled, it is evident

that storm runoff at these two sampling locations contain elevated levels of *E. coli* and Enterococci; the two storm events in KC01 (KC01 #1 and KC01 #2) showing a substantial increase in the median concentrations of *E. coli* and Enterococci, whereas the increase in *E. coli* and Enterococci concentrations for KC02 #1 are modest.



**Figure 3. Land use of five sub-catchments and mean bacteria concentration**



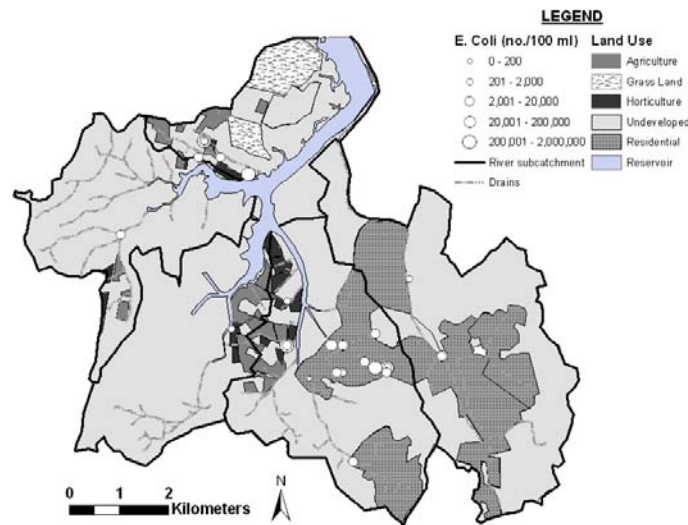
**Figure 4. Box plots of Phase 1 *E. coli* and Enterococci concentrations for storm samples.**

## Phase 2

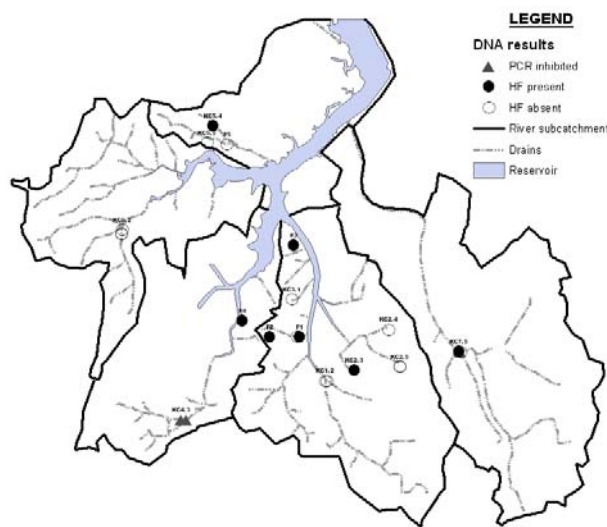
In the second phase of the study, more than 100 non-point source samples were collected predominantly in the KC02 sub-catchment, either via auto-samplers or grab-sampling. Total coliform concentrations ranged from 1,800 in a KC02 drain to  $167 \times 10^6$  CFU/100ml in the runoff from a fish farm. *E. coli* concentrations ranged from 87 in a KC02 drain to  $29 \times 10^6$  CFU/100ml at a location draining directly into the reservoir downstream from KC01. The results for the *E. coli* analysis are shown in Fig. 5a, for both non-point and point source sampling. It is observed that most of samples taken from residential and agricultural and horticultural areas exhibit moderate to high levels of *E. coli*. Only a few sampling points, in the western part of the catchment that is in a relatively undeveloped area, exhibited low to moderate levels of *E. coli*. This apparent distribution of *E. coli* as a function of land use is further elaborated below. The drains upstream of KC02 were progressively sampled in an attempt to trace possible sources of bacteria pollution in the KC02 sub-catchment. This sampling effort led to the identification of a possible source, where an *E. coli* concentration of  $2 \times 10^6$  CFU/100ml, close to that for raw sewage, was measured about 1.4 km along the drainage network, upstream of KC02.

A total of 23 DNA samples were taken at the locations indicated in Fig. 1 (e.g. farms, residential areas, and military facilities) during Phase 2. PCR analysis revealed the presence of the HF marker in

eight out of the 23 samples. Locations where the HF marker was identified are highlighted in Fig. 5b. The presence of the HF marker indicating the occurrence of human-specific *Bacteroides* bacteria generally corresponded with locations at which high coliform concentrations were found and which were known or suspected to be associated with sanitary wastewater. Nonetheless, numerous locations at which coliform counts were well above USEPA water-quality criteria often did not show human factor presence, although it is possible that the HF marker existed below our detection thresholds (i.e.  $<1 \times 10^3$  to  $1 \times 10^4$  targets per mL environmental sample). Although this finding supports previous observations that coliform may not be a specific indicator of human sewage in tropical climates (Rivera, 1988; Jensen, 2001; Winfield, 2003) such as Singapore, additional studies will be required to improve the sensitivity of HF marker detection and to correlate the environmental abundance of the HF marker, total coliforms, *E. coli* and additional tracers of sewage contamination to better understand the relationships between sewage indicators and human risk in tropical environments.

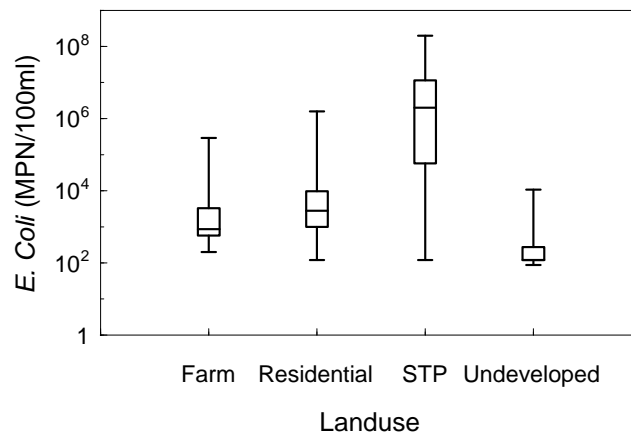


**Figure 5a. Non-point source contribution of *E. coli* in KC02 and contribution of bacteria from STPs.**



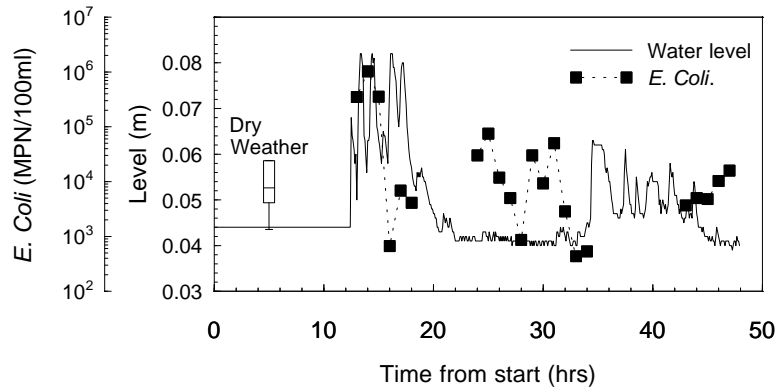
**Figure 5b. Locations where the human factor gene was identified.**

The variation of *E. coli* with land use type is demonstrated more clearly in Fig. 6 where *E. coli* concentrations are plotted as a function of land use. Note that “Farm” indicates vegetable farms but also includes horticultural establishments, “Residential” consists of mainly high intensity public housing areas made up primarily of high-rise apartment blocks, and can also include green areas, schools, commercial establishments and community centres within the housing estate. “STP” refers to the samples taken from the effluent of sewage treatment plants from fish farms, poultry farms and a military installation but also include the wash water running off from the common areas of these establishments as a result of cleaning and housekeeping activities. The figure shows that effluent discharges from the STPs contain the highest *E. coli* concentrations and undeveloped areas have the lowest *E. coli* concentrations. The high level of *E. coli* from the STP areas is not unexpected since the wash water from these establishments is channeled directly into the drainage system. *E. coli* levels in residential areas are also high, with a median concentration of 2,800 MPN/100ml and are probably the result of leaking sewer pipes or illegal connections of sewer pipes to the drainage system, as mentioned previously.



**Figure 6. *E. coli* concentrations as a function of land use**

Continuous flow monitoring and sampling at KC02 was carried out over an approximately 30-hr period in order to assess variations in bacteria level over a continuous period during dry weather. Flow is represented by level measurements obtained from an Isco 750 level and velocity sensor installed at the invert of the dry weather flow channel and samples were collected using an Isco 6712 auto-sampler. The time series of bacteria concentration helps to elucidate diurnal features (if any) in the concentration data and flow monitoring can be used to confirm if the presence of a spike in bacteria concentration is associated with an illegal discharge event. The data are shown in Fig. 7. Gaps in the data for *E. coli* are due to a malfunction of the auto-samplers resulting in samples being missed. Although we could not obtain a continuous dataset, Fig. 7 nevertheless shows that under dry weather flow conditions, background *E. coli* levels are consistent with the *E. coli* concentrations measured under dry weather conditions during Phase 1. This consistency is an indication that temporal variations in dry weather concentrations of *E. coli* at KC02, if any, are minimal. A sharp spike in *E. coli* concentration is observed at the 15-hr mark, and this is accompanied by a discernible increase in the water level implying that the spike in *E. coli* is likely to be associated with an illegal discharge event in the drainage system. A second increase in water level is observed after 40 hrs, however, *E. coli* levels could not be ascertained due to equipment malfunction, as mentioned earlier.



**Figure 7. Continuous sampling at KC02 during dry weather**

## 5. CONCLUSIONS

The following can be concluded from this study:

1. Dry weather *E. coli* levels exceeded USEPA (1986) guidelines at 5 sampling locations, which were monitored for a period in excess of 1 year. *E. coli* and Enterococci concentrations were highest at KC02, which has the sub-catchment with the highest percentage development and elevated concentrations of *E. coli* and Enterococci were found to be contained in storm runoff in KC01 and KC02.
2. There is a positive correlation between *E. coli* concentration and percentage land use development under dry weather conditions.
3. The high levels of *E. coli* and Enterococci at KC02 were traced to a possible source about 1.4 km along the drainage network, upstream of KC02, where an *E. coli* concentration of  $2 \times 10^6$  CFU/100ml was measured.
4. *E. coli* varied according to land use, in increasing order according to the following sequence of land use categories: Undeveloped → Farm → Residential → Sewage Treatment Plant (STP) and DNA analysis revealed that the locations where the human factor marker was positive generally corresponded with locations at which high fecal coliform concentrations were found and which were known or suspected to be associated with sanitary wastewater. Nonetheless, numerous locations at which coliform counts were well above USEPA water-quality criteria often did not show human factor presence. Others have questioned whether coliform may grow naturally in tropical waters and more analyses are needed to investigate the possible contributors to observed coliform counts in Kranji catchment.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and research funding provided by the Public Utilities Board (PUB), Singapore for Phase 1 of the study. We also thank the project officers Yee Woon Kang, Xing Zikun, Lim Lai Wan, Lim Wee Ho, Anuja Padma Gopalakrishnan, Shukla Jayati Jagdishchandra, Ruby Tok Hui Yin, Ng Yen Nie and Ankur Gupta for carrying out the laboratory analyses and their involvement in the Phase 1 fieldwork. In addition, we would also like to acknowledge the support and research funding provided by the Centre for Environmental Sensing and Modeling (CENSAM), Singapore-MIT Alliance for Research and Technology (SMART) and Mr Syed Alwi Bin Sheikh Bin Hussein Alkaff for assistance in the Phase 2 fieldwork. The authors would also like to thank the Planning, Modelling & Water Quality Division, Waterways Health & Limnology Branch – Catchment & Waterways Department of the PUB for their support and assistance rendered during the project.

## REFERENCES

1. Bartram, J., and Rees, G., 2000: Monitoring Bathing Waters: Practical Guide to the Design and Implementation of Assessments and Monitoring Programmes, pp. 113–167. Routledge, New York, N.Y.
2. Bernhard, A. E., and Field, K. G., 2000: Identification of Nonpoint Sources of Fecal Pollution in Coastal Waters by Using Host-Specific 16S Ribosomal DNA Genetic Markers from Fecal Anaerobes. *Applied and Environmental Microbiology*, 66(4), pp. 1587-1594.
3. Eaton, A., Clesceri, L., Rice, E., and Greenburg, A., 2005: Standard Methods for the Examination of Water and Wastewater. American Public Health Association, New York.
4. Fogarty, L.R., and Voytek, M.A., 2005: Comparison of *Bacteriodes-Prevotella* 16S rRNA genetic makers for fecal samples from different animal species. *Applied and Environmental Microbiology*, 71(10), pp. 5999-6007.
5. Hach Customer Service, 2009: Personal communication.
6. Hach Company, 2008: m-ColiBlue24<sup>®</sup>broth. Microbiology procedures. <[www.hach.com](http://www.hach.com)> (Accessed December 1, 2008).
7. Hach Company, 1999: Analytical Methods, Coliforms: Membrane Filtration (simultaneous detection). <<http://www.water-research.net/Waterlibrary/watermanual/MCOLI.PDF>> (Accessed November 20, 2008).
8. IDEXX, 2007. Colilert<sup>®</sup> Test Kit. IDEXX Laboratories, Inc., Westbrook, Maine, USA.
9. IDEXX, 2008. Enterolert<sup>™</sup> Test Kit. IDEXX Laboratories, Inc., Westbrook, Maine, USA.
10. MO BIO Laboratories Inc., 2008: UltraClean<sup>™</sup> soil DNA isolation Kit. Catalogue number: 12800-100, <<http://www.mobio.com/files/protocol/12800.pdf>> (Accessed February 13, 2009).
11. Jensen, P. K., Aalback, B., Aslam, R. and Dalsgaard, A., 2001: Specificity for Field Enumeration of *Escherichia coli* in Tropical Surface waters. *Journal of Microbiological Methods*, 45, 135 – 141.
12. Oregon State Department of Environmental Quality, 2001: Quality Assurance Guidelines–NPDES & WPCF Self-Monitoring Laboratories. <<http://www.deq.state.or.us/lab/techrpts/docs/NPDESWPCF.pdf>> (Accessed April 1, 2009).
13. Peterson, T. M., Rifai, H. S., Suarez, M. P. and Stein, R. A., 2005: Bacteria Loads from Point and Nonpoint Sources in an Urban Catchment. *Journal of Environmental Engineering*, 131(10), pp. 1414-1425.
14. Public Utilities Board, Singapore. 2008: ABC Water Masterplan (Western Catchment). <[http://www.pub.gov.sg/abcwaters/ABCWaterMasterPlan/Documents/FMP4fastviewing\\_Sept2007.pdf](http://www.pub.gov.sg/abcwaters/ABCWaterMasterPlan/Documents/FMP4fastviewing_Sept2007.pdf)> (Accessed November 30, 2008).
15. Rivera, S. C., Hazen, T. C. and Toranzos, G. A., 1988: Isolation of Fecal Coliforms from Pristine Sites in a Tropical Rain Forest. *Applied and Environmental Microbiology*, 54(2), 513-517.
16. Santoro, A. E., and Boehm, A. B., 2007: Frequent Occurrence of the Human-Specific *Bacteriodes* Fecal Marker at an Open Coast Marine Beach: Relationship to Waves, Tides and Traditional Indicators. *Environmental Microbiology*, 9(8), pp. 2038-2049.
17. Schilling, K. E., Zhang, Y. K., Hill, D. R., Jones, C. S. and Wolter, C. F., 2009: Temporal Variations of *Escherichia Coli* Concentrations in a Large Midwestern River. *Journal of Hydrology*, 365, pp. 79-85.
18. Schueler, T. R. (1994): The Importance of Imperviousness. In T. R. Schueler and H. K. Holland (eds.), 2000, *The Practice of Catchment Protection, Catchment Protection Techniques*, Ellicott City, MD, Center for Catchment Protection, pp. 100-111.
19. Stein, E. D., Tiefenthaler, L. L., 2005: Dry-Weather Metals and Bacteria Loading in an Arid, Urban Catchment: Ballona Creek, California. *Water, Air and Soil Pollution*, 164(1), pp. 367-382.
20. USEPA, 1986: Ambient Water Quality Criteria for Bacteria. Report Number EPA440/5-84-002. United States Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.
21. Winfield, M. D. and Groisman E. A., 2003: Role of Nonhost Environments in the Lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology*, 69(7), 3687-3694.