# Addressing Sewage Contamination of Nawiliwili Streams and Kalapaki Beach

# I. Problem and Research Objectives

Identification of the Problem. EPA requires every state to use EPA approved methods to assay water samples to determine whether the water used will meet drinking water or recreational water quality standards. When these water quality standards are exceeded, EPA directives conclude that the water samples are contaminated with sewage. However, previous studies conducted by Hawaii's Water Resources Research Center (WRRC) and confirmed by research microbiologists throughout the nation, have shown that the EPA standards results in data that are suggestive of but do not confirm the presence of sewage contamination. This problem is most apparent in Hawaii because of the islands tropical environment which is characterized by relatively warm temperatures and high humidity. These same conditions allow bacteria to grow more readily in tropical environments (e.g. soil) than in temperate environments. In this regard, studies conducted by WRRC have shown that the EPA fecal indicator bacteria (total coliform, *Escherichia coli*, enterococci), which are used to establish water quality standards, will grow naturally in the soil environments of Hawaii and are washed into all streams in Hawaii at concentrations that exceed EPA standards. The WRRC studies have concluded that the presence of fecal indicator bacteria in Hawaii's environmental waters is more likely due to soil contamination than sewage contamination. Additional studies by WRRC laboratories have shown that analyses of drinking and recreational water samples for other human sewage microorganisms called "alternative fecal indicators" such as Clostridium perfringens, a bacterium and coliphages or viruses that infect fecal bacteria provide more reliable data for determining the presence and absence of sewage contamination.

Dr. Roger Fujioka of WRRC completed a water quality research project for the Nawiliwili watershed on the island of Kauai where cesspools are extensively used. The conclusions of that study were as follows: 1) Many of the microbial water monitoring data obtained from the island of Kauai were similar to data previously obtained from the island of Oahu. 2) FIB (fecal coliforms, *E. coli*, enterococci) are naturally present in high concentrations in soil and represent a major environmental, non-sewage source of FIB. 3) Under ambient conditions, concentrations of FIB in streams routinely exceed current water quality standards and the predominating source of FIB is soil rather than sewage. 4) FIB are unreliable indicators of fecal contamination for streams and coastal waters receiving land based discharges on the islands of Kauai and Oahu. 5) Monitoring for F+ coliphages provided reliable data to detect subsurface contamination of streams by cesspool waste because the small size of these viruses enabled their movement through soil. 6) Although *Clostridium perfringens* was previously shown to be a good indicator of surface sources of sewage pollution on the island of Oahu, this fecal bacteria was not a good indicator for subsurface contamination of streams by cesspool waste because the large size of this bacterium prevented their movement through soil 7) Identifying and genotyping FRNA coliphages recovered from environmental water samples provided additional data to show that human sewage was a source of contamination in the Nawiliwili watershed. 8) The detection of elevated levels of FRNA coliphages of human sources (genotypes II, III) in streams on Kauai indicate that these streams are contaminated with cesspool waste and are likely to be contaminated with human sewage-borne viruses.

Project Objectives. The first objective was to train the new WRRC microbiologist because Dr. R. Fujioka of WRRC retired in 2009 after completing 38 years of research for WRRC. Dr. Marek Kirs was recently hired to replace Dr. Fujioka. For this training objective, Dr. Fujioka participated in the training of Dr. Kirs in the use of established methods at WRRC and to introduce Dr. Kirs to the leaders of Hawaii's water agencies to include the Hawaii Department of

Health, the City and County of Honolulu, Environmental Services and the Honolulu Board of Water Supply. The second proposed research objectives were to confirm previous findings that Nawiliwili watershed is being contaminated by use of cesspools and whether this contamination compromises the quality of the water at Kalapaki Beach which is one of the most popular beaches on Kauai.

## II. Methodology and Experimental Design

To implement the stated objectives of this study, the following experimental designs were completed: 1) Dr. Gayatri Vithanage, who completed the first WRRC study for the Nawiliwili watershed was hired as a post-doctoral researcher to complete most of the water quality assays. 2) Arrangements were made with the Hawaii State Department of Health (HDOH) to participate in this study. 3) Mr. Gary Ueunten of HDOH, who works on the island of Kauai was consulted to establish the sampling sites at the Nawiliwili Watershed (see Figure 1). An agreement was made with Gary Ueunten to collect water samples, to assay the quality of water for selected parameters (temperature, turbidity, pH, salinity) at the time of collection and to send the water samples from Kauai to Oahu. 4) Dr. Vithanage was assigned to pick up the water samples at the airport and proceeded to assay the samples within 6 hours of collection. 5) Dr. Fujioka supervised the training of Dr. Kirs and introduced Dr. Kirs to the all the leaders of the agencies (Hawaii Department of Health, City and County of Honolulu, Honolulu Board of Water Supply, Other researchers at the University of Hawaii as well as researchers from other universities. 6) After Dr. Kirs was sufficiently trained, he was authorized to become the Principle Investigator for this project and assumed responsibilities to implement the research objectives of this study, 7) Dr. Kirs completed the writing of this final report.

To implement the water monitoring design of this study, culture based methods were used to assay for *E. coli*, enterococci, *C. perfringens*, somatic coliphages and male (F+) coliphages. Selected subsamples were assayed for Human enteric viruses (enteroviruses, adenoviruses, noroviruses,) using molecular based method (PCR method).

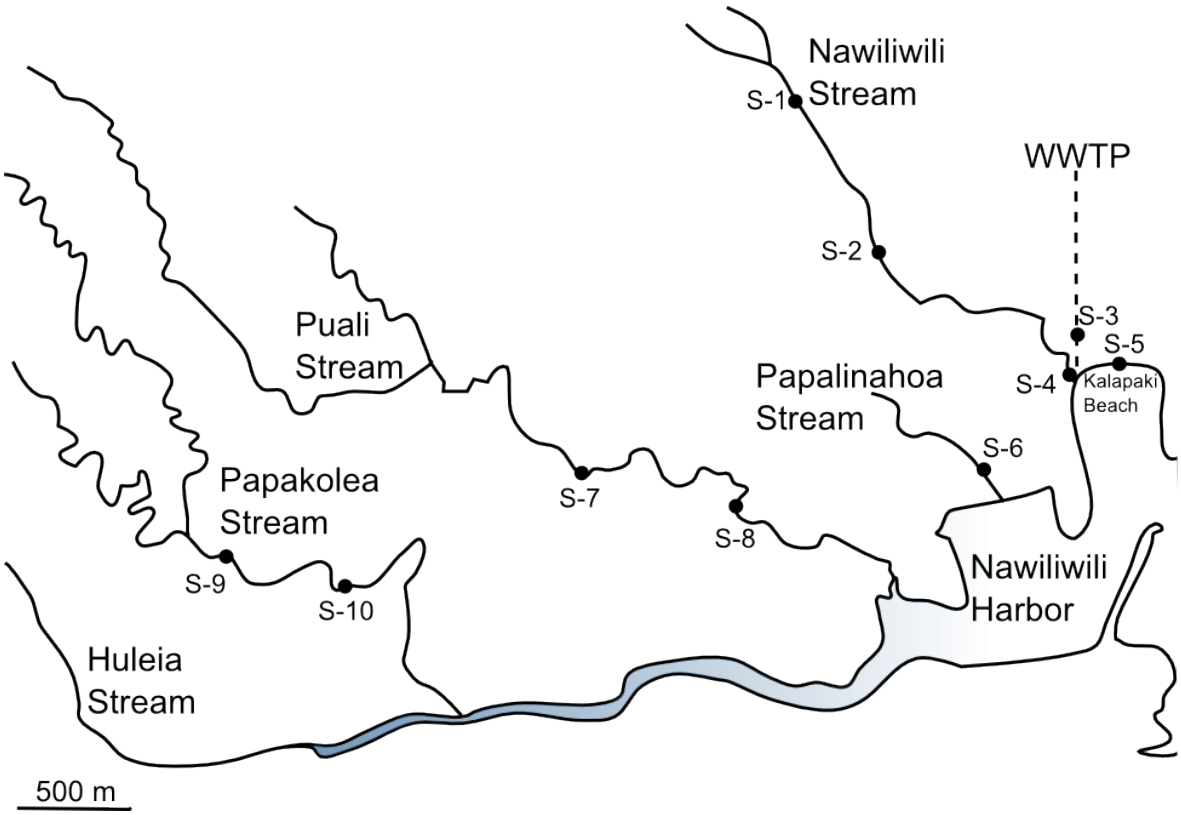


Figure 1. Nawiliwili watershed sample sites. S-1 Upper Nawiliwili Stream, S-2 Lower Nawilwili Stream, S-3 Mariott Culvert, S-4 Pine Trees, S-5 Kalapaki Beach, S-6 Papalinahoa Stream, S-7 Upper Puali Stream, S-8 Lower Puali Stream, S-9 Upper Papakolea Stream, S-10 Lower Papakolea Stream.

A total of 117 water samples were collected by Mr Gary Ueunten (Kauai Branch of Hawaii State Department of Health) from ten sites at the Nawiliwili watershed and Kalapaki Beach located at the eastern section of Kauai Island (Fig. 1), as well as from one additional relatively pristine control site at the Lawai Stream located in the middle section of Kauai Island. Samples were collected roughly fortnightly from June to December 2011. After collection, samples were cooled on ice and shipped by air to the microbiology laboratory at the WRRC, University of Hawaii at Mānoa. All samples were analyzed within 6-9 hours after sample collection for fecal indicator bacteria (enterococci, *E. coli*) and alternative fecal indicators (*C. perfringens*, coliphages). During every second sampling, a subsample was provided for Dr.Yuanan Lu in the Department of Public Health Sciences laboratory at the University of Hawai ʻi at Mānoa for the analyses of noro- entero- and adenoviruses by polymerase chain reaction (PCR) method, a molecular assay that detects for the presence of specific gene of a microorganism but does not determine the infectivity of that microorganism. .

Concentrations of enterococci and *E. coli* were determined using USEPA approved membrane filtration based methods 1106.1 [1] and 1103.1 [2] respectively. In the case of enterococci, the samples were filtered through glass fiber filters (Pall Corporations, Ann Arbor, MI), the filters were placed on mE agar plates and incubated at 41°C for 48 h. After incubation the filters were transferred to pre-warmed EIA plates and incubated for an additional 20 minutes. Colonies with black or reddish brown precipitate on the underside of the membrane were counted as enterococci. In the case of *E. coli* the samples were filtered through glass fiber filters

(Pall Corporations, Ann Arbor, MI), filters were placed on mTEC agar plates and incubated first at 35°C for 2 h and then in a waterbath at 44.5°C for another 22-24 h. After incubation the membranes were transferred to absorbent pads saturated with Urea Substrate Medium for 15-20 minutes. Yellow, yellow-brown and yellow-green colonies were counted as *E. coli.*

Presence of human viruses (adeno-, entero- and noroviruses) was determined by Dr. Lu using endpoint polymerase chain reaction (PCR) technology and group specific primers. PCR products were visualized on agarose gel after electrophoresis. Appropriate negative and positive controls were used during each PCR run.

Water samples to assay for *C. perfringens* spores were pretreated by heating 100ml subsamples for 15 min at 60°C to kill the vegetative cells. Concentrations of *C. perfringens* were assayed for using the SFP media-based Fung double tube (FDT) test [3] during the initial sampling events. Due to the high background signal originating from other species of *Clostridia* in freshwater samples, the conventional mCP agar-based method [4] was used for the rest of samples. After incubation in an anaerobic chamber at 45°C for 24 h, the membranes were exposed to ammonium hydroxide fumes for 20 seconds and pink colonies were counted as *C. perfringens*. Concentrations of somatic and male ($F^+$) specific coliphages were identified in 5ml sample portions using UEPA approved Method 1601 [5] using *E. coli* CN-13 and *E. coli* $F_{amp}$ as hosts respectively. Negative samples were assayed by enrichment using 100 ml sample portions following day.

Somatic and $F^+$ specific coliphages were quantified by assaying five ml sample portions by the double agar layer methodology as in Vithanage et al. [6] using *E.coli* CN13 and *E. coli* $F_{amp}$ as hosts correspondingly. Samples which were negative by the quantitation assay were then assayed by the sensitive presence absence method by assaying 100ml water samples using the initial 24 h at 37°C enrichment method with tryptone broth and appropriate host. After the enrichment procedure, samples were centrifuged (10,000 g for 4 min) and ten µl of supernatant was spotted on fresh bacterial on lawn prepared using double agar methodology. Formation of plaques on the lawns of the bacterial cells indicated the presence of coliphages in that tested water sample.

Concentration of total phosphorus ($PO_4^{3-}$) was determined using acid persulfate digestion (method 8190) on a Chemical Oxygen Demand reactor (Hach, Loveland, CO). Temperature, pH, salinity and weather conditions were recorded in the field by HDOH staff.

# III. Principal Findings and Significance

All freshwater sites, this includes all sites studied except Kalapaki beach (which was a marine site, salinity: 29 - 34 ppm), were characterized by high concentrations of conventional indicator bacteria (*E.coli* and enterococci) throughout the study period. Concentrations of *E. coli* varied from <4 to 7920 CFU per 100 ml with a geometric mean varying from 196 to 1260 CFU per 100ml between sites. Concentrations of enterococci varied from 41 to 6040 CFU per 100 ml with a geometric mean varying from 76 to 1928 CFU per 100 ml between sites, hence concentrations of indicator bacteria were elevated, except for the marine site (S-5 Kalapaki Beach) where concentrations of enterococci were relatively low (4-26 CFU per 100 ml, with a geometric mean of 9 CFU per 100ml) (Table 1). These results are comparable to the earlier study by Vithanage et al. [6], and confirmed again that cesspools and the freshwater streams were the major source for all fecal microbial indicators. The comparative concentrations of these fecal microbial indicators were relatively lower at the harbor sites where the salinity indicated a mixture of freshwater and marine waters. The concentrations of all microbial indicators were

much lower at Kalapaki Beach which was characterized as a marine water based on high salinity and being contaminated with low volumes of fresh stream waters.

Current Hawaii recreational freshwater water quality standards are based on enterococci and state explicitly that no sample should exceed 89 CFU of enterococci per 100ml and the geometric mean of samples collected over 25-30 days should not exceed 33 CFU of enterococci per 100 ml [7]. All samples collected, including the control sample from the pristine environment, exceeded the standard based on the geometric mean throughout the study period. The standard based on the single sample maximum was also exceeded in all freshwater samples, except for one sample collected from Puali Stream and three out of four samples collected at the pristine control site (Lawai Stream). These findings collectively indicate that **current water quality standards are not suitable for Hawaii due to high environmental background of indicator bacteria**, ergo have little relevance to the actual health risk posed by recreational exposure to these waters.

Current Hawaii recreational marine water quality standards are based on enterococci and state explicitly that no sample should exceed 104 CFU of enterococci per 100ml and the geometric mean of samples collected over 25-30 days should not exceed 35 CFU of enterococci per 100 ml [7]. Samples collected at the Kalapaki Beach did not exceed the standards during the study.

Tests for human enteric viruses were conducted at five sites (S-2, S-3, S-4, S-6, S-8, and S-10) only (Table 2). Human norovirus genogroup 1, genogroup 2, enteroviruses, and adenoviruses were detected at all sites, except the norovirus genogroup 2 which was not detected at site S-4 and S-10 (Table 2). While human adenoviruses were detected in all samples tested, PCR signal (group specific DNA fragment) from other groups was inconsistent indicating fluctuating levels of sewage input. It is not clear whether the PCR signal originated from viable viruses and what the associated health risk is, although detection of molecular signal originating from human viruses is a clear indication that **human sewage, likely from adjacent cesspools, is reaching the watershed.**

*C. perfringens* concentrations were low during the study at all sites. No sample exceeded 50 CFU per 100 ml limit, except for one sample collected at Papalinahoa Stream after wet weather. No spores of *C. perfringens* were found at the marine site (Kalapaki Beach). *C. perfringens* concentrations were low likely due to the prevailing dry weather, although the relatively large size of the spores compared to pathogenic viruses can further hamper transport of this bacterium through the soils [6]. While obligate anaerobe *C. perfringens* is a solid indicator of sewage contamination, the viral contamination component might remain undetected using bacterial targets and **monitoring for bacterial and viral indicators simultaneously is warranted**. This is in agreement with earlier studies which indicate that current bacteriologigal water quality standards do not reflect viral contamination component [8].

Concentrations of somatic coliphages (geometric mean 2-289 PFU per 100ml) were comparable to estimates made in an earlier study [6] with the highest concentrations detected at the Papalinahoa Stream and Pine Tree site (2620 PFU and 2680 PFU per 100ml respectively). Also somatic coliphages were more frequently recovered and at higher concentrations compared male ($F^+$) specific coliphages. Somatic coliphages have been proposed as indicators of fecal contamination as they are removed during treatment processes at comparable rates to enteroviruses as well as appear to exhibit similar seasonal variation [9, 10]. Due to their high concentrations in sewage, stability and because they are viruses, somatic coliphages have potential as indicators of fecal contamination, including virus contamination. Furthermore, while

possible replication of somatic coliphages in the environment has been indicated in earlier studies [11, 12], most recent studies have been suggesting that the replication in the natural environment is very unlikely [13, 14]. The results of this study show that the monitoring for somatic coliphages could indicate presence of fecal contamination in the watershed and support PCR based findings for presence of human enteric viruses.

Male ($F^+$) specific coliphages were detected in the watershed at lower levels (geometric mean 1-11 PFU per 100 ml) when compared to earlier report [6]. These phages were detected also at low frequency, with only 30% of the samples analyzed being positive for this phage group. These coliphages cannot replicate in the environment as $F^+$ pili are not formed below 25°C[15] and therefore, in theory, they could also be good indicators of fecal contamination (animal and/or human). It should be noted that only a small percentage of humans and animals carry this group of phages, hence a leaking cesspool from a single family household or input from a small population of animals could remain undetected. Since male ($F^+$) specific coliphages are similar in size to human enteric viruses and smaller than somatic coliphages (25-30 nm vs. (25)80-400 nm) these viruses have been proposed as more specific to human enteric viruses and can be expected to be transported through the soil column. However, recent studies have indicated that they can be accumulated over 100 times more in clayey sediments [16] because the hydrophobic protein coat [17, 18] of these viruses may be favorable for the adsorption to clay particles. In this regard, soils in Hawaii are known to contain high levels of clay. This property of male (F+) specific coliphages may prevent these viruses from being easily transported through soils with high clay contents and may explain the lower detection levels of these viruses in the Nawiliwili watershed. In summary, the concentrations of certain groups of male (F+) specific coliphages in environmental waters are believed to be more specific indicator for human sewage than somatic coliphages. However, a recognized limitation in monitoring for male (F+) coliphages is their lower numbers as compared to somatic coliphages.

A significance of this project was the introduction of Dr. Kirs to many collaborators to include Mr. Joseph Lichwa and Mr. Philip Moravcik of WRRC, University of Hawaii at Mānoa. Also with Dr. Yuanan Lu (Department of Public Health Sciences, University of Hawaii at Mānoa). In addition Dr. Kirs was introduced to Mr. Watson Okubo (Section Chief, Clean Water Branch, HDOH), Mr. Ross Tanimoto (Deputy Director, Department of Environmental Services, CCH), Mr. Ken Tenno (Laboratory Director, Lab. Branch, Department of Environmental Services, CCH) and Mr. Owen Narikawa of Honolulu Board of Water Supply. As a result Dr. Kirs have discussed future collaborative projects with these scientists. Finally, USEPA recently reported that they will continue to use the same recreational water quality standards based on monitoring for standard FIB for the next five years (2012 to 2017). WRRC has already determined that these EPA standards are not reliable in Hawaii and other tropical climates because these FIB can grow in soil environments. One solution proposed by USEPA is to initiate microbial source tracking (MST) methods to confirm for the presence and absence of human sewage versus animal wastes. The need for verification of the usefulness of MST methods is greater in tropical environments such as Hawaii than in other US states. Therefore a new proposal seeking to evaluate current and alternative indicator bacteria using microbial source tracking tools was submitted for funding under the WRIP program for 2012-2013.

Table 1. Nawiliwili watershed sampling sites and their geometric means of *E. coli*, enterococci and alternative indicators at each site.

| Site | *E. coli* CFU/100 ml | Enterococci CFU/ 100ml | *C. perfringens* CFU/100 ml | Somatic coliphages PFU/100 ml | Male ($F^+$) coliphages PFU/100 ml |
|---|---|---|---|---|---|
| S-1 | 286 (4-1560) | 255 (120-480) | 3 (<1-25) | 32 (<1-240) | 2 (<1-40) |
| S-2 | 1151 (640-2040) | 900 (480-1880) | 4 (<1-30) | 108 (20-440) | 8 (<1-260) |
| S-3 | 239 (<4-7280) | 780(284-2880) | 4 (<1-15) | 123 (20-1040) | 7 (<1-160) |
| S-4 | 409 (<4-1440) | 520 (256-1040) | 4 (<1-10) | 272 (80-2680) | 11 (<1-260) |
| S-5 | 7 (<4-106) | 9 (4-26) | 3 (<1-15) | 2 (<1-40) | 1 (<1) |
| S-6 | 1050 (144-7920) | 1928 (1000-3640) | 6 (<1-56) | 289 (<1-2620) | 2 (<1-500) |
| S-7 | 475 (92-5440) | 204 (88-480) | 2 (<1-25) | 87 (20-800) | 2 (<1-140) |
| S-8 | 1260 (560-3920) | 1113 (332-6040) | 4 (<1-15) | 78 (<1-800) | 1 (<1-20) |
| S-9 | 352 (40-1120) | 348 (128-2040) | 3 (<1-35) | 214 (60-600) | 11 (<1-140) |
| S-10 | 741 (280-5600) | 524 (240-1040) | 4 (<1-15) | 101 (<1-360) | 4 (<1-40) |
| Lawai Stream (Control) | 196 (99-305) | 76 (41-140) | 1 (<1-2) | 18 (<1-140) | 9 (<1-260) |

Table 2. Detection of human enteric viruses in the Nawiliwili watershed (number of positive samples / number of samples tested).

| Site | Noroviruses Genogroup I | Noroviruses Genogroup II | Enteroviruses | Adenoviruses |
|---|---|---|---|---|
| S-2 | 2/3 | 1/3 | 3/3 | 3/3 |
| S-3 | 3/3 | 1/3 | 2/3 | 3/3 |
| S-4 | 2/3 | 0/3 | 2/3 | 3/3 |
| S-6 | 3/3 | 1/3 | 3/3 | 3/3 |
| S-8 | 3/3 | 1/3 | 3/3 | 3/3 |
| S-10 | 2/3 | 0/3 | 1/3 | 3/3 |

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