ASSESSING THE SOURCE OF FECAL CONTAMINATION IN STREAMS ON KAUA'I BASED ON CONCENTRATION AND GENOTYPES OF FRNA BACTERIOPHAGES

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ABSTRACT

Extensive data from O'ahu indicate that all streams on this island consistently exceed the USEPA standards (200 fecal coliform/100 ml, 33 enterococci/100 ml) for water quality. Soil was determined to be the source of the elevated counts of these bacteria. In tropical areas, as Hawai'i, these bacteria are able to survive and multiply in the soil. Thus, these bacteria can end up in nearby streams after heavy rains or due to erosion. As a result, the USEPA recommended indicator bacteria (fecal coliform, enterococci) cannot be used to reliably determine when waters in tropical areas are fecally contaminated. Several alternative indicators have been proposed for such areas such as *C. perfringens* and FRNA coliphages. Extensive monitoring data does not exist for the other islands of Hawai'i. Kaua'i differs from O'ahu in that it is older, wetter and contains an abundance of cesspools. The Nawiliwili Watershed, on the island of Kaua'i, was chosen for this study. Sampling was conducted over a period of one year, and all samples were assayed for the traditional USEPA indicators (fecal coliform, enterococci) as well as two alternative indicators (*C. perfringens*, FRNA coliphages). Of the 14 sites sampled, 12 contained levels of fecal coliform and enterococci that exceeded the USEPA standards (200 fecal coliform/100 ml and 33 enterococci/100 ml. This is similar to what has been documented in O'ahu streams. Based on the concentrations of these indicator bacteria, the USEPA would deem these sites as sewage contaminated. However, monitoring of these same sites for *C. perfringens* indicated that there was no sewage contamination (geometric mean values fell below the proposed standard of 50 CFU/100 ml). FRNA coliphage data indicate that cesspools may be leaching into nearby streams. Two streams (Nawiliwili, Papakōlea) had geometric mean levels greater than the 50 PFU/100 ml (based on O'ahu streams). Other streams in the watershed may be sporadically contaminated by cesspool because elevated FRNA coliphage levels were detected on occasion. Genotyping these FRNA coliphage isolates furthered supported the theory that cesspools were contaminating these sites because 98% of the FRNA isolates were typed as human while only 2% were typed as of animal origin. Current USEPA standards (fecal coliform, enterococci) are not reliable indicators of sewage pollution in tropical

areas, thus, alternative indicators such as *C. perfringens* and FRNA coliphages may prove to be better indicators in these areas.

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CHAPTER 1

INTRODUCTION

1.1 Pathogens in Recreational Waters

The recreational uses of fresh and marine waters for contact sports (swimming, surfing, canoeing) and non-contact sports (fishing, picnicking) is increasing worldwide. Waters contaminated by human sewage can cause health problems because of the presence of infectious microorganisms that can cause gastrointestinal infections following accidental ingestion. Furthermore, infections of the upper respiratory tract, ears, eyes, nasal cavity and skin can also result upon exposure to these microorganisms. These illnesses are often mild and may not be readily attributed to water exposure. However, epidemiological studies have shown a correlation between exposure to fecally polluted recreational water and gastrointestinal and respiratory infections. There are several sources of fecal pollution of recreational waters. Among these are accidental sewage discharges, leaching of septic tanks and runoff from rivers that are receiving waters from sewage discharge (WHO, 2003).

A variety of pathogens including bacteria, viruses, protozoa and helminths are found in human sewage (see Table 1.1). The most frequent category of illnesses associated with exposure to fecally contaminated recreational water is gastroenteritis caused by ingestion of pathogens. The second category of illnesses is associated with contact with pathogens in recreational waters, and these infections lead primarily to respiratory illnesses and ear, eye and skin infections.

Gastroenteritis is the most common disease symptom following the unintentional ingestion of fecally contaminated water, and the most frequently identified etiologic

agent of this disease are the Norwalk-like and Hepatitis A viruses (Debartolomeis & Cabelli, 1991). These viruses require a low dose or low numbers of viruses (10-100 viral particles) to cause infection and disease. Other enteric microorganisms capable of causing gastrointestinal diseases are *Giardia*, *Cryptosporidium*, *E. coli* O157:H7, *Shigella* and *Salmonella*. The dose required to cause illness is dependent upon several factors, such as, the type and form of microorganisms, and the susceptibility of the host. Bacterial pathogens generally require a high infectious dose of 10^6 to 10^8 cells, as in the case of *Salmonella*, to cause symptomatic disease. However, some bacterial pathogens such as *E. coli* 0157:H7 require a low dose of only 10 to 100 cells for onset of illness (EPA, 1999).

Table 1.1. Common Pathogens found in Domestic Sewage.

Organism	Disease/Symptoms
Bacteria	
Salmonella spp.	Salmonellosis (food poisoning), typhoid fever
Shigella spp.	Bacillary dysentery
E. coli (pathogenic strains)	Gastroenteritis
Enteric Viruses	
Hepatitis A virus	Infectious hepatitis
Norwalk and Norwalk-like viruses	Epidemic gastroenteritis with severe diarrhea
Polioviruses	Poliomyelitis
Protozoa	
Cryptosporidium spp.	Gastroenteritis
Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps, weight loss)
Toxoplasma gondii	Toxoplasmosis
Helminth Worms	
Ascaris lumbricoides	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms
Taenia saginata	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances

(Table modified from EPA, 1999)

1.2 Bacteria as Water Quality Indicator System

Because of the potential for illness from exposure to fecally contaminated recreational waters, a method for the routine analysis of these waters is necessary to assess their level of contamination. Though most gastrointestinal illnesses have been attributed to specific viral families, the specific detection of these pathogenic viruses has not been adapted to routine analysis. Cell culture, which has been the traditional method for virus detection and cultivation, is time consuming and does not allow for the detection of all pathogenic viruses such as Norwalk virus. More recent molecular techniques, such as PCR, can yield an overestimation of viral load because no information is obtained concerning viral infectivity (Skraber *et al*, 2004 and Gantzer *et al*, 1998). Furthermore, it is neither realistic nor feasible to assay for all pathogenic viruses that may be present in water.

Due to the limitation of viral assays, a more practical indicator of fecal pollution is needed. In order for this fecal indicator to be useful, it must fulfill the following five criteria: 1. it must be present whenever pathogens are present and should be associated with sewage, 2. the indicator should not be able to multiply in the environment, 3. it should be more resistant to disinfection than pathogens, 4. there should be a positive correlation between the indicator and health hazards caused by fecal pollution and 5. the assay methods should be easy and rapid. Bacterial assays, specifically fecal indicator bacteria (fecal coliform, *E. coli*, enterococci), have been used as a means of assessing when waters have been fecally contaminated. These fecal indicator bacteria are found in high concentrations in the feces of all warm blooded animals (Fujioka, 1988). Thus, their occurrence in environmental waters would lead to the conclusion that these waters are

fecally contaminated and, that these waters may contain other pathogenic microorganisms. These fecal indicator bacteria fulfill most of the criteria for an ideal indicator and most importantly they are easier to isolate and enumerate than viruses. Because of this, the U.S. Environmental Protection Agency (USEPA) has approved the use of these fecal indicator bacteria (*E. coli*, enterococci) in environmental waters as a measure of its hygienic quality and to determine whether these waters are suitable for recreational uses such as swimming and bathing (WHO, 2003).

1.3 History of Recreational Water Quality Standards

Prior to the approval by the USEPA of *E. coli* and enterococci as indicators of fecal pollution, several epidemiological studies were conducted to establish the validity of these microorganisms in establishing whether recreational waters were fecally contaminated, and whether the presence of these indicators correlated with illness in humans who came into contact with these waters.

Initially, the microbiological criteria for bathing waters was based on studies conducted during the late 1940's and early 1950's by the U.S. Public Health Service (USPHS). These early epidemiological studies consisted of the routine monitoring of bathing waters using total coliform bacteria and the questioning of families about their bathing activities and illness. The study was conducted over the summer months, when bather loads are the highest, and it was noted that an excess of gastrointestinal illnesses was observed when the geometric mean of total coliform density was 2,300 per 100 ml. As a result, the first set of recreational water quality standards used by states (federal standards were not set at this time) was set at 1,000 total coliform per 100 ml. In the mid-1960's the USPHS translated this coliform index into a fecal coliform index because

the habitats (soil, plants, insects) of many total coliforms are not fecal. Also, by culturing all coliform bacteria at 44.5°C, thermotolerant fecal coliforms, which were more specific to fecal contamination, grew while most other total coliform bacteria were not able to grow. The fecal coliform standard was based on the total coliform standard of 1,000 total coliform per 100 ml. Since the ratio of fecal coliform to total coliforms was known to be 1:5, the first calculated standard was 400 fecal coliform per 100 ml. However, this index was further halved (200 fecal coliform per 100 ml) because a detectable risk was undesired. As a result, the USEPA first approved the hygienic bacterial water quality standard at a geometric mean concentration of 200 fecal coliforms per 100 ml (EPA, 1986) from 1972 to 1986.

After the establishment of the USEPA in 1970, this agency concluded that water quality standards should be set based on well-designed epidemiological studies to determine the relationship between concentrations of fecal indicator bacteria in the water and rates of swimming-associated illness. The USEPA conducted several extensive epidemiological studies and the concentrations of several potential fecal indicator bacteria (total coliform, fecal coliform, *E. coli*, enterococci, *Klebsiella*, *P. aeruginosa*, Staphylococci) were measured in the water at the time the swimmers were using that body of water. Epidemiological surveys were conducted on weekends because the numbers of swimmers were greatest at that time. For marine beaches the study was conducted at bathing beaches in New York City, Boston and on Lake Pontchartrain. For fresh water beaches a lake in Ohio was used. Two beaches were studied at each site. One beach from each location contained low concentrations of fecal coliforms, while the second beach site had water quality that was barely acceptable based on concentrations of

fecal coliform (200 fecal coliform per 100 ml). The participants were asked about illness symptoms on the day of the test and for 14 days after the swimming event to determine the percent of swimmers who became ill, and the degree of illness. The results of these studies determined that gastroenteritis rates were significantly higher in swimmers from the more polluted of the two sites and concluded that there was an increased risk of illness from swimming in fecally contaminated water. Of the indicators, enterococci was the only fecal indicator that showed a strong relationship to gastroenteritis in seawater. In fresh water sites both *E. coli* and enterococci showed strong relationships to gastroenteritis.

Based on the results of the epidemiological studies, the USEPA, in 1986, established the current recreational water quality standards which were related to acceptable gastroenteritis disease rates. For fresh water the acceptable disease rate was established as 8 out of 1,000 people and the standard was set at geometric mean of 126 *E. coli* per 100 ml or 33 enterococci per 100 ml. For marine waters, the acceptable disease rate of 19 out of 1,000 people was established and the standard was set at a geometric mean of 35 enterococci per 100 ml (USEPA, 1986). A body of water is considered non-compliant if these standards are not met. Individual states have the choice of using any of the fecal indicators that have been approved by the USEPA. Whereas the majority of states have switched to using *E. coli*, fecal coliform and enterococci standards, some states continue to use the total coliform standard (USEPA, 2003).

1.4 Bacterial Indicators in Tropical Environments

Two assumptions were made when fecal indicator bacteria were adopted by the USEPA as indicators of fecal pollution. The first assumption was that the only sources of

these bacteria are the feces of humans and other warm-blooded animals and sewage. The second assumption was that these indicator bacteria do not multiply in the environment. Unfortunately, these two assumptions are not applicable to tropical environments such as Hawai'i and Puerto Rico (Rivera *et al*, 1988 and Hazen, 1988) where these fecal indicator bacteria are naturally present and multiply in the environment. Because of this, the routine monitoring of waters in tropical areas will lead to consistently elevated levels of these indicator bacteria, which do not represent a health risk because they are associated with environmental (non-point) sources rather than a sewage source. Numerous studies (Fujioka *et al*, 1999; Fujioka & Byappanahalli, 1998; Rivera *et al*, 1988) have shown that *E. coli* and enterococci persist in tropical soils and environmental waters. Thus, the current USEPA recreational water quality standards, based on concentrations of fecal indicator bacteria, are not reliable standards for measuring fecal contamination in Hawai'i and other tropical areas.

It should be noted that the study sites sampled by the USEPA to establish water quality standards were all located in temperate regions of the continental U.S. These water quality standards based on concentrations of fecal indicator bacteria were then applied to all regions (temperate and tropical) of the U.S. However, Hawai'i is located in the tropical region of the world where fecal indicator bacteria are naturally present in soil under ambient soil environments. In this regard, tropical soil environments in Hawai'i provide favorable conditions of temperature (22-30°C), moisture and nutrients, which allow for the proliferation of these microorganisms (Fujioka & Byappanahalli, 2000).

It should also be noted that the USEPA conducted one epidemiological study at a Connecticut recreational pond on the continental U.S. The results of this study showed

that when recreational waters were contaminated with non-point sources (not sewage but land run-off), the concentrations of fecal indicator bacteria in the water were not correlated to illness rates among swimmers (Calderon *et al*, 1991). Unlike the swimming sites chosen by the USEPA for their epidemiological study, the conditions of the Connecticut study are more closely related to conditions in Hawai'i. However, the USEPA has chosen to ignore the results of this study for application to the rest of the country.

1.5 Alternative Water Quality Indicators

Due to high concentrations of USEPA approved fecal indicator bacteria in all streams, these bacteria represent a non-point source (soil) of pollution of waterways in tropical climates such as Hawai'i. Thus, a more reliable indicator of point source (sewage) pollution is needed. Two alternative indicators that have been proposed are *Clostridium perfringens* and FRNA coliphages. Both *C. perfringens* and FRNA coliphages are found in high concentrations in sewage and do not multiply under ambient conditions. They are found in low concentrations in streams under ambient conditions and increase when sewage contamination occurs. Furthermore, *C. perfringens* and FRNA coliphages meet four of the five recommended criteria for an ideal indicator (see Section 1.2). Both microorganisms are associated with sewage and do not multiply in the environment. They are also present when pathogens are present and are more resistant to disinfection than these pathogens. However, because extensive epidemiological studies have not been conducted in Hawai'i to show a correlation between elevated concentrations of *C. perfringens* or FRNA coliphages in recreational waters and

increased illness rate in humans, the USEPA has yet to approve water quality standards based on concentrations of *C. perfringens* and FRNA coliphages in tropical areas.

1.5.1 *Clostridium perfringens* as Alternative Water Quality Indicator Used in Hawai'i

C. perfringens is an anaerobic, Gram-positive rod shaped bacterium found in the intestinal tract of humans and other warm blooded animals. It is consistently associated with sewage, though its densities are lower in human fecal material than other indicators, and is present in high concentrations in sewage (10^4 CFU per 100 ml). Because these microorganisms have the ability to sporulate they are more resistant to environmental stresses and disinfection. The presence of spores is useful in detecting past contamination events that can be missed by the traditional fecal bacterial indicators. Because *C. perfringens* cannot multiply in the soil, its numbers will not increase, like that of fecal coliforms, after a heavy rain event. In uncontaminated streams on O'ahu, the numbers of *C. perfringens* can range from 0 to 46 CFU per 100 ml while in sewage discharge sites the numbers can range from 56 to 2100 CFU per 100 ml (Shizumura, 1982). Fujioka had recommended that recreational water quality standards based on *C. perfringens* are more reliable in determining when streams and coastal waters are contaminated with sewage than the USEPA recommended standards. The Hawai'i State Department of Health has accepted, and currently uses, the *C. perfringens* standard based on a geometric mean of 50 CFU/100 ml for stream water and 5 CFU/100 ml for coastal marine waters (Roll & Fujioka, 1997).

1.5.2 Somatic Coliphages: The First Alternative Fecal Water Quality Indicator

The effectiveness of using coliforms as indicators has come into question because most enteric viruses (Poliovirus, Coxsackie virus, Hepatitis virus, Norwalk virus) are able to survive wastewater chlorination better than most bacteria. Therefore, the absence of these indicator bacteria may not necessarily mean that pathogenic viruses, and more resistant pathogenic bacteria, are absent. Though coliform levels in environmental waters have been shown to be poorly correlated with levels of enteroviruses (Debartolomeis & Cabelli, 1991), the routine monitoring for enteric viruses is not feasible because their enumeration is labor intensive, expensive and time consuming.

Bacteriophages are viruses that infect bacteria, and coliphages are a class of bacteriophages that infect fecal bacteria. Somatic coliphages are those that attach to the outer cell wall of coliform bacteria. They were the first coliphages detected by scientists and were the first coliphages proposed as an alternative to fecal indicator bacteria because they are more similar to human enteric viruses than fecal bacteria. Moreover, their detection methods are far more rapid, inexpensive and easy to perform than detection methods for human enteric viruses. Somatic coliphages, like other viruses, are obligate intracellular parasites that use their bacterial hosts' biosynthetic machinery to replicate. They are composed of a core nucleic acid (double stranded DNA) covered by a complex protein configuration. Most somatic coliphages range in size from (20 to 200 nm) in length and infect their bacterial host by attaching to receptor sites on the surface of the bacterium and injecting their nucleic acid into the host cell (Adams, 1959 and Black, 1996). Once this takes place, the coliphage takes over the biosynthetic machinery of the

host and uses it to synthesize viral components and to form infectious viruses which are then released into the environment as the host cell lyses.

Somatic coliphages were the first group of coliphages evaluated as alternative fecal indicators. However, monitoring data for somatic coliphages were inconsistent with many other water quality parameters and reflected the fact that somatic coliphages are very heterogeneous, in terms of sources, and some have been reported to multiply under environmental conditions (Gerba, 1987). Furthermore, somatic coliphages have been reported to be variable in human feces and have been found more often in the feces of animals than in that of humans (Calci *et al*, 1998; Gerba, 1987 and Havelaar *et al*, 1986). Because somatic coliphages are comprised of a heterogeneous population, their reported stability in the environment has also been variable. While some coliphages are capable of persisting as long as enteric viruses, studies have shown that many can become inactivated more easily than human enteric viruses (Muniesa *et al*, 1999). This may explain why somatic coliphages have not been isolated even in the presence of pathogenic viruses (Gerba, 1987). As a result, state and federal agencies, including the USEPA, have been reluctant to use somatic coliphages as markers of fecal pollution.

1.5.3 Male-Specific RNA Coliphages (FRNA coliphage)

Male-specific RNA (FRNA) coliphages are by definition those coliphages that infect the F pilus of male strains of fecal bacteria such as *E. coli*. After somatic coliphages were not supported as good fecal indicators, several water microbiologists from around the world began to evaluate FRNA coliphages as reliable indicators for the presence or absence of human enteric viruses in environmental waters. The use of FRNA coliphages as indicators has been proposed for several reasons: 1. they are more resistant

to chlorination and inactivation by environmental factors than fecal bacteria, 2. the methods for their enumeration is direct, rapid and cost effective, 3. they are found in high densities in both untreated and treated sewage, 4. they are unlikely to multiply in the environment (this is because the likelihood of encountering an *E. coli* host strain containing F-pili in the environment is rare) and 5. studies have shown a high correlation between FRNA coliphages and viral concentrations in raw and treated sewage, raw and treated drinking water and surface and recreational waters (Cole *et al*, 2003 and IAWPRC, 1991). Many scientists support the use of FRNA coliphages as indicators of the presence or absence of human enteric viruses because these coliphages are similar in size and structure to human enteric viruses.

In summary, FRNA coliphages contain a single strand of RNA contained within an icosahedral capsid similar to human enteric viruses. They are about 25 nm in diameter and infect via the adsorption and penetration of the virus into the F-pili produced by susceptible *E. coli* host strains. These pili are coded by the F gene which can be located either on a plasmid or within the chromosome of male *E. coli* cells (Brinton, 2002). There may be several types of pili on one host cell, but of these only one to four may be F pili. There are several *E. coli* strains that can serve as host to FRNA bacteriophages. Ideal host strains should only support the growth of male-specific bacteriophages and not serve as host to somatic phages. One such *E. coli* strain is HS(pFamp)R. This strain was created to be resistant to somatic coliphages (T2 and T4). This strain was also made to be resistant to streptomycin and ampicillin (this antibiotic resistance is very useful as it will suppress the growth of many other bacteria which can interfere with the FRNA coliphage assays, including enrichment procedures). At temperatures less than 25°C, pili formation

will not occur in the *E. coli* host and studies have shown that under environmental conditions, *E. coli* hosts will not produce pili. Additional studies have shown that this strain of *E. coli* will support the growth of more than 95% of male specific bacteriophages from sewage (Debartolomeis & Cabelli, 1991).

Two relevant findings regarding FRNA coliphages have been reported. First, FRNA coliphages cannot be recovered from most human fecal matter and only from feces of a few animals. Second, studies in Japan have shown that human fecal matter may contain an inhibitory agent that may decrease the efficiency of plating of coliphages (Kamiko *et al*, 1994). This inhibitory agent has yet to be identified and characterized. However, FRNA coliphages are consistently present at high densities in sewage. As a result, it is an excellent indicator of sewage pollution.

1.6 Sero-Typing of FRNA Coliphages

A serious limitation of most fecal indicators is that the source of the fecal indicator cannot be determined. Sero-typing of FRNA bacteriophages has been established as a useful method to distinguish human from animal fecal contamination in environmental waters (Gerba, 1987). The method of serotyping was the first, and traditional, method used to differentiate between human and animal FRNA coliphage isolates. This method is based on the ability of specific antisera, developed based on the four serotypic groups (I, II, III, IV) of FRNA coliphages, to neutralize, and thus differentiate, between human and animal FRNA isolates. However, reliable antisera are not readily available, and it has been reported that several FRNA coliphage isolates, particularly those from surface waters, have given ambiguous results because they have been neutralized by more than one antiserum (Hsu *et al*, 1995).

1.7 Genotyping of FRNA Coliphages

Genotyping of FRNA coliphages was developed as a method to provide more consistent results. This is because reagents for genotyping are much easier to obtain and the quality of genotyping reagents are much more consistent as compared to antisera used in serotyping. The method of genotyping employs specific gene probes to differentiate between the four genotypic groups (I, II, III, IV) of FRNA coliphages. Groups I and IV have been found associated with animal feces while groups II and III have been associated with human feces and sewage (Hsu *et al*, 1995 and Beekwilder *et al*, 1996). However, there have been several studies that show FRNA isolates from pig feces cross react with probes of the human group (Hsu *et al*, 1995). This cross reactivity may be due to the fact that the digestive tracts of humans and pigs are similar, and thus, may share the same microflora.

Genotyping of FRNA isolates is based on their ability to hybridize with probes that correspond to the four sero-groups of FRNA coliphages. These probes were constructed based on conserved sequences in representative FRNA coliphages (Table 1.2). Extensive testing of these probes with FRNA isolates from sewage and various animal feces has shown the ability of the probes to differentiate between these two sources with the exception of cross reactions seen between pig fecal isolates and the human probes (Hsu *et al*, 1995).

Table1.2. Oligonucleotide probes used for the genotypic identification of FRNA isolates.

Probe	Locus	Length (bases)	Source Phage
I	Maturation protein	26	MS2
II	Maturation protein	27	GA
III	5' end nontranslated region	25	Qβ
IV	5' end nontranslated region	25	SP

The risk of viral gastroenteritis and hepatitis in humans resulting from ingestion of water contaminated with human fecal material is higher than the risk from contamination with fecal matter from animals (Hsu *et al*, 1995). In order to reduce this public health risk, it is important to be able to distinguish between human and animal fecal pollution events so that control measures can be taken. Additional genetic information about coliphage strains found in human and animal impacted environments may be useful in identifying more specific markers capable of distinguishing between the two sources. Though studies have shown no significant difference between serotyping and genotyping, the unavailability of reliable antisera has made the method of genotyping the preferred choice for source tracking.

CHAPTER 2

THE PROPOSED STUDY

2.1 Identification of the Problem

It has been previously reported that all streams on the island of O'ahu routinely exceed the recreational water quality standards based on concentrations of all USEPA-recommended fecal indicators (fecal coliform, enterococci, *E. coli*) (Fujioka, 1998). Soil was determined the source of these fecal bacteria in stream water and the concentrations could not be related to sewage contamination (Hardina & Fujioka, 1991). One solution was to monitor streams for alternative fecal indicators (*C. perfringens*, FRNA coliphages) which do not multiply under environmental conditions, and whose numbers increase when sewage contamination occurs.

One limitation of these studies is that most of the water quality studies were conducted on the island of O'ahu, and it was assumed similar results would be observed on the other islands of Hawai'i. However, soil composition and geology of the various islands can be expected to differ because the age of the islands differs.

2.2 The Problem

Although extensive water monitoring studies have been conducted on O'ahu, similar studies have not been conducted on the other neighbor islands. Therefore, results obtained on O'ahu cannot be assumed to be applicable to all islands without conducting similar studies on each island. The islands of Kaua'i and O'ahu differ in three respects. First, Kaua'i is older (5 million years) than O'ahu (3 million years) (Hazlett & Hyndman, 1996). Second, Kaua'i has higher rainfall than O'ahu, which results in larger streams and rivers, as well as higher moisture in soil. Third, cesspools are more abundant on the

island of Kaua'i (Appendix C) than on O'ahu, and may discharge inadequately treated sewage into soil and surrounding streams.

2.3 Goal and Objectives of the Study

The goal of this study was to determine whether the fecal bacteria monitoring data established for the island of O'ahu are applicable to the island of Kaua'i. The specific objectives of the study were:

1. to evaluate whether the USEPA-approved fecal indicator bacteria (fecal coliform, enterococci) are naturally present in high concentrations in Kaua'i streams.
2. to determine whether these bacteria could be used to reliably determine when the streams are contaminated by sewage/cesspools.
3. to evaluate whether monitoring of these streams using alternative fecal indicators (FRNA coliphages and *C. perfringens*) could provide more reliable data when streams are contaminated by sewage/cesspools.
4. to determine if genotyping of FRNA coliphage isolates could distinguish between human and animal contamination.

2.4 Sample Sites and Sample Collection

The Nawiliwili watershed (Appendix D) consists of Nawiliwili Bay which is fed by four streams (Hulē'ia, Papakōlea, Puali and Nawiliwili). The Hawai'i Department of Health has listed Nawiliwili Bay, Nawiliwili Stream and Hulē'ia Stream as water quality limited bodies of water due to excessive turbidity (El-Kadi *et al*, 2002). Pollution of these streams by non-point sources, resulting in high turbidity, has been previously documented. These streams can also be expected to be contaminated with inadequately treated wastewater from cesspools. Many of these streams are used for various

recreational activities (swimming, kayaking) by local residents and tourists, and these streams discharge and carry pollution to Nawiliwili Bay. Water samples were collected from this watershed between October 31, 2001 and November 25, 2002 by trained personnel on Kaua'i using USEPA-approved methods. Briefly, sterile plastic containers were used to collect the water samples, which were placed into a cooler with ice and promptly shipped to O'ahu (flight time between Kaua'i and O'ahu is approximately 20 minutes). Personnel from our lab picked up the cooler at the O'ahu airport and transported it to the lab. The samples were assayed within six hours of collection. Ten to fourteen samples were sampled and shipped on a bimonthly basis from the following sampling sites (See Appendix E):

Site 1. Nawiliwili Stream Upper. This site is located in Rice Camp and is expected to contain urban and agricultural contaminants.

Site 2. Nawiliwili Stream Lower. This site is located approximately 100 m downstream of Site 1.

Site 3. Marriott Culvert. This site is a combination of stream water and storm drain discharge. It also includes runoff from a nearby golf course. Samples were collected from under a foot bridge before discharge into Nawiliwili Stream.

Site 4. Pine Trees: Estuarine site. This site is downstream of Site 3 and is located where the mouth of Nawiliwili Stream enters into Kalapakī Beach. At this location there is a mixing of stream water and ocean water. Children like to play in this location because sand from the beach creates a bank between the stream and the ocean forming a "wading pool".

Site 5. Kalapakī Beach. This site is located at the center of Kalapakī Beach and is the primary beach site. It is located approximately 0.2 km from the mouth of Nawiliwili Stream.

Site 6. Seaflite Jetty. This site is located at the Nawiliwili Harbor Jetty in Nawiliwili Bay.

Site 7. Small Boat Harbor. This site is located in Nawiliwili Harbor where water from Hulē'ia Stream is discharged into the Bay.

Site 8. Papalinahoa Stream. This sampling site is located near the mouth of the stream before it enters the culvert and flows under the road to Nawiliwili Harbor.

Site 9. Puali Stream Upper. This site is located approximately 30 m upstream from Site 10.

Site 10. Puali Stream Lower. This site is located where water is taken for irrigation purposes by a resident of the area. This stream flows into Nawiliwili Bay near the mouth of Hulē'ia Stream.

Site 11. Papakōlea Stream Upper. This site is located under the bridge on Hulemalu Road.

Site 12. Papakōlea Stream Lower. This site is located about 100 m downstream of Site 11.

Site 13. Hulē'ia Stream Upper. This site is upstream of Alekoko Fishpond. Samples were collected at the stone bridge at the end of Haiku Road.

Site 14. Hulē'ia Stream Lower. This site is located about 30 m downstream of Site 13.

Raw sewage samples were obtained by Kaua'i personnel from the Lihue Wastewater Treatment Plant (WWTP) in sterile one liter bottles, packed in ice and shipped to O'ahu. Samples were assayed within six hours of collection for all fecal indicator bacteria and somatic and male specific coliphages. A total of three sewage samples were obtained. Two cesspool samples were obtained from Hanapepe Salt Pond Park (located approximately 18 miles from the Lihue Airport). One sample was obtained from the women's restroom and the other was obtained from the men's restroom. Two cesspool samples were also collected from a restroom at Hanalei Bay Pavilion which is located on the northern part of Kaua'i. All samples were collected into sterile one liter bottles and shipped in the same manner as the sewage samples. Three soil samples were collected from the banks of Nawiliwili, Hulē'ia and Puali Streams. The samples were collected by Dr. Roger Fujioka with sterile wooden spatulas and placed into sterile Whirlpak bags. Dr. Fujioka then transported the samples in a cooler to O'ahu where they were assayed the following day.

2.5 Materials and Methods

2.5.1 Fecal Indicator Bacteria in Water, Sewage and Cesspool Samples

The Membrane Filtration Procedure was used for the enumeration of fecal coliform, enterococci (Standard Methods 20th Edition, Sections 9222 & 9230) and *C. perfringens* (Bisson & Cabelli, 1979). A phosphate buffer solution (PBS), containing 68g potassium dihydrogen phosphate (KH_2PO_4) and 38g magnesium chloride ($MgCl_2$) in 1 L distilled water, was used to dilute the water samples prior to filtration. Water samples were diluted as needed into sterile PBS, and 25 ml portions were filtered through a 0.45 μm Gelman filter and placed on various media (mFC, mE and mCP) to enumerate

fecal coliforms, enterococci and *C. perfringens*, respectively. Upon filtration, mFC plates were incubated in a 44.5°C water bath for 24 hours, mE plates were incubated at 41°C for 48 hours and mCP plates were incubated at 42°C in an anaerobic chamber for 24 hours. After incubation, target colonies were counted following the guidelines for each of the procedures.

2.5.2 Fecal Indicator Bacteria in Soil Samples

Bacterial assays for the soil samples were done by the 5-tube most-probable-number (MPN) method (Standard Methods, Sections 9230B and 9221B). 10g of soil was added to 90 ml of PBS and shaken vigorously for 20 minutes. A dilution series (10^{-1} to 10^{-5}) was made, and 1 ml aliquots were placed into MPN tubes containing 10 ml of Lauryl Tryptose Broth (LTB) with inverted Durham tubes (coliforms) and Azide Dextrose Broth (ADB) for enterococci. The tubes were incubated at 37°C for 48 hours. Growth from positive LTB tubes were inoculated into EC broth and incubated at 44.5°C for 24 hours. Positive ADB tubes were confirmed for the presence of enterococci was confirmed by growth on Bile Esculin Agar and Brain Heart Infusion broth. Concentrations based on MPN/g of soil were determined following the procedure as reported by Fujioka & Byappanahalli, 2000.

2.5.3 Coliphage Enumeration in Water, Sewage and Cesspool Samples

2.5.3.1 Host Strain Preparation

E. coli HS(pFamp)R was used as the host strain for the enumeration of F-specific coliphages (Debartolomeis & Cabelli, 1991), and *E. coli* CN13 (EPA Method 1602) was used for the enumeration of somatic coliphages. Both strains were grown in Tryptic Soy Broth (TSB) from frozen bead (ATCC) in the presence of ampicillin and nalidixic acid,

respectively. Stock concentrations of both antibiotics were made by dissolving 1.5 g of the antibiotic in 100 ml of distilled water and filter sterilizing through a 0.45 μm filter membrane. This stock constitutes a 100X concentration of the antibiotic. *E. coli* HS(pFamp)R was grown in TSB containing a 1X concentration of ampicillin (1 ml of 100X stock ampicillin in 100 ml TSB) while *E. coli* CN13 was grown in the presence of 1X nalidixic acid (1 ml of 100X stock nalidixic acid in 100 ml TSB). A stock plate of each host *E. coli* culture was made by streaking onto Tryptic Soy Agar (TSA) plates containing a 1X concentration of the appropriate antibiotic. A well isolated colony from each plate was streaked onto another plate to create a working plate for each host. Working plates were kept for one month and discarded and a new plate was created from the stock plate.

For the coliphage assays, an isolated colony of *E. coli* from the working plate was inoculated into 5 ml of TSB containing either ampicillin or nalidixic acid (depending on the host strain) and incubated overnight at 37°C. A three-hour host culture was made by inoculating 0.5 ml of the overnight culture into a fresh tube of TSB (5 ml) and incubating it for three hours at 37°C. This assures that the *E. coli* are in their log phase of growth and pili formation in the HS(pFamp)R has occurred.

2.5.3.2 Double Layer Plaque Assay

The double layer plaque assay was used for the enumeration of somatic and male specific bacteriophages (Debartolomeis & Cabelli, 1991). This method allows for the enumeration of viable bacteriophages and is fairly rapid and simple. However, one major drawback is that only a small volume of sample (5 ml) can be used for the assay. This

may lead to coliphages being undetected, particularly in samples where their concentrations are low.

The double layer plaque assay is a fairly simple assay that consists of using two layers of agar, top and bottom agar, to enumerate plaques. Top agar consisted of 20 g tryptone, 2 g dextrose, 10 g sodium chloride, 10 g yeast extract, 0.015 g calcium chloride and 15 g agar in one liter of deionized water. The bottom agar consisted of 10 g tryptone, 1 g dextrose, 5 g sodium chloride and 15 g agar in one liter of deionized water (Debartolomeis & Cabelli, 1991). Both agar media were sterilized by autoclaving for 15 minutes at 121°C. After both agars had cooled to 55°C, 4 ml of the top agar was dispensed into sterile tubes and stored until used. A 5X concentration of ampicillin (5 ml of 100X stock ampicillin in 100ml bottom agar) was added to the cooled bottom agar and the agar was poured into sterile Petri plates to make the 5X ampicillin bottom agar. For somatic coliphage enumeration, a 1X concentration of nalidixic acid (1 ml of 100X stock nalidixic acid in 100 ml bottom agar) was added to the bottom agar to make the 1X nalidixic acid bottom agar. All agar reagents were stored at 4°C until used.

The plaque assay consisted of adding 5 ml of sample into a tube containing 4 ml of melted top agar, adding 0.3 ml of a three-hour host, and pouring the entire contents of the tube onto a solidified plate of bottom agar. Once the top agar solidified, the plate was incubated for 24 hours at 37°C, and clear, round spots (plaques) are counted. Presumptive FRNA plaques from the double layer plaque assay were picked using sterile Pasteur pipettes and placed into individual sterile tubes containing 0.5 ml of PBS containing 15% glycerol (Hsu *et al*, 1995). The bacteriophage isolates were stored at 4°C until ready for use.

2.5.3.3 Enrichment for Presence/Absence of Coliphages

Because only 5 ml of sample can be assayed with the double layer plaque assay, coliphages may go uncounted in samples with low concentrations. For samples that did not yield visible plaques, a method to test 100 ml of water sample for the presence of absence of coliphage was done (EPA, Method 1601). This method is called enrichment because the host bacteria is allowed to grow to high concentrations in 100 ml of water sample, and even one virus in the 100 ml water sample will now have the chance to infect a host cell and release thousands of coliphage into the test sample. Once these viruses reach a high concentration they can be easily detected by the plaque assay method.

This procedure consisted of two steps. First, a 100 ml aliquot of sample was enriched by the addition of 10 ml of 10X TSB and 1 ml of a three-hour host culture. Incubation was done for 24 hours at 37°C. Second, a 35 ml aliquot of the enrichment was centrifuged and the double layer plaque assay was performed using 1 ml of the enriched supernatant. Plates are scored as positive or negative for plaques.

2.5.4 Genotyping of FRNA Isolates

Genotyping of the FRNA bacteriophage isolates was done to determine whether the stream samples were being impacted by human or animal contamination. This method is a modified version of that used by Hsu *et al*, 1995.

Prior to the isolates being genotyped it was necessary to determine whether they were FRNA or FDNA coliphages. In order to accomplish this, a 0.1% solution of RNase was added to top agar tubes containing the *E. coli* HS(pFamp)R host and poured onto bottom agar plates. After the plate solidified, 2 µl of each coliphage isolate was spotted using a micro-pipette onto the plate, which was then incubated at 37°C for 24 hours.

After incubation, plates were checked for zones of lysis (plaques) where spots were made. Isolates that exhibited lysis were classified as FDNA because RNase will specifically destroy RNA but not DNA. Thus, this method allows FDNA coliphage but not FRNA coliphage to form plaques. Isolates that did not form lysis were classified as FRNA and genotyping was performed on these isolates.

Genotyping of FRNA coliphages was done by first extracting the coliphages from the double-layer agar plate via a Pasteur pipette and homogenizing it in PBS to make a suspension of each coliphage isolate. Only 2 μl of this FRNA coliphage suspension was spotted onto 0.45 μm nylon membranes and allowed to be absorbed for two minutes. A 10X solution of SSC consisting of 9.73 g NaCl and 4.90 g sodium citrate in 100 ml distilled water was made and used in various steps as part of buffers and washing solutions. The coliphages on the membranes were denatured with blotting buffer (7.5X SSC and 4.6 M formaldehyde) and the released coliphage RNA fixed to the membrane by UV irradiation and baking at 80°C. The probes (I, II, III, IV) were labeled at the 3' end with digoxigenin (DIG)-ddUTP using a DIG Oligonucleotide 3'-end labeling kit and were allowed to hybridize with the coliphage RNA overnight at 45°C. After hybridization the membranes were washed (6X SSC and 0.1% SDS) and an anti-DIG sheep antibody was added to the membrane. This antibody will bind to the probes that have hybridized with the RNA. Non-specific hybridization of probes was prevented by using a high concentration of probe (5pmol/ml), a high hybridization temperature of 45°C and a concentrated washing solution (6X SSC and 0.1% SDS). Two substrates, 5-bromo-4-chloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium chloride (NBT), were used for the colorimetric detection of the DNA:RNA hybrids. A detection buffer consisting of

0.1M Tris, 0.1M NaCl, 0.019% (w/v) BCIP and 0.04g (w/v) NBT at pH 9.5 was added to the membranes and incubated at room temperature for 16 hours. Specific hybrids, demonstrating the presence of FRNA coliphage, appeared as dark bluish-purple spots on the membrane.

2.5.5 Chemical Parameters (Phosphate, Nitrate, Turbidity, Salinity) of the Water Samples

Several physical parameters including phosphate, nitrate, turbidity and salinity were measured in the water samples. Phosphate levels were measured using the ascorbic acid method (Hach DR/3000 Procedure Code P.4) which measures low range (0-2.0 mg/L) levels of phosphates. Nitrate levels were measured using the cadmium reduction method (Hach DR/3000 Procedure Code N.6) which measures medium range (0-5.0 mg/L) levels of nitrates. Turbidity was measured with a Hach Turbidometer and salinity was measured using a refractometer. A suggested standard of 0.01 mg/L phosphate and 0.1 mg/L nitrate was used to determine when nutrient loads were in excess at the 14 sampling sites. These values were based on studies conducted on unpolluted streams on O'ahu (El-Kadi, 2003). A turbidity value of 5 NTU (Nephelometric Turbidity Units) was used to determine if excess runoff was present in the sampling sites. Salinity measurements were taken of water samples to characterize the site as fresh water, estuarine or ocean water.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Assessment of Water Quality at Each of the 14 Sites in the Nawiliwili Watershed

Up to now most conclusions on recreational water quality for the state of Hawai'i have been based on monitoring data from the island of O'ahu. On O'ahu, all streams contain all fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) at concentrations well above the standards set forth by the USEPA. Extensive monitoring of streams on the other Hawaiian islands have not been done. To determine if the same results obtained on O'ahu can be expected on the island of Kaua'i, water samples from 14 sites within the Nawiliwili Watershed (see Appendix E) were assayed for various microbial indicators (fecal coliform, enterococci, *C. perfringens*, FRNA coliphages, somatic coliphages) as well as selected physical (turbidity, salinity) and nutritional (phosphate, nitrates) parameters at the frequency of twice a month for approximately one year. A total of 131 samples were analyzed and the results of each assay for each site is summarized in Appendices A and B. The results of all assays are summarized as geometric mean concentrations in Tables 3.1 and 3.3.

A. Nawiliwili Stream

Site 1: Upper Nawiliwili Stream. Water samples from this site were characterized with a salinity of less than 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (10 NTU) of water at this site was high and exceeded the 5 NTU standard supporting previous reports (El-Kadi *et al*, 2002) that sediments are entering this stream. The geometric mean nitrate (0.4 mg/L) exceeded the suggested standard of 0.1 mg/L and the geometric mean phosphate (0.04 mg/L) exceeded

the suggested standard of 0.01 mg/L. These results (Table 3.1) indicate that organic components such as wastewater or fertilizers are entering this stream at this site. In these same water samples, the geometric mean value of 7,740 CFU/100 ml fecal coliform greatly exceeded the USEPA standard of 200 CFU/100 ml, and the geometric mean value of 2,914 CFU/100 ml enterococci greatly exceeded the USEPA standard of 33 CFU/100 ml (Table 3.1). According to the USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

Site 2: Lower Nawiliwili Stream. Water samples from this site were characterized with a salinity of less than 1 ppt, confirming that this water represents fresh stream water. The geometric mean turbidity (7 NTU) of water at this site exceeded the 5 NTU standard supporting previous reports (El-Kadi *et al*, 2002) that sediments are entering this stream. The geometric mean nitrate (0.3 mg/L) and phosphate (0.07 mg/L) values exceeded the suggested nitrate standard of 0.1 mg/L and the suggested phosphate standard of 0.01 mg/L. These results (Table 3.1) indicate that organic components such as wastewater or fertilizers from run-off are entering this stream at this site. The geometric mean value of 3,036 CFU/100 ml fecal coliform greatly exceeded the USEPA standard of 200 CFU/100 ml, and the geometric mean value of 923 CFU/100 ml enterococci also

greatly exceeded the USEPA standard of 33 CFU/100 ml (Table 3.1). According to the USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

Site 3: Marriott Culvert. Water samples from this site were characterized as fresh stream water because geometric mean salinity levels were less than 1 ppt. The geometric mean turbidity level of 5 NTU matched the 5 NTU standard, indicating that elevated levels of sediments were being contributed from this site. Geometric mean phosphate (0.14 mg/L) and nitrate (0.7 mg/L) levels at this site greatly exceeded the suggested standards of 0.01 mg/L phosphate and 0.1 mg/L nitrate. These results (Table 3.1) are indicative that organic components such as wastewater or fertilizers from run-off are entering this site. Geometric mean values for both fecal coliform (4,915 CFU/100 ml) and enterococci (1,939 CFU/100 ml) greatly exceeded the USEPA standards of 200 fecal coliform/100 ml and 33 fecal coliform/100 ml (Table 3.1). According to the USEPA this site would be considered contaminated by sewage. However, fecal indicator bacteria levels for this site are similar to what has been reported for the island of O'ahu (Roll & Fujioka, 1997). The source of these bacteria in O'ahu streams have been determined to be soil, where these bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted

that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

Site 4: Pine Trees (Estuarine). Water samples for this site were characterized as primarily fresh stream water with a geometric mean salinity value of 1 ppt. However, on occasion (see Table B-4 in Appendix B), salinity levels did increase to as high as 5.4 ppt, indicating that some mixing of ocean water from Kalapakī Beach (Site 5) was present. The geometric mean turbidity level (8 NTU) exceeded the 5 NTU standard indicating that sediments were entering this site. Geometric mean phosphate (0.08 mg/L) and nitrate (0.3 mg/L) levels at this site exceeded the suggested standard of 0.01 mg/L phosphate and 0.1 mg/L nitrate. These results (Table 3.1) indicate that water at this site is primarily fresh stream water but occasionally mixes with ocean water and the quality of water is characterized by elevated concentrations of turbidity, phosphate and nitrates. In these same water samples, the geometric mean values for both fecal coliform (3,971 CFU/100 ml) and enterococci (1,918 CFU/100 ml) greatly exceeded the USEPA standard of 200 fecal coliform/100 ml and 33 enterococci/100 ml (Table 3.1). Based on USEPA guidelines, this site is consistently contaminated with sewage. However, fecal indicator bacteria levels for this site are similar to what has been reported for the island of O'ahu (Roll & Fujioka, 1997). The source of these bacteria in O'ahu streams has been determined to be soil, where these bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

B. Ocean Sites

Site 5: Kalapakī Beach (marine beach). Water samples from this site were characterized with a geometric mean salinity of 32 ppt, confirming that water at this site is primarily ocean water but contains some fresh water influence. Water at this site was characterized by geometric mean turbidity level of 5 NTU, 0.02 mg/L phosphate and 0.3 mg/L nitrate. These results indicate that a significant volume of Nawiliwili Stream, which empties into Nawiliwili Bay approximately 0.2 km from this site, is being transported to Kalapakī Beach. In these same water samples the geometric mean values for both fecal coliform (11 CFU/100 ml) and enterococci (14 CFU/100 ml) fell below the USEPA standards of 200 fecal coliform/100 ml and 35 enterococci/100 ml (Table 3.1). However, the geometric mean level of 14 enterococci/100 ml exceeded the state of Hawai'i standard of 7 enterococci/100 ml. These results show that the drainage of Nawiliwili Stream into Nawiliwili Bay is being transported to Kalapakī Beach and lowering the quality of water at Kalapakī Beach below State of Hawai'i marine recreational water quality standards.

Site 6: Seaflight Jetty (Harbor). This is a harbor site designated for harbor uses such as boating and fishing but not for primary contact such as swimming. Most harbors are expected to be impacted by land run-off, including streams. Water samples from this site were characterized with a geometric mean salinity of 30 ppt, confirming that this site represents an ocean water site receiving stream water input. Turbidity for this site (4 NTU) fell below the recommended standard of 5 NTU indicating that sediments flowing into this site were generally low. Phosphate levels for this site had a geometric mean value of 0.02 mg/L which is slightly above the recommend standard of 0.01 mg/L. The

geometric mean nitrate level at this site was 0.3 mg/L which exceeded the standard of 0.1 mg/L. Elevated levels of phosphate and nitrate suggest that organic components such as wastewater or fertilizers from run-off may be entering Nawiliwili Bay via Papalinahoa Stream (Site 8). Geometric mean values of both fecal coliform (3 CFU/100 ml) and enterococci (3 CFU/100 ml) at this site were low and met the USEPA standards of 200 fecal coliform/100 ml and 35 CFU/100 ml. Similar to the Kalapakī Beach Site, this site is an ocean site, thus indicator bacteria are being diluted and dispersed by tides as they enter from Papalinahoa Stream. Thus, it is not surprising levels of these bacteria fell below the USEPA standards.

Site 7: Small Boat Harbor. This is a harbor site designated for harbor uses such as boating and fishing but not for primary contact such as swimming. Most harbors are expected to be impacted by land run-off, including streams. Water samples from this site were characterized with a geometric mean salinity of 7 ppt, confirming that this harbor site is primarily fresh water with stream input from Hulē'ia and Puali Streams. The geometric mean turbidity level (9 NTU) at this site exceeded at the 5 NTU standard, indicating that sediments were entering this site, most likely from run-off from Hulē'ia and Puali Streams. The geometric mean value of phosphate (0.04 mg/L) at this site exceeded the 0.01 mg/L phosphate standard, and barely met the nitrate standard of 0.1 mg/L. These results (Table 3.1) are indicative that run-off from Hulē'ia and Puali Streams are dumping organic components such as wastewater or fertilizers at this site and into Nawiliwili Bay. These same water samples were characterized by geometric mean concentrations of both fecal coliform (259 CFU/100 ml) and enterococci (155 CFU/100 ml) which exceeded the USEPA standards of 200 fecal coliform/100 ml and 35

enterococci/100 ml. Based on USEPA guidelines, this site is contaminated with sewage. These elevated levels can be attributed to these bacteria being washed into this site from Hulē'ia and Puali Streams. Elevated concentrations of fecal coliform and enterococci in these two streams are similar to what has been reported for the island of O'ahu (Roll & Fujioka, 1997). The source of these bacteria in O'ahu streams has been determined to be soil, where these bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

C. Papalinahoa Stream.

Site 8: Mouth of Papalinahoa Stream. This site represents lower Papalinahoa Stream just before this stream discharges into Nawiliwili Harbor. Water samples from this site were characterized with a salinity of less than 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (9 NTU) of water at this site was high and exceeded the 5 NTU standard supporting previous reports (El-Kadi *et al*, 2002) that sediments are entering this stream. The geometric mean nitrate (0.1 mg/L) barely met the suggested standard of 0.1 mg/L and the geometric mean phosphate (0.05 mg/L) exceeded the suggested standard of 0.01 mg/L. These results (Table 3.1) indicate that organic components such as wastewater or fertilizers are entering this stream at this site. In these same water samples, the geometric mean value of 2,557 CFU/100 ml fecal coliform greatly exceeded the USEPA standard of 200 CFU/100 ml, and the geometric mean value of 2,093 CFU/100 ml enterococci greatly exceeded the USEPA standard of 33 CFU/100 ml (Table 3.1). According to the USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with

sewage. However, the concentrations of these two fecal indicators at this site are similar to the levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

D. Puali Stream

Site 9: Upper Puali Stream. Water samples from this site were characterized with a salinity of less than 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (4 NTU) of water at this site met the 5 NTU standard indicating that in general only low levels of sediments were entering Puali Stream at this upper stream site. The geometric mean nitrate (0.2 mg/L) exceeded the suggested standard of 0.1 mg/L and the geometric mean phosphate (0.06 mg/L) greatly exceeded the suggested standard of 0.01 mg/L. These results (Table 3.1) indicate that organic components such as wastewater or fertilizers are entering this stream at this site. The geometric mean value of 697 CFU/100 ml fecal coliform exceeded the USEPA standard of 200 CFU/100 ml, and the geometric mean value of 756 CFU/100 ml enterococci exceeded the USEPA standard of 33 CFU/100 ml (Table 3.1). According to the USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to the levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka

et al, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

Site 10: Lower Puali Stream. Water samples from Lower Puali were characterized with a salinity of less than 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (5 NTU) of water at this site fell at the 5 NTU standard supporting previous reports (El-Kadi *et al*, 2002) that sediments are entering this stream at this site. The geometric mean nitrate level (0.3 mg/L) exceeded the suggested standard of 0.1 mg/L and the geometric mean phosphate level (0.05 mg/L) greatly exceeded the suggested standard of 0.01 mg/L. These results (Table 3.1) indicate that organic components such as wastewater or fertilizers are entering this stream at this site. The geometric mean value of 837 CFU/100 ml fecal coliform and the geometric mean value of 936 CFU/100 ml enterococci greatly exceeded the USEPA standard of 200 CFU of fecal coliform/100 ml and 33 CFU of enterococci /100 ml (Table 3.1). These levels are higher than the upstream site indicating greater contributions of fecal indicator bacteria at this lower Puali Stream site. According to the USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to the levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on

Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

E. Papakōlea Stream

Site 11: Upper Papakōlea Stream. Water samples from at this site were characterized with a salinity of less than 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (<1 NTU) of water at this site was low and well below the 5 NTU standard indicating that lower levels of sediments were entering this upper stream site (Table 3.1). However, the geometric mean nitrate level (0.4 mg/L) greatly exceeded the suggested standard of 0.1 mg/L and the geometric mean phosphate level (0.05 mg/L) also greatly exceeded the suggested standard of 0.01 mg/L. These results (Table 3.1) are indicative that organic components such as wastewater or fertilizers are entering this stream at this site. In these same water samples, the geometric mean fecal coliform value (1,324 CFU/100 ml) and geometric mean enterococci value (1,085 CFU/100 ml) greatly exceeded the USEPA standards of 200 fecal coliform/100 ml and 33 enterococci/100 ml. According to USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to the levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

Site 12: Lower Papakōlea Stream. Water samples from this site were characterized with a salinity of 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (8 NTU) of water at this site greatly exceeded the 5 NTU standard, and was higher than the Upper Papakōlea site indicating that more sediments were entering the stream at this site as compared to the upper stream site. The geometric mean nitrate level (0.2 mg/L) exceeded the suggested standard of 0.1 mg/L and the geometric mean phosphate level (0.04 mg/L) also exceeded the suggested standard of 0.01 mg/L. These results (Table 3.1) are indicative that organic components such as wastewater or fertilizers are entering this stream at this site. The geometric mean fecal coliform value (725 CFU/100 ml) and geometric mean enterococci value (608 CFU/100 ml) greatly exceeded the USEPA standards of 200 fecal coliform/100 ml and 33 enterococci/100 ml but were lower than the Upper Papakōlea Stream site (Table 3.1). According to the USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to the levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams. In this regard, the higher levels of fecal indicator bacteria at the Upper Papakōlea Stream site may be indicative of greater input of cesspool waste at that stream site.

Table 3.1. Geometric mean values of fecal coliform, enterococci and chemical and physical parameters in stream and marine sites on Kaua'i sampled over a period of ten months.

Sample Site (n)	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)[a]	Salinity (ppt)[b]
1 Nawiliwili Stream Upper (10)	7,740	2,914	0.04	0.4	10	<1
2 Nawiliwili Stream Lower (8)	3,036	923	0.07	0.3	7	<1
3 Marriott Culvert (10)	4,915	1,939	0.14	0.7	5	<1
4 Pine Trees (10)	3,971	1,918	0.08	0.3	8	1
5 Kalapakī Beach (10)	11	14	0.02	0.3	5	32
6 Seaflite Jetty (10)	3	3	0.02	0.3	4	30
7 Small Boat Harbor (10)	259	155	0.04	0.1	9	7
8 Papalinahoa Stream (9)	2,557	2,093	0.05	0.1	9	<1
9 Puali Stream Upper (8)	697	756	0.06	0.2	4	<1
10 Puali Stream Lower (10)	837	936	0.05	0.3	5	<1
11 Papakōlea Stream Upper (10)	1,324	1,085	0.05	0.4	<1	<1
12 Papakōlea Stream Lower (8)	725	608	0.04	0.2	8	1
13 Hulē'ia Stream Upper (10)	340	335	0.02	0.1	6	<1
14 Hulē'ia Stream Lower (8)	230	343	0.05	0.1	5	<1

[a] NTU = Nephelometric Turbidity Units
[b] ppt = parts per thousand

F. Hulē'ia Stream

Site 13: Upper Hulē'ia Stream. Water samples from Upper Hulē'ia Stream were characterized with a salinity of less than 1 ppt confirming observations that this water represents fresh stream water. The geometric mean turbidity (6 NTU) of water at this site exceeded the 5 NTU standard supporting previous reports (El-Kadi *et al*, 2002) that sediments are entering this stream. The geometric mean nitrate level (0.1 mg/L) just met the standard of 0.1 mg/L and the geometric mean phosphate level (0.02 mg/L) exceeded the suggested standard of 0.01 mg/L. These results (Table 3.1) indicate that some organic components such as wastewater or fertilizers are entering this stream at this site. In these

same water samples, the geometric mean value of 340 CFU/100 ml fecal coliform exceeded the USEPA standard of 200 CFU/100 ml, and the geometric mean value of 335 CFU/100 ml enterococci greatly exceeded the USEPA standard of 33 CFU/100 ml (Table 3.1). According to USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to the levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

Site 14: Lower Hulē'ia Stream. This site was characterized by a salinity of less than 1 ppt indicating this site represents fresh stream water. Geometric mean turbidity at this site (5 ppt) just met the standard of 5 NTU indicating that some sediments were entering Hulē'ia Stream at this site and supporting previous reports (El-Kadi *et al*, 2002). The geometric mean phosphate level (0.05 mg/L) exceeded the recommended standard of 0.01 mg/L at this site. The geometric mean nitrate level (0.1 mg/L) barely met the standard of 0.1 mg/L. These results (Table 3.1) indicate that this stream was being polluted by organic components such as wastewater or fertilizers at this site. In these same water samples, both geometric mean values of fecal coliform (230 CFU/100 ml) and enterococci (343 CFU/100 ml) exceeded the USEPA standards of 200 fecal coliform/100 ml and of 33 enterococci/100 ml (Table 3.1). According to USEPA, these levels of fecal coliform and enterococci indicate that this stream is extensively

contaminated with sewage. However, the concentrations of these two fecal indicators at this site are similar to levels previously reported for streams on O'ahu (Roll & Fujioka, 1997). The primary source of these fecal bacteria in the streams of O'ahu has been identified as soil, where these fecal bacteria are able to multiply (Fujioka *et al*, 2000). It should be noted that unlike O'ahu, cesspools are extensively used on Kaua'i and these cesspools can be expected to contribute nutrients and fecal bacteria to nearby streams.

3.2 Assessment of Water Quality Based on USEPA Bacterial Water Quality Standards

Fecal Coliform Standard. The USEPA recreational water quality standards based on concentrations of fecal coliform is a geometric mean of 200 CFU/100 ml. The geometric mean values for fecal coliform at all 14 sites are plotted on Figure 3.1. The results show that geometric mean concentrations of fecal coliform at only two of the 14 sites were below the fecal coliform standard. These two sites (Sites 5 and 6) were characterized as marine waters based on high salinity and were not significantly contaminated with stream water. These sites are subject to dilution with ocean water and dispersion by tidal action. It should be noted that 12 of the 14 sites exceeded the 200 CFU/100 ml standard, and based on salinity, these 12 sites were characterized as stream water or estuarine waters with significant input of stream water. These sites were further characterized by contribution primarily of fresh stream water flowing from the upper watershed toward the sea. The expected sources of the elevated concentrations of fecal coliform at these stream sites are from soil and from cesspool waste.

Enterococci Standard. The USEPA recreational water quality standards based on concentrations of enterococci is a geometric mean of 33 CFU/100 ml in fresh water and

35 CFU/100 ml in marine waters. The geometric mean values for enterococci of the 14 sampling sites are plotted on Figure 3.2. Of the 14 sites only two fell below the USEPA enterococci standard. These two sites (Sites 5 and 6) were characterized as marine sites based on high salinity and were not significantly contaminated with stream water. Furthermore, these two sites are subject to dilution with ocean water and dispersion by tidal action. It should be noted that twelve of the 14 sites exceeded the 33 CFU/100 ml standard and based on salinity; these 12 sites were characterized as stream water or estuarine waters with significant input of stream water. These sites were further characterized by contribution primarily of fresh stream water flowing from the upper watershed toward the sea. The expected sources of the elevated concentrations of enterococci at these stream sites are from soil and from cesspool waste.

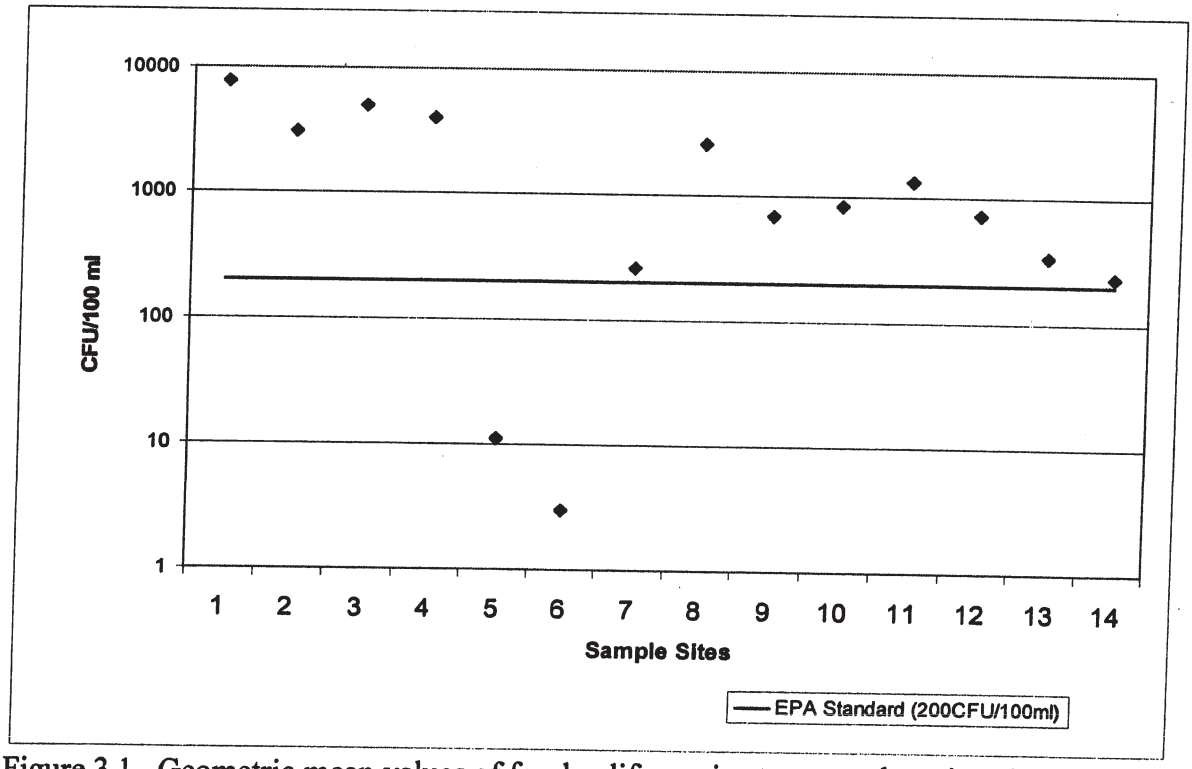


Figure 3.1. Geometric mean values of fecal coliforms in stream and marine sites on Kaua'i sampled over a period of ten months plotted with the current USEPA standard for fecal coliforms (200 CFU/100ml).

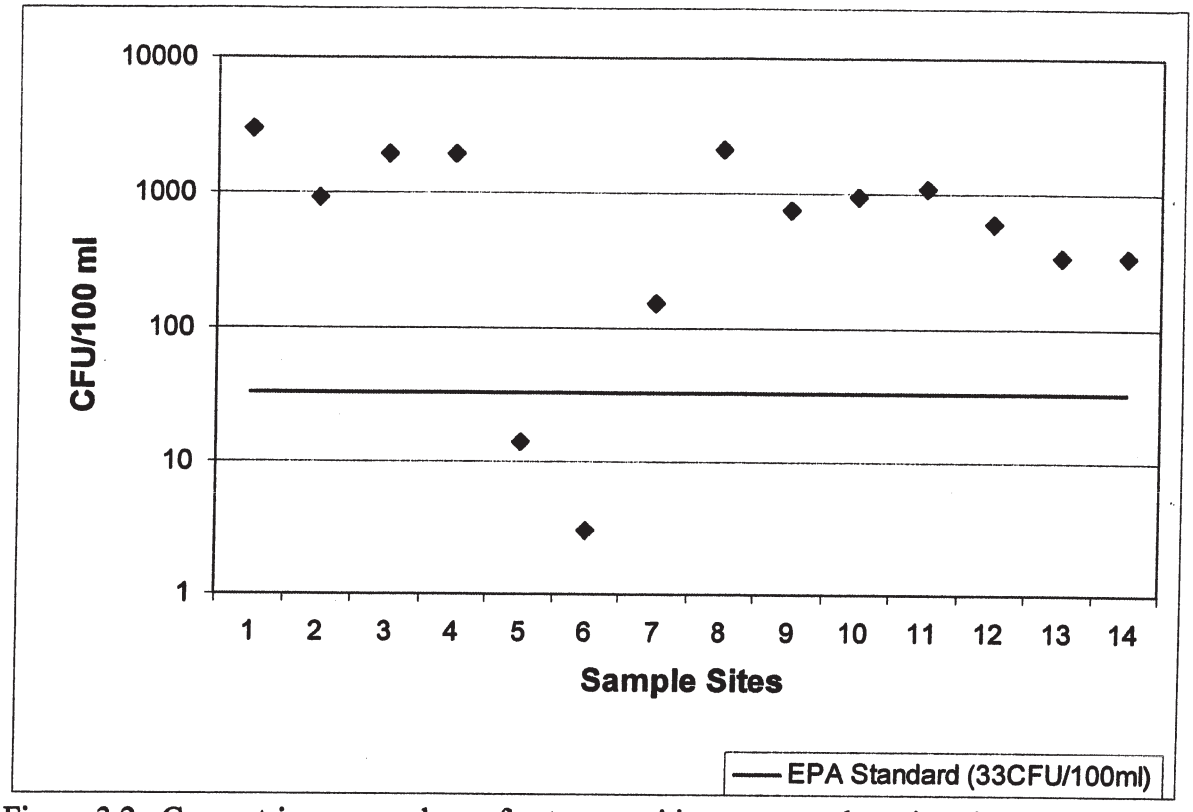


Figure 3.2. Geometric mean values of enterococci in stream and marine sites on Kaua'i sampled over a period of ten months plotted with the current USEPA standard for enterococci (33 CFU/100ml).

3.3 Soil as a Primary Source of Fecal Indicator Bacteria in the Nawiliwili Watershed

Soil is thought to be the cause of elevated levels of fecal indicator bacteria in stream water. Studies on O'ahu have shown that fecal indicator bacteria (fecal coliform, *E. coli*, enterococci) are able to grow and multiply in various soils based on the amount of moisture that is present (Fujioka *et al*, 2000). Because Kaua'i receives more rainfall than O'ahu, and is considered one of the wettest places on earth, we would expect soils on Kaua'i to retain moisture better than soils on O'ahu. Based on this we can predict that soils on Kaua'i will provide a good environment for the persistence of fecal indicator bacteria (El-Kadi *et al*, 2003).

Studies of soils sampled along streams of O'ahu indicate the presence of high levels of fecal coliforms and enterococci. Soils sampled along the bank of Mānoa Stream on O'ahu have yielded *E. coli* concentrations of 10^3 and enterococci counts of 10^2 (Hardina & Fujioka, 1991). Often during rain events, soil, along with these microorganisms, is washed into streams. This leads to a high elevation of these indicator bacteria in the streams and subsequently in beaches where the streams flow.

Table 3.2. Fecal coliform and enterococci concentrations found in soils near the banks of three streams on Kaua'i.

Soil Sample	Fecal Coliforms (MPN/g Dry Soil	Enterococci (MPN/g Dry Soil)
Hulē'ia Stream Bank Soil	1.78×10^3	3.88×10^2
Puali Stream Bank Soil	2.43×10^2	7.72×10^4
Nawiliwili Stream Bank Soil	9.51×10^4	4.23×10^3

To provide data that soil is a major source of fecal indicator bacteria on Kaua'i, soil samples from the Nawiliwili watershed were assayed for fecal coliform and enterococci. The results of these assays are summarized in Table 3.2 and show that fecal coliform and enterococci counts in three soils along the banks of three streams (Hulē'ia, Puali and Nawiliwili Streams) contained highly elevated levels of both fecal coliforms (10^2-10^4 MPN/g of soil) and enterococci (10^2-10^4 MPN/ g of soil). These levels are similar to what has been documented in soils on O'ahu (Hardina & Fujioka, 1991), and it is presumable that rain washes these fecal indicator bacteria into surrounding streams and leads to their elevated numbers in these waterways.

3.4 Impact of Rain on Contaminant Levels in the Nawiliwili Watershed

During the sampling period of this study, a particularly high rain event was noted on May 10th, 2002 (Appendix F). Over nine inches of rain was dumped into the

Nawiliwili watershed on this date which resulted in elevated levels of indicator bacteria (fecal coliforms and enterococci) in many of the samples (Appendix A, Tables A-1, A-3, A-4, A-5, A-6, A-7, A-10, A-13) obtained during the May 14th, 2002 sampling date. These heavy rains resulted in an increase of one to three log concentrations of both fecal coliform and enterococci at these sites. The highest counts (an increase of three logs) of both indicators on this sampling date were seen at the Kalapakī Beach site. This is not unexpected because soil, along with both fecal coliform and enterococci, are expected to be carried via Nawiliwili Stream, and empty approximately 0.2 km from this site, into Nawiliwili Bay. Previous studies conducted on O'ahu have shown rain to be the mechanism responsible for transporting fecal bacteria growing in soil to the streams and coastal waters of O'ahu (Hardina & Fujioka, 1991). This type of occurrence can be quite common in tropical areas, and can lead to the misinterpretation of microbial water quality data. The USEPA fecal indicator bacteria cannot be used in these areas because of the persistence and multiplication of these microorganisms in tropical environments such as soil. For this reason, a more reliable indicator is needed to assess fecal pollution in tropical areas.

3.5 The Presence of the Alternative Fecal Indicators *C. perfringens* and Coliphages in the Nawiliwili Watershed

To evaluate the usefulness of alternative fecal indicators, the concentrations of *C. perfringens*, FRNA coliphages and somatic coliphages, were measured in each of the water samples obtained from the 14 sites. Table 3.3 summarizes the geometric mean concentrations of *C. perfringens* and FRNA and somatic coliphages found in the 14 sample sites in the Nawiliwili watershed. In this assessment, a standard for *C.*

perfringens of 50 CFU/100 ml for fresh water and 5 CFU/100 ml for marine water ,as proposed by Fujioka (Roll and Fujioka, 1997) in determining if waters are contaminated with sewage and adopted by Hawai'i state Department of Health, was used. This standard is based on ambient concentrations of this organism in pristine waters of O'ahu. In this study, *C. perfringens* counts were used as indicators of surface sewage pollution. This is because *C. perfringens* is not found in soil and elevated concentrations in streams can be attributed to surface run-off of sewage (El-Kadi *et al*, 2003).

Currently no standard for coliphages exist, but based on studies of uncontaminated streams on O'ahu (Luther, 1995) a baseline of <50 PFU/100 ml was used in this study to determine sewage/cesspool contamination. The presence of FRNA coliphages in Kaua'i streams was used to determine when cesspool contamination is present. Studies have shown f2 coliphages (such as FRNA), unlike bacteria, adsorb poorly to a wide variety of soil types (Goyal and Gerba, 1979). Thus, the presence of FRNA coliphages in streams in areas where cesspools are prevalent may be indicative of cesspool leaching into these waters.

A. Nawiliwili Stream

Site 1: Upper Nawiliwili Stream. The geometric mean value of *C. perfringens* at this site was 19 CFU/100 ml (Table 3.3). This value is much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). These results indicate no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 6 out of the 10 samples at this site, with a geometric mean value of 73 PFU/100 ml (Table 3.3). FRNA coliphage concentrations

ranged from undetectable to 6,460 PFU/100 ml. Somatic coliphage concentrations ranged from undetectable to 3,180 PFU/100 ml with a geometric mean value of 234 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 9 out of 10 samples. Both FRNA and somatic coliphages were detected at higher concentrations in this stream than in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995). All sources of somatic coliphages have not yet been specifically determined but sewage is a source of somatic coliphages and FRNA coliphages. Other studies have shown that f2 coliphages, like FRNA, attach poorly to soil particles and are able to pass through various types of soil (Goyal & Gerba, 1979). Thus, it can be assumed that these coliphages pass from cesspools, into the soil, and contaminate nearby streams. The high geometric mean value of FRNA and somatic coliphages in water from Upper Nawiliwili Stream is indicative that this site is being contaminated by cesspools on a continuous basis.

Site 2: Lower Nawiliwili Stream. The geometric mean value of *C. perfringens* for this site was 6 CFU/100 ml. This value is much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 5 out of the 8 samples at this site, with a geometric mean value of 30 PFU/100 ml. FRNA coliphage concentrations ranged from undetectable to 460 PFU/100 ml. Somatic coliphage concentrations ranged from undetectable to 460 PFU/100 ml with a geometric mean value of 41 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 6 out of 8 samples. These sporadically elevated levels of FRNA and somatic coliphages were not detected in Mānoa and Nu'uanu Streams on O'ahu

(Luther, 1995) and indicate that cesspool waste discharge into Lower Nawiliwili occurs less frequently than in Upper Nawiliwili or that there is some dilution of water in Lower Nawiliwili Stream which is not contaminated with cesspool wastes.

Site 3: Marriott Culvert. The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml (Table 3.3). This value is much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). However, the results shown in Figure 3.3 and Appendix Table A-3 show that there was one sampling day (May 14, high rainfall) when the concentrations of all fecal indicators (fecal coliform, enterococci) as well as alternative fecal indicators *C. perfringens* (100 CFU/100 ml), FRNA coliphages (1080 PFU/100 ml, somatic coliphage (1600 PFU/100 ml) were elevated and most likely represented the washing of all of these fecal indicators, as well as alternative fecal indicators, from soil in a golf course, because sewage is used to irrigate a golf course in this area. At this site, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 2 out of the 10 samples with a geometric mean value of 4 PFU/100 ml. FRNA coliphage concentrations ranged from undetectable to 1,080 PFU/100 ml. In these same water samples, somatic coliphage concentrations ranged from undetectable to 1,600 PFU/100 ml with a geometric mean value of 44 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 5 out of 10 samples. In summary, the consistently low concentration of *C. perfringens* from this site is indicative that continuous surface sewage contamination is not occurring. However, since sewage is used to irrigate a golf course in this area, rain will occasionally transport alternative fecal

indicators such as *C. perfringens*, FRNA coliphages, somatic coliphages which can be expected to persist in soil of the golf course area.

Site 4: Pine Trees. This site is characterized by the mixing of stream water and ocean water. It is a site where sediments from Nawiliwili stream tend to accumulate. Water samples from this site revealed an elevated geometric mean value of *C. perfringens* (111 CFU/100 ml). This value is much higher than at the upper and lower Nawiliwili Stream sites and even from Marriot Culvert site. The best explanation for the high level of *C. perfringens* at this site is the expected accumulation of *C. perfringens* in the sediments at this site and resuspension due to tidal action of ocean water. As shown earlier, the source of *C. perfringens* at this site is via Marriott culvert which receives run-off from a nearby golf course that uses reclaimed wastewater for irrigation. Since *C perfringens* spores can last for long periods of time in the environment, it is possible to detect this microorganism long after a sewage spill has taken place. Elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 5 out of the 10 samples at this site, with a geometric mean value of 21 PFU/100 ml (Table 3.3). FRNA coliphage concentrations ranged from undetectable to 1,220 PFU/100 ml. Somatic coliphage concentrations ranged from undetectable to 1,900 PFU/100 ml with a geometric mean value of 250 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 8 out of 10 samples. The best explanation for the results obtained is that the primary source of *C. perfringens*, FRNA coliphages and somatic coliphages at this site is the sediment at this site. However, the original source is sewage from the sewage treatment plant used to irrigate a golf course in this area.

B. Ocean Sites

Site 5: Kalapakī Beach (marine site). The geometric mean value of *C. perfringens* for this site was 1 CFU/100 ml. This value is much lower than the proposed 5 CFU/100 ml standard and is similar to what has been documented in uncontaminated sites on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. No FRNA coliphages were detected in any of the 10 samples at this site, while somatic coliphages were detected in only one sample (300 PFU/100 ml). Geometric mean values for both FRNA and somatic coliphages were less than 1 PFU/100 ml. The low levels of *C. perfringens*, FRNA coliphages, and somatic coliphages at this site represents the relatively lower levels of these alternative fecal indicators as compared to fecal indicator bacteria in the discharge of Nawiliwili Stream into Nawiliwili Bay. Dilution and tidal forces serve to disperse microorganisms flowing into Nawiliwili Bay via Nawiliwili Stream into the Kalapakī Beach site.

Site 6: Seaflite Jetty (Harbor). The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml. This value is much lower than the proposed 5 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. No FRNA coliphages were detected in any of the 10 samples at this site, while somatic coliphages were detected in two of the 10 samples (300 PFU/100 ml). Geometric mean values for both FRNA and somatic coliphages were less than 1 PFU/100 ml. No cesspool contamination appears to be occurring at this site, most likely due to dilution and tidal forces at this marine site that served to disperse microorganisms flowing into Nawiliwili Bay via Nawiliwili Stream.

Site 7: Small Boat Harbor. The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml. This value is much lower than the proposed 5 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. No FRNA coliphages were detected in any of the 10 samples at this site, while somatic coliphages were detected at elevated concentrations (>50 PFU/100 ml) in 2 of the 10 samples (300 PFU/100 ml). The geometric mean value of FRNA coliphage was less than 1 PFU/100 ml while the geometric mean value of somatic coliphages was 5 PFU/100 ml. Based on this data, no cesspool contamination appears to be occurring at this site, most likely due to dilution and tidal forces at this marine site that served to disperse these microorganisms flowing into Nawiliwili Bay via Puali and Hulē'ia Streams.

C. Papalinahoa Stream.

Site 8: Mouth of Papalinahoa Stream. The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml (Table 3.3) This value was much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 5 out of the 9 samples at this site, with a geometric mean value of 20 PFU/100 ml (Table 3.3). FRNA coliphage concentrations ranged from undetectable to 1,160 PFU/100 ml. Somatic coliphage concentrations ranged from undetectable to greater than 4,500 PFU/100 ml with a geometric mean value of 208 PFU/100 ml, and were detected at elevated concentrations

(>50 PFU/100 ml) in 8 out of 9 samples. Unlike the study in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995), FRNA coliphages were periodically detected at very elevated levels (Appendix A, Table A-8). The source of somatic coliphages has not yet been specifically determined but the presence of FRNA coliphages have been shown to be sewage associated. Though the geometric value of FRNA coliphage at this site was below the baseline of 50 PFU/100 ml, sporadic cesspool contamination at this site may be occurring due to the periodic presence of elevated concentrations (>50 PFU/100 ml) of FRNA coliphage.

D. Puali Stream

Site 9: Upper Puali Stream. The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml (Table 3.3). This value was much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 3 out of the 8 samples at this site, with a geometric mean value of 10 PFU/100 ml (Table 3.3). Unlike the study in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995), FRNA coliphages were periodically detected at very elevated levels. FRNA coliphage concentrations ranged from undetectable to 500 PFU/100 ml. Somatic coliphage concentrations ranged from 20 to 500 PFU/100 ml with a geometric mean value of 149 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 7 out of 8 samples. The source of somatic coliphages has not yet been specifically determined but the presence of FRNA coliphages have been shown to be sewage associated. Though the geometric value of FRNA coliphage at this site was

below the baseline of 50 PFU/100 ml, sporadic cesspool contamination at this site may be occurring due to the periodic presence of elevated concentrations (>50 PFU/100 ml) of FRNA coliphage.

Site 10: Lower Puali Stream. The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml. This value was much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 4 out of the 10 samples at this site, with a geometric mean value of 25 PFU/100 ml. FRNA coliphage concentrations ranged from undetectable to 540 PFU/100 ml. Somatic coliphage concentrations ranged from 19 to 680 PFU/100 ml with a geometric mean value of 146 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 8 out of 10 samples. Unlike the study in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995), FRNA coliphages were periodically detected at very elevated levels. These results indicate that sporadic cesspool contamination at this site may be occurring.

E. Papakōlea Stream

Site 11: Upper Papakōlea Stream. The geometric mean value of *C. perfringens* at this site was less than 1 CFU/100 ml. This value is much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 7 out of the 10 samples at this site, with a geometric mean

value of 182 PFU/100 ml. FRNA coliphage concentrations ranged from undetectable to 4,140 PFU/100 ml. Somatic coliphage concentrations ranged from 31 to 5,740 PFU/100 ml with a geometric mean value of 521 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in 9 out of 10 samples. Both FRNA and somatic coliphages were detected at higher concentrations in this stream than in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995). The high geometric mean value of FRNA coliphage is indicative that this site is being contaminated by cesspools on a continuous basis. The source of somatic coliphages have not yet been specifically determined, however, because the presence of FRNA coliphages have been shown to be sewage associated, this stream may contain cesspool wastes.

Site 12: Lower Papakōlea Stream. The geometric mean value of *C. perfringens* at this site was less than 1 CFU/100 ml. This value is much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) were detected in 7 out of the 8 samples at this site, with a geometric mean value of 85 PFU/100 ml. FRNA coliphage concentrations ranged from undetectable to 500 PFU/100 ml. Somatic coliphage concentrations ranged from 180 to 720 PFU/100 ml with a geometric mean value of 375 PFU/100 ml, and were detected at elevated concentrations (>50 PFU/100 ml) in all 10 samples. Both FRNA and somatic coliphages were detected at higher concentrations in this stream than in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995). The high geometric mean value of FRNA coliphage is indicative that this site is being contaminated by cesspools on a continuous basis. The

source of somatic coliphages have not yet been specifically determined, however, because the presence of FRNA coliphages have been shown to be sewage associated, this stream may contain cesspool wastes.

E. Hulē'ia Stream

Site 13: Upper Hulē'ia Stream. The geometric mean value of *C. perfringens* for this site was 1 CFU/100 ml. This value was much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is occurring at this site. However, elevated concentrations of FRNA coliphages (>50 PFU/100 ml) was detected in 1 out of the 10 samples at this site, with a geometric mean value of 2 PFU/100 ml. FRNA coliphage concentrations ranged from undetectable to 100 PFU/100 ml. Somatic coliphage concentrations ranged from undetectable to 1000 PFU/100 ml with a geometric mean value of 22 PFU/100 ml, and were detected at elevated levels (>50 PFU/100 ml) in 4 of the 10 samples. FRNA coliphages were detected at concentrations in this stream at levels similar to what has been documented in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995). The source of somatic coliphages has not yet been specifically determined but the presence of FRNA coliphages have been shown to be sewage associated. Since this site contains a very low concentration (2 PFU/100 ml) of FRNA coliphage, cesspools are not contaminating this stream at this site.

Site 14: Lower Hulē'ia Stream. The geometric mean value of *C. perfringens* for this site was less than 1 CFU/100 ml. This value was much lower than the proposed 50 CFU/100 ml standard and is similar to what has been documented in uncontaminated streams on O'ahu (Shizumura, 1982). Based on this, no surface sewage contamination is

occurring at this site. The geometric mean value for FRNA coliphages was less than 1 PFU/100 ml, and was detected in only one of the 10 samples at a concentration of 20 PCFU/100 ml. Somatic coliphage concentrations ranged from undetectable to 80 PFU/100 ml with a geometric mean value of 9 PFU/100 ml, and were detected at elevated levels (>50 PFU/100 ml) in 3 of the 8 samples at concentrations greater than the baseline of 50 PFU/100 ml. FRNA coliphages were detected at concentrations in this stream at

Table 3.3. Geometric mean concentrations of the alternative indicators *C. perfringens* and coliphages in stream and marine sites on Kaua'i sampled over a period of ten months.

Sample Site	Number of Samples (n)	*C. perfringens* (CFU/100ml)	Male Specific Coliphages (PFU/100ml)	Somatic Coliphages (PFU/100ml)
1 Nawiliwili Stream Upper	10	19	73	234
2 Nawiliwili Stream Lower	8	6	30	41
3 Marriott Culvert	10	<1	4	44
4 Pine Trees	10	111	21	250
5 Kalapakī Beach	10	1	<1	<1
6 Seaflite Jetty	10	<1	<1	1
7 Small Boat Harbor	10	<1	<1	5
8 Papalinahoa Stream	9	<1	20	208
9 Puali Stream Upper	8	<1	10	149
10 Puali Stream Lower	10	<1	25	146
11 Papakōlea Stream Upper	10	<1	182	521
12 Papakōlea Stream Lower	8	<1	85	375
13 Hulē'ia Stream Upper	10	1	2	22
14 Hulē'ia Stream Lower	8	<1	<1	9

levels similar to what has been documented in Mānoa and Nu'uanu Streams on O'ahu (Luther, 1995). The source of somatic coliphages has not yet been specifically

determined but the presence of FRNA coliphages have been shown to be sewage associated. Since this site contains a very low concentration (<1 PFU/100 ml) of FRNA coliphage, cesspools are not contaminating this stream at this site.

3.6 Assessment of Water Quality Based on *C. perfringens* and FRNA Coliphages

C. perfringens. Currently no standard exists for *C. perfringens*. However, based on studies conducted on clean streams on O'ahu, a standard of 50 CFU/100 ml has been proposed for fresh water and 5 CFU/100 ml for marine water. In this study, *C. perfringens* was used to determine when surface sewage contamination was present in the Nawiliwili Watershed. Of the 14 sites, *C. perfringens* levels at 13 sites fell below the proposed standard of 50 CFU/100 ml. The geometric mean *C. perfringens* values of the 14 sites are plotted in Figure 3.3 along with the 50 CFU/100 ml proposed standard. Only one site (Pine Trees) had an elevated geometric mean level (111 CFU/100 ml) of this proposed indicator. It was determined that elevated levels of *C. perfringens* at this site were due to the fact that this site receives run-off from a nearby golf course that uses reclaimed wastewater for irrigation. Based on the proposed *C. perfringens* standard, the remaining 13 sites in this watershed are not being contaminated by surface sewage run-off.

FRNA Coliphages. The role somatic coliphages play in determining sewage contamination has not been specifically determined. However, FRNA coliphages have been shown to be specifically related to sewage (and cesspool) waste (Hsu *et al,* 1995). Thus, these coliphages may be better determinants of point source pollution as compared to somatic coliphages. Studies conducted on clean streams on O'ahu (Luther, 1995),

indicate that FRNA coliphages are present at ambient levels at concentrations less than 50 PFU/100 ml. Thus, a baseline of 50 PFU/100 ml of FRNA coliphage was used to determine when streams in the Nawiliwili Watershed were contaminated with cesspool waste. The geometric mean FRNA coliphage values of the 14 sampling sites are plotted

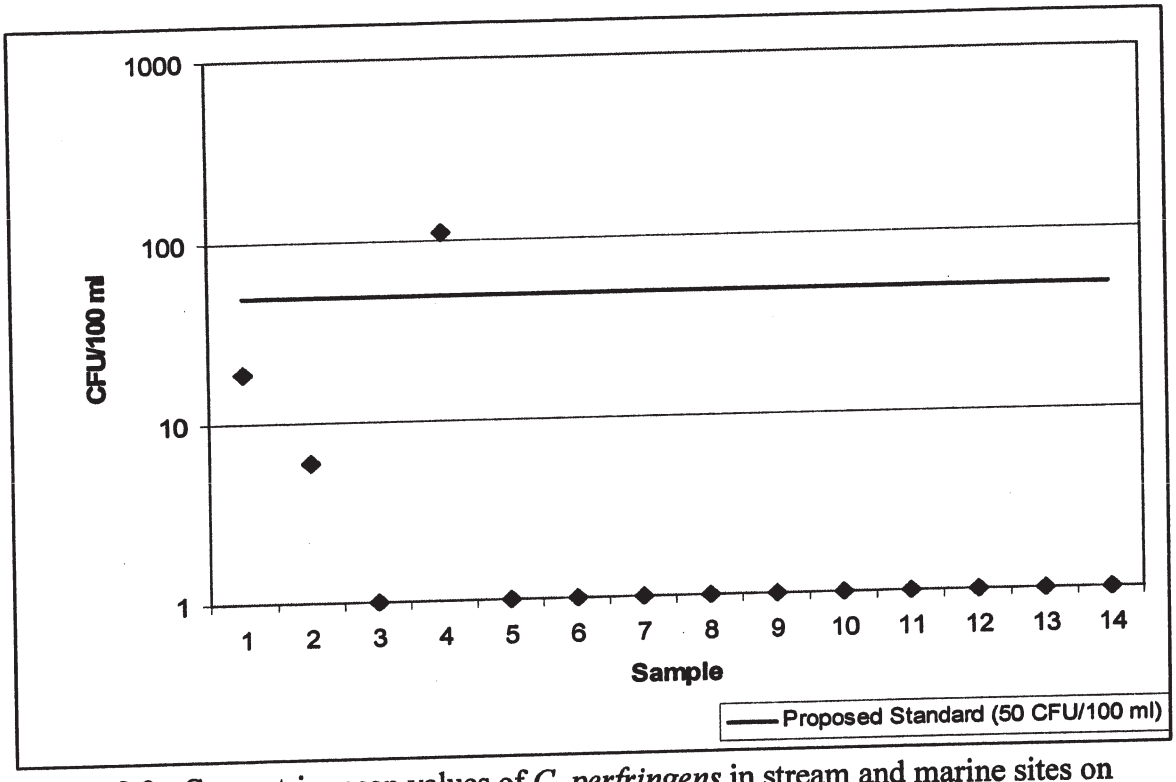


Figure 3.3. Geometric mean values of *C. perfringens* in stream and marine sites on Kaua'i sampled over a period of ten months plotted with the proposed standard for *C. perfringens* (50 CFU/100ml).

in Figure 3.4. Of the 14 sites, the geometric mean values of this proposed indicator exceed the baseline of 50 PFU/100 ml at three sites. Studies have shown that f2 phages, like FRNA coliphages, are able to travel through various soil types, particularly when moisture is present (Goyal & Gerba, 1979). The reported movement of FRNA coliphages through soil, and because the use of cesspools is prevalent on Kaua'i, it is reasonable to conclude that these three sites (Upper Nawiliwili Stream and Upper and Lower Papakōlea Stream) are being impacted by cesspool waste. Though the remaining

11 sites had low geometric mean values of FRNA coliphage, sporadic contamination of these sites by cesspools must be occurring because elevated concentrations of these coliphages are occasionally detected.

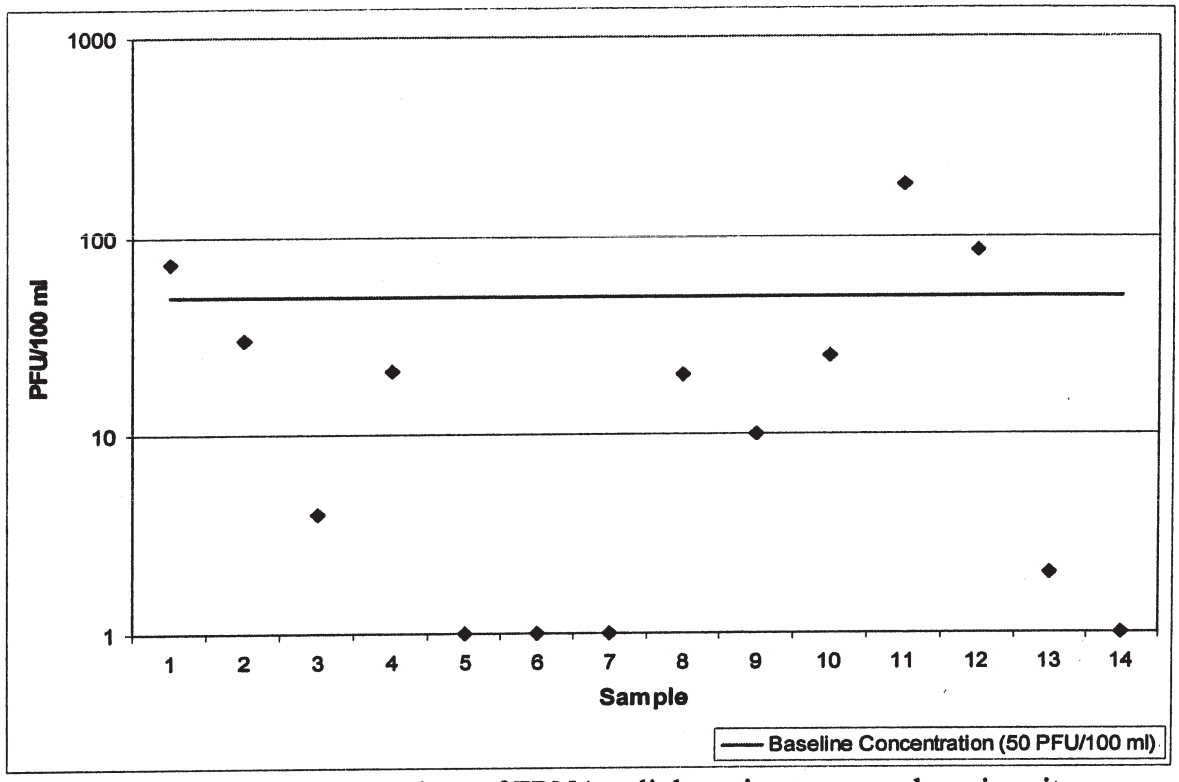


Figure 3.4. Geometric mean values of FRNA coliphage in stream and marine sites on Kaua'i sampled over a period of ten months plotted with the baseline for FRNA coliphage (50 CFU/100ml).

3.7 The Presence of FRNA Coliphages in Raw Sewage and Cesspools on Kaua'i

Raw sewage samples were obtained on three occasions from the Lihue Wastewater Treatment Plant (WWTP). As expected, FRNA coliphage counts, summarized in Table 3.4, were high (10^3 PFU/100 ml) in these samples. Of the four cesspool samples obtained, only one sample yielded FRNA coliphage plaques via the direct plaque assay. It was interesting to note that this sample came from a men's restroom while FRNA levels in the women's restroom was undetectable, even after

enrichment. In general, cesspool samples seem to contain less FRNA coliphage concentrations than sewage. This may be because cesspools tend to contain feces from a small population of the community (particularly cesspools of single family homes) while

Table 3.4. Coliphage concentrations in raw sewage and cesspools on Kaua'i.

Sample	Male Specific Coliphages (PFU/100ml)
Lihue WWTP Raw Sewage 1	2060
Lihue WWTP Raw Sewage 2	1780
Lihue WWTP Raw Sewage 3	1940
Hanapepe Salt Pond Women's Restroom	0 enrichment: -
Hanapepe Salt Pond Men's Restroom	140
Hanalei Bay Pavilion 1	0 enrichment: +
Hanalei Bay Pavilion 2	0 enrichment: +

sewage is a mixture of feces from a large population of the community. Thus, since FRNA coliphages are found only in a small population of humans, it is assumable that sewage will contain a higher concentration of FRNA coliphages than cesspools.

3.8 Source Tracking of Male Specific Coliphages by Genotyping

In order to prevent fecal contamination it is useful to know its source. Once the source is known, preventive steps can be taken in order to minimize it. Source tracking of FRNA isolates is a good method to determine whether contamination is coming from a human or animal source. Previously, the method of serotyping was used in order to accomplish this. However because of the unavailability of antisera, and because some isolates were neutralized by two different antisera, this method was abandoned for the newer method of genotyping (Hsu *et al*, 1995).

Genotyping uses specific gene probes to differentiate between the four groups of FRNA bacteriophages. Groups I and IV have been primarily associated with animals while groups II and III are predominantly seen in humans and sewage. This method is highly sensitive, though cross contamination has been known to occur between human and pig isolates (Hsu *et al*, 1995).

Table 3.5 summarizes the genotyping results from this experiment. 92% of total FRNA isolates were grouped as either human or animal. Of these, 98% of the genotyped FRNA isolates fell into either group II or III while the remaining 2% into group I or IV. The high percentage of group II and III isolates is indicative that contamination in the Nawiliwili Watershed may have come from a human source. However, feral pigs are known to be present on Kaua'i. These animals may also be contributing to the high percentage of group II and III FRNA coliphages. The animal isolates may be due to ducks and other birds that nest near streams and contaminate the water with their feces.

Previous studies (Griffin *et* al, 2000) have shown that the majority of FRNA coliphage isolates from raw sewage are genotyped as group II rather than group III. This was indeed the case with FRNA coliphages isolated from the Lihue WWTP and cesspools. Since the majority of FRNA isolates for both sewage and cesspool isolates were typed as group II, and since the majority (98%) of stream isolates were also typed as group II, the source of contamination of these stream sites may be highly likely due to cesspool contamination. Though no piggeries are present in the Nawiliwili watershed, feral pigs are present. These pigs are know to cause erosion problems to streams in the watershed (El-Kadi *et al,* 2003), and may be a potential source of FRNA contamination in these streams.

Table 3.5. Genotyping of male specific coliphage isolates to determine the likely source (human vs. animal) of contamination.

Isolate Site	Number of FRNA Isolates	Number of genotyped FRNA Isolates	Number of Group I Isolates[a]	Number of Group II Isolates[b]	Number of Group III Isolates[b]	Number of Group IV Isolates[a]
Nawiliwili Stream Upper	343	312	7	289	16	0
Nawiliwili Stream Lower	131	127	6	109	9	3
Marriott Culvert	121	112	0	112	0	0
Pine Trees	173	157	0	149	8	0
Kalapakī Beach	100	92	0	92	0	0
Seaflite Jetty	100	87	0	85	2	0
Papalinahoa Stream	138	129	14	115	0	0
Small Boat Harbor	100	95	0	95	0	0
Puali Stream Upper	127	122	5	117	0	0
Puali Stream Lower	120	105	7	96	2	0
Papakōlea Stream Upper	451	419	2	408	9	0
Papakōlea Stream Lower	69	61	0	61	0	0
Hulē'ia Stream Upper	103	93	0	93	0	0
Hulē'ia Stream Lower	0	0	0	0	0	0
Lihue WWTP Raw Sewage 1	28	26	0	26	0	0
Lihue WWTP Raw Sewage 2	24	21	0	21	0	0
Lihue WWTP Raw Sewage 3	44	40	0	40	0	0
Hanapepe Salt Pond Men's Restroom	7	7	0	5	2	0
Hanalei Bay Pavilion 1	50	38	1	26	12	0
Hanalei Bay Pavilion 2	50	42	0	34	8	0

[a] Associated with animal feces
[b] Associated with human feces

CHAPTER 4

SUMMARY AND CONCLUSIONS

4.1 Summary

Fecally contaminated recreational waters are a great public health risk. Contaminated waters can lead to gastrointestinal illness, as well as skin, eye and ear infections. A variety of bacteria, viruses, protozoa and helminths are known to be associated with sewage. A self limiting gastroenteritis is the most frequent result of exposure to fecally contaminated water, and the most frequently identified agents are the Norwalk-like and Hepatitis A viruses.

In order to prevent such infections, a method for the routine analysis of recreational waters is needed. Because the assay methods for viruses are cumbersome and expensive, levels of contamination are routinely assessed with bacterial indicators. A reliable bacterial indicator should be present when pathogens are present, be associated with sewage, shouldn't multiply in the environment and is just as resistant to disinfection as the pathogens are. In addition, a positive correlation between the indicator bacteria and illness in exposed people should be present. From 1972 to1986, the USEPA recommended that fecal coliform be used to establish recreational water quality standards instead of the unreliable total coliform standard (USEPA, 1986). The fecal coliform standard (200 CFU/100 ml) represented the first national water quality standard established by the USEPA. To address the limitations of the fecal coliform standards, USEPA conducted a 10 year study which evaluated many fecal indicators to establish water quality in beaches contaminated with sewage and measurements of health effects of people swimming in those beaches using epidemiological methods. Based on these

epidemiological/water quality studies, the USEPA, in 1986, established new recreational water quality standards, which was specific for marine and for fresh waters. For marine waters the only standard was based on enterococci (35 CFU/100 ml). For fresh waters, the standards were based either on *E. coli* (126 CFU/100 ml) or enterococci (33 CFU/100 ml). These standards were based on reliably predicting disease rates among swimmers (USEPA, 1986).

The USEPA water quality standards were based on data collected only in temperate regions of the USA, only at beaches contaminated with sewage and based on the assumption that the only significant source of *E. coli* and enterococci in environmental waters is sewage and direct input of feces from mammals. However, monitoring data from several tropical regions of the world such as in Hawai'i, Guam, Puerto Rico, south Florida (Fujioka & Byappanahalli, 2003) have shown that the USEPA approved fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) grow in tropical environments (soil, plant, sediments). These environmental sources of fecal indicator bacteria are washed into environmental waters (streams, rivers, storm drains, coastal waters) primarily by rain but also by anthropogenic practices (irrigation, non-point discharges). For example, monitoring data from Hawai'i have shown that all streams routinely exceed concentrations of fecal coliform, *E. coli* and enterococci well above the USEPA standards (200 fecal coliform/100 ml, 126 *E. coli*/100 ml, 33 enterococci/100 ml). The primary source of these elevated levels of fecal indicator bacteria in streams and in coastal waters which receive discharge of streams and storm drains was determined to be soil and not from sewage (Hardina & Fujioka, 1991). In 2001, the Tropical Indicator Workshop, which was sponsored by the USEPA and by the Hawai'i

State Department of Health, reviewed all the studies conducted in tropical regions of the world. The first conclusion of this workshop indicated that in some tropical regions of the world, the use of USEPA approved fecal indicators to assess the hygienic quality of recreational waters is unreliable. The second conclusion was that more appropriate fecal indicators for tropical climates should be developed (Fujioka & Byappanahalli, 2003).

Two alternative indicators that have been proposed for Hawai'i are *Clostridium perfringens* (Shizumura, 1982) and more recently FRNA coliphages (Luther, 1995). Both organisms are present in high concentrations in sewage and are more resistant to disinfection than the traditional indicator bacteria. In addition, these two microorganisms do not multiply under ambient conditions, are found at low concentrations in streams under ambient conditions and increase following sewage contamination. However, because no epidemiological studies have been conducted in Hawai'i to show a correlation between the increasing concentrations of these microorganisms in recreational waters and increased illness rates of *C. perfringens* and FRNA coliphages, the USEPA has not approved the use of these fecal indicators to establish water quality standards in Hawai'i.

One limitation in the data collected in Hawai'i is that most of the studies were conducted only on the island of O'ahu and not on other islands of the state of Hawai'i. The results of all the studies, which reported the consistently high concentrations of fecal indicator bacteria in all streams on O'ahu were reviewed (Fujioka, 1988 and Roll & Fujioka, 1997). In these studies, soil was determined to be the consistent source of this contamination. Similar studies have not been done on the other islands of the Hawaiian chain. It has just been assumed that the quality of the water on these islands would be similar to that of O'ahu. Kaua'i is the oldest island of the major islands that make up the

State of Hawai'i. Two major differences between the islands of O'ahu and Kaua'i are higher rainfall and extensive use of cesspools on Kaua'i. Increased rainfall leads to increased moisture in soil, which in turn provides ideal conditions for the multiplication of fecal indicator bacteria in soil. Increased rainfall also leads to increased run-off of soil into streams and eventually into the ocean. If fecal indicator bacteria also grow in the soil environment of Kaua'i, the weather conditions on Kaua'i can be expected to lead to elevated concentrations of these indicator bacteria (fecal coliform, *E. coli,* enterococci) in streams on Kaua'i and to the misinterpretation that these waters are sewage contaminated. A complicated factor for Kaua'i is the extensive use of cesspools, which discharge inadequately-treated sewage into soil. Cesspools on Kaua'i, many in close proximity to streams, can also be expected to serve as potential sources of fecal contamination of these streams.

A major objective of the present study was to obtain monitoring data from Kaua'i and to compare the results of this study with existing data from the island of O'ahu. The Nawiliwili watershed on the island of Kaua'i was selected as the study site. For this study a total of 14 water sites from four streams (Hulē'ia, Nawiliwili, Puali, Papakōlea) a storm drain, a beach site, two estuarine sites where stream waters discharge into coastal waters, and – harbor sites were monitored for USEPA-approved fecal indicator bacteria (fecal coliform, enterococci) and for alternative fecal indicators (*C. perfringens*, FRNA coliphages) approximately twice a month for over the period of one year. The monitoring results from this watershed on Kaua'i resulted in similar data when compared to monitoring results from O'ahu. Concentrations of the USEPA approved fecal indictors (fecal coliform, enterococci) consistently exceeded their water quality standards at all

stream sites, at the storm drain sites and were also elevated at the estuarine sites. The only two sites that met the USEPA standards for both fecal coliform and enterococci were two marine sites. This is not surprising because these bacteria are subject to dilution and dispersion once they enter the ocean. Soil samples taken along the banks of three rivers (Nawiliwili, Hulē'ia and Puali) that flowed in this watershed showed elevated counts of both fecal coliform and enterococci. These results confirm the results obtained from O'ahu that soil is a primary source of these fecal indicator bacteria (Hardina & Fujioka, 1991) and rain is the mechanism for transporting these soil bound bacteria to surrounding streams and then to oceans. This mechanism was confirmed based on monitoring data after a day of heavy rain.

These monitoring data from Kaua'i indicate that USEPA indicator bacteria (fecal coliform, enterococci) cannot be reliably used to determine when sewage contamination has occurred in environmental waters on all islands in the state of Hawai'i. Two alternative indicators, *C. perfringens* and FRNA coliphages were used in this study to determine if sewage and cesspool contamination was occurring in this watershed. Geometric mean values of *C. perfringens* were generally low (<50 CFU/100 ml) for 13 of the 14 sample sites. This indicated that these sites were not subject to surface sewage contamination. The only site (Pine Trees) that exceeded the 50 CFU/100 ml proposed standard was influenced by run-off from a nearby golf course that uses reclaimed sewage water for irrigation. Because *C. perfringens* spores can survive in the environment for long periods of time, it was concluded that the spores of *C. perfringens* from this reclaimed water accumulated in the sediment at the Pine Tree site and were resuspended by tidal action.

While *C. perfringens* was used as a means of determining surface sewage contamination in this study, FRNA coliphages were used as a means of determining cesspool contamination in the Nawiliwili watershed. Cesspools function by collecting fecal wastes into a pit and allowing it to degrade naturally, which includes its movement into the surrounding soil so that more waste can enter the pit. Studies have shown that f2 coliphages (i.e., FRNA coliphages) are poorly absorbed by a wide variety of soils (Goyal & Gerba, 1979). Thus it is reasonable to expect that these viruses can pass from cesspools through the surrounding soil and will eventually end up in streams (El-Kadi *et al*, 2003). A recreational water quality standard based on concentrations of FRNA coliphages has not been determined. However, the first proposed standard for this study was set at a conservative level of a geometric mean of 50 PFU/100 ml. Several stream sites on Nawiliwili and Papakōlea Streams exceeded this proposed standard and indicated that these stream sites were being consistently contaminated with cesspool wastes. Elevated concentrations of FRNA coliphages, well above the levels observed in streams on O'ahu, were sporadically observed in Papalinahoa and Puali Streams, indicating sporadic contamination with cesspool wastes into these stream sites. Genotyping of FRNA isolates further solidified the source of contamination in the watershed. The majority (98%) of the genotyped isolates were typed to be of human origin. This strongly suggests that cesspools may indeed by leaching into the surrounding streams. However, studies have shown that FRNA coliphage isolated from pigs tended to cross-react with the human probes (Hsu *et al*, 1995) due to similar digestive systems and microflora. Feral pigs are known to be present in the Nawiliwili

watershed. Thus, the high numbers of FRNA isolates from streams in this watershed classified as human may also reflect contamination from pig feces.

In summary, fecal indicator bacteria cannot reliably be used to assess when fecal contamination has occurred in tropical areas such as on Kaua'i. These bacteria are capable of persisting and multiplying in the environment, and assaying for these microorganisms would give erroneous results regarding the quality of water in these areas. Thus an alternative indicator should be used to evaluate the quality of recreational waters in tropical waters. In this study, *C. perfringens* and FRNA coliphages have been shown to be more reliable indicators of sewage and cesspool contamination. *C. perfringens* is a good indicator of surface sewage contamination because it is not naturally present in soils, thus rain events are not likely to increase *C. perfringens* concentrations in streams. On the other hand, FRNA coliphages appear to be a good indicator of cesspool contamination in streams because they have been shown to travel through a wide variety of soils, especially when excess moisture is present (Goyal & Gerba, 1979). Based on this and other studies, it is imperative that the USEPA revise the current fecal indicator standards to include such alternative indicators as *C. perfringens* and FRNA coliphages in areas where the traditional indicators cannot be used to reliably determine when recreational waters are contaminated with sewage.

4.2 Conclusions

1. USEPA-approved fecal indicator bacteria are present in high concentrations in Kaua'i streams. Streams in the Nawiliwili Watershed have elevated levels of fecal coliforms and enterococci. These streams do not meet the USEPA standards for recreational waters (200 fecal coliform/100 ml, 33 enterococci/100 ml). These monitoring results

are similar to those reported for O'ahu.

2. On O'ahu, soil was found to be the cause of elevated levels of fecal indicator bacteria in streams. Soil sampled near the banks of three streams on Kaua'i showed elevated levels of fecal indicator bacteria (fecal coliform and enterococci). These bacteria get washed into streams along with the soil during rain events. The elevated presence of these fecal indicator bacteria in streams due to a non-point source of pollution such as soil does not necessarily constitute a health risk. Thus, the traditional USEPA standards may not be reliable in determining when Kaua'i streams are contaminated by sewage.
3. A more reliable, indicator is needed for tropical areas such as Hawai'i. Indicators such as *C. perfringens* and FRNA coliphages have been proposed as alternatives to the USEPA fecal indicators. A *Clostridium perfringens* standard of 50 CFU/100ml is currently used by the state of Hawai'i based on data collected on O'ahu streams. Based on this proposed standard, no surface sewage contamination is occurring to Kaua'i streams. The Pine Trees site was the only site with elevated levels of *Clostridium*. This site consists of stagnant water caused by flow of stream water out to the bay and the ocean water flowing into the stream. This conditions allows for sediments to accumulate. The spores of *C. perfringens* from wastewater used for irrigation persists in this sediment and become resuspended due to tidal action at this site. This study evaluated the use of FRNA coliphages to determine when sewage/cesspool contamination of stream occurs. FRNA coliphages have been associated with sewage and cesspools, which is the most prevalent mode of waste disposal on Kaua'i. No standard currently exists for FRNA coliphages, but based on

uncontaminated O'ahu streams, a baseline of approximately 50 PFU/100ml was used to asses contamination in Kaua'i streams by sewage. Nawiliwili and Papakōlea streams were found to contain elevated levels of FRNA coliphage. Therefore these two streams appear to be impacted by cesspools. Furthermore, Papalinahoa and Puali streams may be periodically impacted by cesspools because of the sporadic presence of elevated levels of FRNA coliphages. While *C. perfringens* may be a better indicator of surface sewage contamination, FRNA coliphages may be better suited for Kaua'i to assess cesspool contamination. This is because FRNA coliphages are smaller compared to *Clostridium* which may facilitate its passage from cesspools through the soil and into streams.

4. FRNA coliphages can be differentiated as human vs. animal based on a gene probe test which selectively hybridizes to four specific genetic sequences of FRNA coliphages, which correlated to the previously determined four serotypes of FRNA coliphages. Genogroups I and IV are associated with animals while genogroups II and III are associated with humans. FRNA isolates obtained from the Nawiliwili Watershed were primarily typed (98%) as either group II or III indicating human contamination. Only 2% of FRNA isolates were typed as group I or IV, indicating fewer animal strains of FRNA coliphages. This is further evidence that Kaua'i streams may be impacted by cesspool contamination. FRNA isolates from pigs have shown to be frequently typed as of human origin. This may be because they share similar digestive tracts and microflora as humans. The presence of feral pigs on Kaua'i may also have been a contributing factor to the high number of group II & III FRNA isolates in this watershed.

4.3 Future Work

Future work should start with monitoring of the remaining islands of the Hawaiian island chain, which thus far has been limited to O'ahu, and in this study to include Kaua'i. It would be interesting to see whether differences in the quality of surface water exist between the various islands, particularly between the islands of Hawai'i and Kaua'i. Hawai'i is the youngest island, and differences may exist between its geology as compared with the oldest island, Kaua'i. These differences may lead to differences in the quality of water as well.

Collecting animal feces and assaying it for the presence of FRNA coliphage and subsequent typing of these isolates would give us more insight as to how animals impact the watershed. Typing of isolates from pig feces in particular would tell us if the typing of 98% of stream isolates as humans is accurate. In this regard it would also be important to find a site where cesspools are present but pigs are not.

One of the lingering questions about the use of FRNA coliphages as an indicator is why they are present in high concentrations in sewage but virtually nonexistent in human feces. The use of FRNA as an indicator may gain wider acceptance if this issue can be resolved. One of the major drawbacks of the double layer plaque assay is the size of the sample inoculum. If a method was developed for larger sample volumes, the sensitivity of this method could be increased.

APPENDIX A

RAW MICROBIAL DATA FOR STREAMS AND MARINE SITES IN THE NAWILIWILI WATERSHED

Table A-1. USEPA approved indicator bacteria and alternative indicator concentrations in Nawiliwili Stream Upper.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	6,400	3,600	12	520	2,100		
11/28/01	6,800	2,080	32	1,060	1,420		
2/27/02	5,160	2,200	68	6,460	3,180		
3/20/02	3,440	3,760	32	100	60		
4/22/02	14,400	11,200	228	20	200		
5/14/02	27,200	1,560	72	600	600		
6/24/02	3,080	1,640	16	0	180	+	
7/24/02	6,760	1,680	0	0	120	+	
10/16/02	3,480	1,680	16	0	0	+	-
11/25/02	35,200	8,800	20	1,040	3,340		

* shaded areas indicate than an enrichment was not done.

Table A-2. USEPA approved indicator bacteria and alternative indicator concentrations in Nawiliwili Stream Lower.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
3/19/02	4,240	4,400	16	40	120		
4/24/02	1,104	1,040	4	460	200		
5/16/02	2,560	1,320	0	120	80		
6/26/02	2,640	720	8	160	200		
7/16/02	4,360	32	12	160	460		
8/21/02	3,560	1,440	8	0	0	+	-
9/25/02	5,400	1,680	8	120	0		+
11/20/02	2,720	1,560	24	0	440	-	

* shaded areas indicate than an enrichment was not done.

Table A-3. USEPA approved indicator bacteria and alternative indicator concentrations in Marriott Culvert.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	9,080	4,400	0	0	140	+	
11/28/01	4,240	2,160	0	0	80	+	
2/27/02	4,160	2,200	0	0	40	-	
3/20/02	968	1,320	0	0	20	+	
4/22/02	2,400	720	4	20	20		
5/14/02	72,400	108,000	100	1,080	1,600		
6/24/02	3,000	1,120	40	0	0	+	+
7/24/02	9,000	1,160	0	0	420	+	
10/16/02	4,040	960	0	0	0	+	+
11/25/02	2,800	280	0	120	220		

* shaded areas indicate than an enrichment was not done.

Table A-4. USEPA approved indicator bacteria and alternative indicator concentrations in Pine Trees.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	5,560	1,440	120	0	80	+	
11/28/01	3,200	188	48	220	940		
2/27/02	2,080	3,200	960	1,220	1,580		
3/20/02	2,160	1,560	528	40	320		
4/22/02	3,320	2,400	800	100	400		
5/14/02	48,800	27,200	0	280	1,900		
6/24/02	1,600	1,160	336	0	40	+	
7/24/02	6,440	11,200	920	0	320	-	
10/16/02	2,200	640	92	0	4	+	
11/25/02	3,320	920	44	660	640		

* shaded areas indicate than an enrichment was not done.

Table A-5. USEPA approved indicator bacteria and alternative indicator concentrations in Kalapakī Beach.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	0	4	0	0	0	-	+
11/28/01	304	244	16	0	0	+	+
2/27/02	0	4	0	0	0	-	-
3/20/02	16	4	12	0	0	-	-
4/22/02	44	80	20	0	0	-	+
5/14/02	22,400	14,800	0	0	300	-	
6/24/02	16	20	16	0	0	+	-
7/24/02	80	60	12	0	0	+	+
10/16/02	0	0	0	0	0	+	+
11/25/02	4	0	0	0	0	-	-

* shaded areas indicate than an enrichment was not done.

Table A-6. USEPA approved indicator bacteria and alternative indicator concentrations in Seaflite Jetty.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	0	0	0	0	0	+	+
11/28/01	372	232	4	0	380	-	
2/27/02	4	0	0	0	0	-	-
3/20/02	0	4	0	0	0	-	-
4/22/02	0	4	0	0	0	-	+
5/14/02	404	348	0	0	20	+	
6/24/02	20	16	0	0	0	+	+
7/24/02	4	0	4	0	0	-	+
10/16/02	0	0	0	0	0	+	+
11/25/02	12	4	0	0	0	-	-

* shaded areas indicate than an enrichment was not done.

Table A-7. USEPA approved indicator bacteria and alternative indicator concentrations in the Small Boat Harbor.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	116	48	0	0	0	+	+
11/28/01	5,080	2,440	0	0	1,380	+	
2/27/02	172	64	0	0	0	-	+
3/20/02	72	36	0	0	60	+	
4/22/02	76	64	16	0	0	-	+
5/14/02	1,240	1,080	0	0	20	-	
6/24/02	244	312	4	0	40	+	
7/24/02	160	92	0	0	0	-	+
10/16/02	176	108	0	0	3	+	
11/25/02	288	140	0	0	0	-	-

* shaded areas indicate than an enrichment was not done.

Table A-8. USEPA approved indicator bacteria and alternative indicator concentrations in Papalinahoa Stream.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	3,320	6,400	0	0	140	-	
11/28/01	244	11,200	0	1,160	560		
2/27/02	1,120	1,400	8	20	>4,500		
3/20/02	632	6,400	0	400	260		
4/22/02	1,200	2,200	12	0	160	-	
5/14/02	7,560	8	8	280	740		
6/24/02	3,560	2,280	0	0	20	+	
7/24/02	5,720	6,800	0	360	600		
11/25/02	44,080	4,400	24	60	120		

* shaded areas indicate than an enrichment was not done.

Table A-9. USEPA approved indicator bacteria and alternative indicator concentrations in Puali Stream Upper.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
3/19/02	708	1,960	0	500	500		
4/24/02	304	760	0	60	120		
5/16/02	828	328	8	0	240	-	
6/26/02	604	520	0	20	20		
7/16/02	2,960	3,120	0	0	240	+	
8/21/02	856	920	40	0	220	+	
9/25/02	556	280	0	20	80		
11/20/02	368	520	0	60	200		

* shaded areas indicate than an enrichment was not done.

Table A-10. USEPA approved indicator bacteria and alternative indicator concentrations in Puali Stream Lower.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	596	840	4	0	40	-	
11/28/01	744	920	0	20	100		
2/27/02	640	840	0	400	440		
3/20/02	592	960	0	540	680		
4/22/02	736	840	0	220	320		
5/14/02	4,040	2,600	4	20	160		
6/24/02	1,064	840	0	20	100		
7/24/02	1,056	1,440	0	20	240		
10/16/02	512	560	0	0	19	+	
11/25/02	584	560	0	120	160		

* shaded areas indicate than an enrichment was not done.

Table A-11. USEPA approved indicator bacteria and alternative indicator concentrations in Papakōlea Stream Upper.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	804	920	0	2,160	5,740		
11/28/01	524	1,480	0	3,900	4,500		
2/27/02	1,160	1,320	20	4,140	1,160		
3/20/02	1,052	1,080	8	660	800		
4/22/02	2,720	1,280	4	240	420		
5/14/02	3,360	2,320	8	560	640		
6/24/02	640	536	0	0	60	+	
7/24/02	676	800	0	40	100		
10/16/02	2,600	760	0	0	31	+	
11/25/02	3,120	1,200	0	320	1,240		

* shaded areas indicate than an enrichment was not done.

Table A-12. USEPA approved indicator bacteria and alternative indicator concentrations in Papakōlea Stream Lower.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
3/19/02	1,080	1,560	4	460	720		
4/24/02	788	360	0	400	600		
5/16/02	1,168	568	4	420	600		
6/26/02	664	680	0	60	220		
7/16/02	1,000	1,320	0	500	180		
8/21/02	472	600	0	140	220		
9/25/02	408	272	0	80	360		
11/20/02	604	400	8	0	480	-	

* shaded areas indicate than an enrichment was not done.

Table A-13. USEPA approved indicator bacteria and alternative indicator concentrations in Hulē'ia Stream Upper.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
10/31/01	212	144	8	0	20	+	
11/28/01	2,200	1,360	8	100	460		
2/27/02	200	152	16	0	180	+	
3/20/02	120	164	8	20	120		
4/22/02	180	288	8	0	40	+	
5/14/02	1,760	2,440	0	20	1,000		
6/24/02	316	376	4	0	40	+	
7/24/02	148	260	0	0	0	-	+
10/16/02	232	148	0	0	0	+	+
11/25/02	528	360	0	0	0	-	-

* shaded areas indicate than an enrichment was not done.

Table A-14. USEPA approved indicator bacteria and alternative indicator concentrations in Hulē'ia Stream Lower.

Sample Date	Fecal Coliforms (CFU/100ml)	Enterococci (CFU/100ml)	*C. perfringens* (CFU/100ml)	Coliphage		Enrichment of 100 ml	
				Male Specific (PFU/100ml)	Somatic (PFU/100ml)	Male Specific	Somatic
3/19/02	272	720	0	20	60		
4/24/02	108	356	0	0	0	-	+
5/16/02	692	396	0	0	40	-	
6/26/02	212	360	0	0	0	-	+
7/16/02	296	760	0	0	80	-	
8/21/02	280	440	0	0	0	-	-
9/25/02	184	176	0	0	20	-	
11/20/02	120	88	0	0	80	-	

* shaded areas indicate than an enrichment was not done.

APPENDIX B

RAW CHEMICAL AND PHYSICAL DATA FOR STREAMS AND MARINE SITES IN THE NAWILIWILI WATERSHED.

Table B-1. Chemical and physical parameters for Nawiliwili Stream Upper.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.035	1.9	5.7	1.0
11/28/01	0.129	1.2	8.45	0.2
2/27/02	0.004	0.3	10.0	0.0
3/20/02	0.017	0.3	10.5	0.2
4/22/02	0.016	0.4	8.85	0.1
5/14/02	0.030	0.2	63.40	0.2
6/24/02	0.102	0.3	17.5	0.5
7/24/02	0.292	0.5	10.1	0.1
10/16/02	0.132	0.3	9.37	0.1
11/25/02	0.025	0.2	2.86	0.4

Table B-2. Chemical and physical parameters for Nawiliwili Stream Lower.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
3/19/02	0.022	0.4	5.7	1.0
4/24/02	0.031	0.4	6.5	1.0
5/16/02	0.038	0.3	7.0	2.0
6/26/02	0.202	0.6	7.0	1.0
7/16/02	0.128	0.3	8.5	1.0
8/21/02	0.156	0.3	6.4	2.0
9/25/02	0.187	0.3	6.7	0.5
11/20/02	0.034	0.2	4.9	0.0

Table B-3. Chemical and physical parameters for Marriott Culvert.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.091	1.9	4.8	1.0
11/28/01	0.039	2.4	4.35	0.3
2/27/02	0.056	1.0	3.1	0.0
3/20/02	0.079	1.0	2.2	0.2
4/22/02	0.062	0.7	3.65	0.2
5/14/02	0.274	0.1	163	0.1
6/24/02	0.305	1.0	3.68	0.3
7/24/02	0.450	0.8	2.47	0.3
10/16/02	0.301	1.0	3.05	0.3
11/25/02	0.253	0.1	4.02	1.3

Table B-4. Chemical and physical parameters for Pine Trees.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.052	0.9	2.6	3.0
11/28/01	0.166	1.8	3.75	1.3
2/27/02	0.042	0.2	6.8	2.0
3/20/02	0.039	0.3	4.22	0.3
4/22/02	0.028	0.2	7.49	0.2
5/14/02	0.130	0.2	66.2	0.4
6/24/02	0.104	0.2	9.05	0.6
7/24/02	0.153	0.3	8.49	5.4
10/16/02	0.123	0.2	5.82	1.6
11/25/02	0.132	0.2	13.2	3.2

Table B-5. Chemical and physical parameters for Kalapakī Beach.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.011	1.4	2.7	28.0
11/28/01	0.043	1.7	5.93	32.7
2/27/02	0.001	0.2	3.8	35.0
3/20/02	0.015	0.2	2.82	34.8
4/22/02	0.019	0.1	4.01	34.0
5/14/02	0.053	0.3	51.9	21.5
6/24/02	0.095	0.3	3.75	31.8
7/24/02	0.032	0.3	5.82	33.6
10/16/02	0.045	0.3	3.90	34.4
11/25/02	0.041	0.2	1.97	34.4

Table B-6. Chemical and physical parameters for Seaflite Jetty.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.015	2.5	3.7	28.0
11/28/01	0.042	0.9	5.37	28.4
2/27/02	0.001	0.2	9.0	35.0
3/20/02	0.012	0.2	2.18	34.6
4/22/02	0.012	0.1	4.84	34.0
5/14/02	0.016	0.2	10.2	27.7
6/24/02	0.103	0.2	3.14	21.7
7/24/02	0.049	0.2	4.01	29.5
10/16/02	0.235	0.2	1.98	34.8
11/25/02	0.013	0.2	1.58	34.4

Table B-7. Chemical and physical parameters for Papalinahoa Stream.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.044	0.8	7.30	2.0
11/28/01	0.051	0.7	9.54	0.2
2/27/02	0.006	0.1	9.40	1.0
3/20/02	0.046	0.1	7.88	0.3
4/22/02	0.034	0.0	7.84	0.1
5/14/02	0.042	0.1	38.9	0.2
6/24/02	0.194	0.1	5.71	0.2
7/24/02	0.212	0.1	6.67	0.1
11/25/02	0.039	0.1	5.36	4.1

Table B-8. Chemical and physical parameters for Small Boat Harbor.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.013	1.2	2.70	24.0
11/28/01	0.316	0.3	29.4	6.8
2/27/02	0.019	0.1	4.20	16.0
3/20/02	0.016	0.1	4.33	20.1
4/22/02	0.026	0.1	23.0	19.8
5/14/02	0.026	0.0	29.3	3.1
6/24/02	0.101	0.0	12.8	4.0
7/24/02	0.088	0.1	12.2	9.7
10/16/02	0.093	0.1	6.05	23.7
11/25/02	0.023	0.1	2.95	0.1

Table B-9. Chemical and physical parameters for Puali Stream Upper.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
3/19/02	0.044	0.2	4.1	0.5
4/24/02	0.022	0.1	3.9	2.0
5/16/02	0.031	0.2	7.5	0.0
6/26/02	0.188	0.3	4.6	1.0
7/16/02	0.103	0.2	5.0	1.0
8/21/02	0.091	0.1	3.8	1.0
9/25/02	0.101	0.2	3.5	1.0
11/20/02	0.035	0.1	3.0	1.0

Table B-10. Chemical and physical parameters for Puali Stream Lower.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.023	0.7	2.4	2.0
11/28/01	0.032	1.2	4.06	0.1
2/27/02	0.005	0.2	4.40	0.0
3/20/02	0.027	0.1	4.09	0.2
4/22/02	0.033	0.2	2.92	0.1
5/14/02	0.066	0.3	14.7	0.1
6/24/02	0.225	0.4	3.37	0.1
7/24/02	0.182	0.4	10.1	0.1
10/16/02	0.214	0.4	5.67	0.2
11/25/02	0.079	0.2	9.96	0.1

Table B-11. Chemical and physical parameters for Papakōlea Stream Upper.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.026	1.0	6.5	1.0
11/28/01	0.684	1.7	13.2	1.7
2/27/02	0.006	0.2	11.0	0.2
3/20/02	0.033	0.4	18.3	0.4
4/22/02	0.046	0.4	9.95	0.4
5/14/02	0.048	0.3	64.2	0.3
6/24/02	0.089	0.3	14.1	0.3
7/24/02	0.090	0.2	13.1	0.2
10/16/02	0.074	0.3	17.1	0.3
11/25/02	0.029	0.2	3.72	0.2

Table B-12. Chemical and physical parameters for Papakōlea Stream Lower.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
3/19/02	0.019	0.4	11.0	0.5
4/24/02	0.021	0.3	10.0	2.0
5/16/02	0.024	0.4	17.0	1.0
6/26/02	0.075	0.3	7.5	1.0
7/16/02	0.090	0.2	7.6	1.0
8/21/02	0.086	0.1	8.0	1.0
9/25/02	0.083	0.1	5.3	1.0
11/20/02	0.021	0.2	6.0	1.0

Table B-13. Chemical and physical parameters for Hulē'ia Stream Upper.

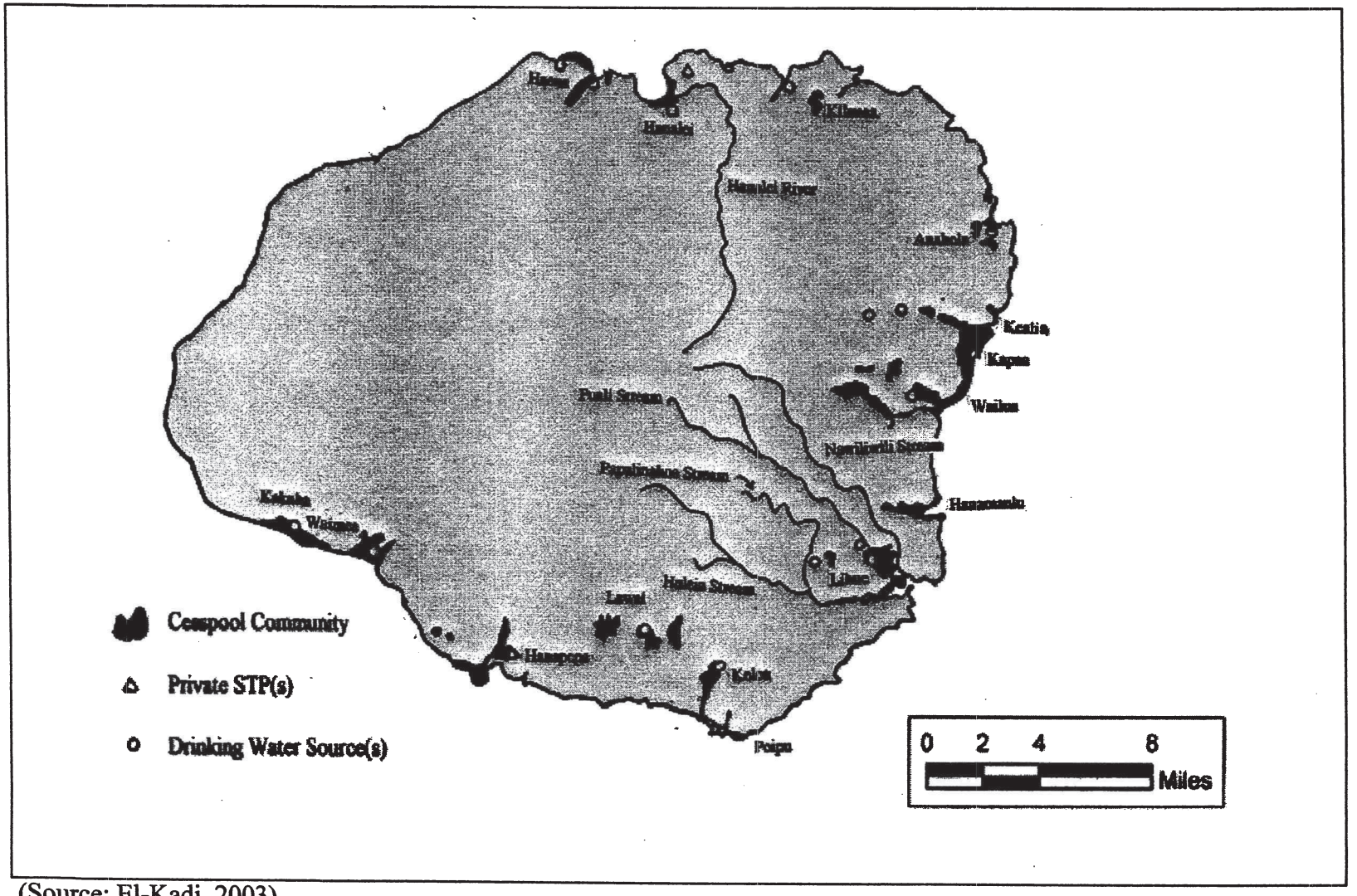
Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
10/31/01	0.015	1.5	2.6	3.0
11/28/01	0.029	0.7	21.8	0.1
2/27/02	0.001	0.1	5.5	0.0
3/20/02	0.010	0.1	9.6	0.1
4/22/02	0.013	0.1	3.4	0.1
5/14/02	0.023	0.0	25.6	0.1
6/24/02	0.106	0.0	6.2	0.1
7/24/02	0.066	0.1	3.5	0.1
10/16/02	0.083	0.1	2.1	0.1
11/25/02	0.021	0.1	3.6	0.1

Table B-14. Chemical and physical parameters for Hulē'ia Stream Lower.

Sample Date	Phosphate (mg/L)	Nitrate (mg/L)	Turbidity (NTU)	Salinity (ppt)
3/19/02	0.031	0.1	12.0	0.5
4/24/02	0.037	0.1	5.9	1.0
5/16/02	0.022	0.1	8.5	1.0
6/26/02	0.079	0.1	5.3	0.0
7/16/02	0.097	0.1	2.3	0.0
8/21/02	0.109	0.1	4.4	1.0
9/25/02	0.084	0.1	2.2	0.0
11/20/02	0.021	0.1	3.8	0.0

APPENDIX C

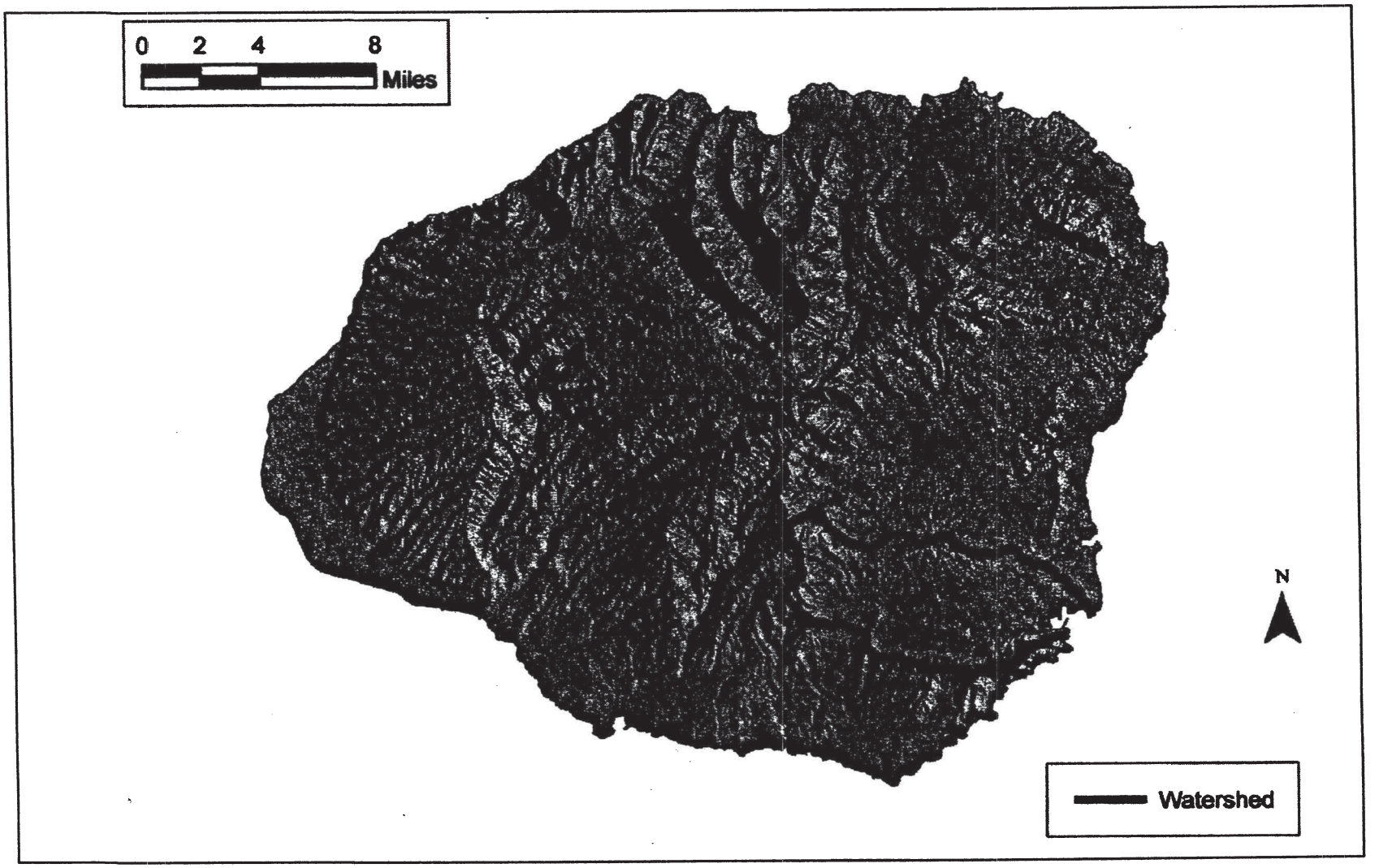
GENERAL LOCATION OF CESSPOOLS ON KAUA'I



(Source: El-Kadi, 2003).

APPENDIX D

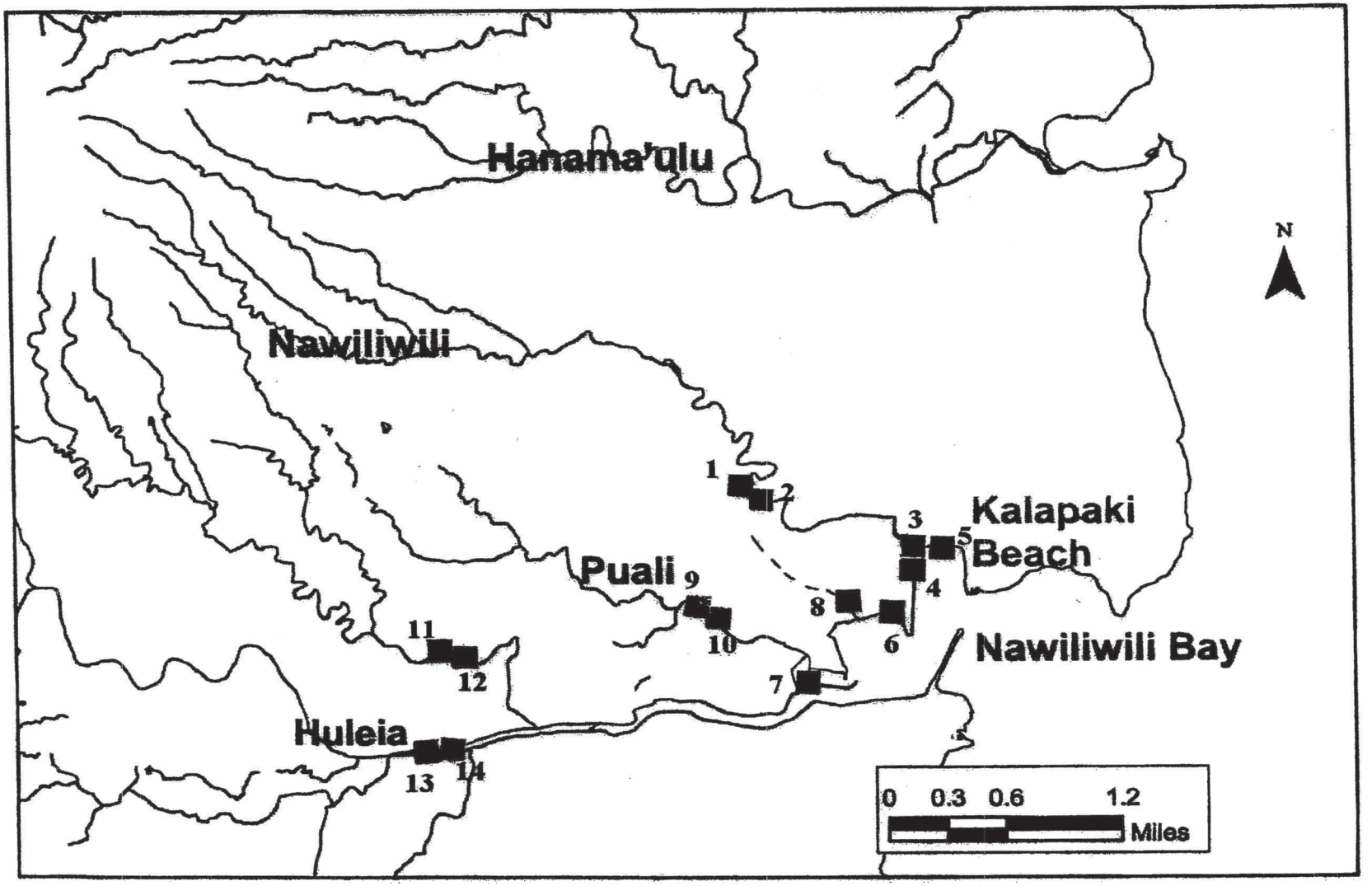
GENERAL LOCATION OF THE NAWILIWILI WATERSHED



(Source: El-Kadi, 2003).

APPENDIX E

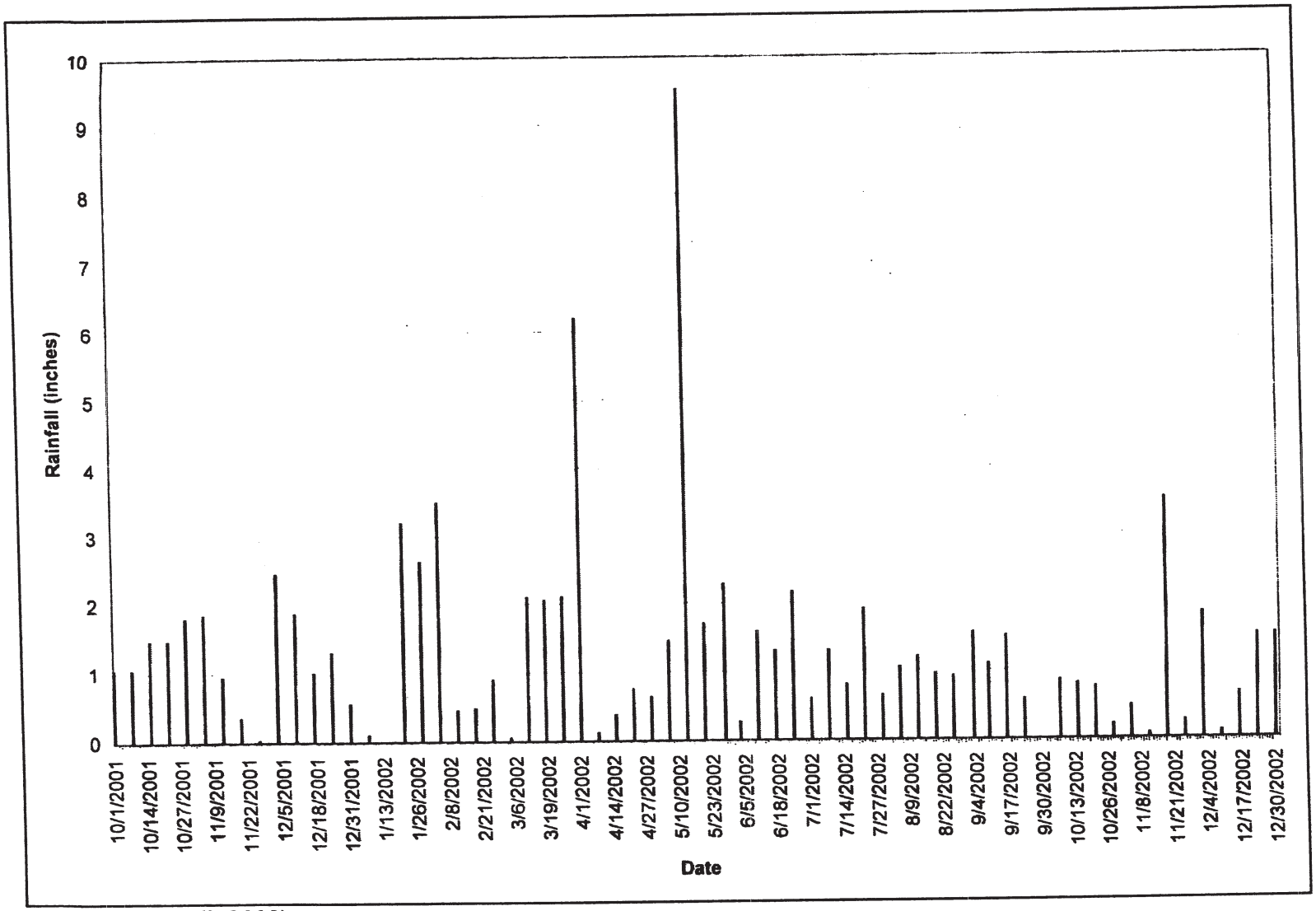
MAP OF SAMPLING LOCATIONS IN THE NAWILIWILI WATERSHED



(Source: El-Kadi, 2002).

APPENDIX F

RAINFALL DATA FOR NAWILIWILI WATERSHED



(Source: El-Kadi, 2003).

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