MICROBIOLOGICAL ASSESSMENT OF KAELEPULU STREAM AND THE IMPACT OF DISCHARGE IN KAILUA BAY (KB-3)

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[12] ABSTRACT (PURPOSE, METHOD, RESULTS, CONCLUSIONS)

Kaelepulu Pond is an inland brackish water pond (20 ppt salinity) which is under tidal influence and is fed by rainfall. Water from this pond flows via canals and streams (Kaelepulu Stream) for approximately 2 miles through a residential community (Kailua) and discharges into the ocean at Kailua Beach, the most popular beach on the windward side of Oahu, Hawaii. Water in the Kaelepulu pond and stream system has been classified for recreational use and must meet the State standard of 200 fecal coliform/100 ml. A sewage pumping station located next to this stream has been documented to occasionally discharge untreated sewage into the stream. The bacterial quality of the water in this stream system has been previously determined to be poor, and citizens of this community have concluded that the sewage from the pumping station is responsible for the poor water quality. The objective of this study was to determine the sources of fecal indicator bacteria entering the Kaelepulu Stream system and to assess the impact of this stream on the water quality of water at Kailua Beach. Water from throughout the stream system, soil, and duck feces were analyzed for indicator bacteria (fecal coliform, enterococci, *E. coli,* and *C. perfringens*). Storm drains and tributary streams (especially during rainfall, soil, and duck feces) were the major sources of fecal indicator bacteria Kaelepulu Stream. Analysis of stream water samples showed that, of the three recreational water quality standards, the enterococci standard was exceeded most frequently, followed by the *E. coli* and the fecal coliform standard.

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Abstract

Kaelepulu Pond is an inland brackish water pond (20 ppt salinity) which is under tidal influence and is fed by rainfall. Water from this pond flows via canals and streams (Kaelepulu Stream) for approximately 2 miles through a residential community (Kailua) and discharges into the ocean at Kailua Beach, the most popular beach on the windward side of Oahu, Hawaii. Water in the Kaelepulu pond and stream system has been classified for recreational use and must meet the State standard of 200 fecal coliform/100 ml. A sewage pumping station located next to this stream has been documented to occasionally discharge untreated sewage into the stream. The bacterial quality of the water in this stream system has been previously determined to be poor, and citizens of the Kailua community have concluded that the sewage from the pumping station is responsible for the poor water quality. The objective of this study was to determine the sources of fecal indicator bacteria entering the Kaelepulu Stream system and to assess the impact of this stream on the water quality at Kailua Beach. Water from throughout the stream system, soil, and duck feces were analyzed for indicator bacteria (fecal coliform, enterococci, *E. coli,* and *C. perfringens).* Storm drains and tributary streams (especially during rainfall), soil, and duck feces were the major sources of fecal indicator bacteria in Kaelepulu Stream. Analysis of stream water samples showed that, of the three recreational water quality standards, the enterococci standard was exceeded most frequently, followed by the *E. coli* and the fecal coliform standards.

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I. Types of Diseases Transmitted by Recreational Waters

Recreational waters surrounding the islands of Hawaii are an important natural resources for the residents and tourists of Hawaii. Many leisure time activities associated with these recreational waters, whether it be swimming, boating, sailing, scuba diving or fishing, draw an abundant number of participants. With the increase in the resident and tourist populations this natural resource is threatened by both known and unknown sources of pollution. These types of pollution may pose a health risk to those individuals who contact contaminated recreational waters. This risk comes from contact and ingestion of contaminated water. The risk of contracting an illness from polluted water is dependent on the type of exposure. Activities such as swimming, surfing and scuba diving are often termed primary exposure activities since the individual is in intimate contact with the water. Other activities such as sailing, boating and fishing pose less of a risk since exposure is not as frequent. The risk of contracting illness from these forms of exposure is dependent on the health of the individual, duration of exposure, concentration, and type of disease causing organism present in the water. As shown in Table 1 and 2 there are a variety of bacterial, viral and protozoan pathogens capable of causing diseases. Illnesses obtained from recreational waters can be divided into two categories, water contact diseases and waterborne diseases from the ingestion of contaminated waters.

Table 1 Water Transmitted Infectious Bacteria

Organism	Disease
A. Water Contact Diseases	
Aeromonas hydrophila	Wound infection
A. sobria	Wound infection
A. caviae	Wound infection
Citrobacter spp.	Wound infection
Acinetobacter calcoaceticus	Nosocomial infections
Enterobacter spp.	Nosocomial infections
Francisella tularensis	Tularemia
Klebsiella pneumoniae	Nosocomial, pneumonia
Fusobacterium necrophorum	Liver abscesses
Klebsiella pneumoniae	Nosocomial, pneumonia
Leptospira icterohemorrhagia	Leptospirosis
Legionella pneumophila	Legionellosis
Mycobacterium tuberculosis	Tuberculosis
M. marinum	Granuloma
Morganella morganii	Nosocomial
Pseudomonas pseudomallei	Meliodosis
Staphylococcus aureus	Wound infections
Vibrio alginolyticus	Wound infections
Vibrio vulnificus	Wound infections
B. Waterborne Diseases	
Aeromonas hydrophila	Enteritis
A. sobria	Enteritis
A. caviae	Enteritis
Campylobacter jejuni	Enteritis
C. coli	Enteritis
Chromobacterium violaceum	Enteritis
Citrobacter spp.	Nosocomial infections
Clostridum perfringens type C	Enteritis
Enterobacter spp.	Nosocomial
Escherichia coli serotypes	Enteritis
Flavobacterium meningosepticum	Nosocomial, meningitis
Plesiomonas shigelloides	Enteritis
Salmonella enteritidis	Enteritis
S. montevideo B	Salmonellosis
S. paratyphi A & B	Paratyphoid fever
S. typhi	Typhoid fever
S. typhimurium	Salmonellosis
Serratia marcescens	Nosocomial
Shigella dysenteriae	Dysentary
Staphylococcus aureus	Food poisoning
Vibrio cholerae	Cholera dysentery
V. fluvialis	Enteritis
V. mimicus	Enteritis
V. parahaemolyticus	Enteritis
Yersinia enterocolitica	Enteritis

(adapted from Hazen, 1988)

Table 2 Other Water Transmitted Pathogens

Organism	Disease
Viruses	
A. Water Contact Diseases	
Adenovirus	Pharyngitis, eye infections
B. Waterborne Diseases	
Adenovirus	Enteritis
Calicivirus	Enteritis
Norwalk virus	Enteritis
Coronavirus	Enteritis
Coxsackievirus A & B	Meningitis, myocrditis
Echo virus	Enteritis,meningitis
Hepatisis A	Hepatitis
Poliovirus	Poliomyelitis
Rotavirus	Enteritis
Astrovirus	Enteritis
Protozoa	
A. Water Contact Diseases	
Nalegleria fowleri	Meningoencephalitis
Acanthmoeba spp.	Meningoencephalitis
B. Waterborne Diseases	
Balantidium coli	Balantidiosis
Cryptosporidium spp.	Cryptosporidiosis
Giardia lamblia	Giardiasis
Entamoeba histolytica	Dysentery

(Hazen, 1988)

A. Water Contact Diseases

The first category, water contact diseases, are those illnesses contracted from contact with recreational waters. Most of the diseases that fall into this category are characterized by diseases that cause skin and wound infections. Pathogens that causes

these types of diseases have both human and environmental sources. The pathogens associated with human sources are generally opportunistic pathogens and are considered normal inhabitants of humans. One example, *Staphylococcus aureus,* has been found to be the most numerous pathogen shed by swimmers (Robinton and Mood, 1966). *S. aureus* is a common organism found as a normal inhabitant of many individuals. Among swimmers, *S. aureus* is capable of causing a variety of infections usually targeting the skin (e. g. scratches boils, and other skin infection) and less frequently the eyes and throat. Another example of a pathogen having human sources is *Pseudomonas aeruginosa* which is the most frequently pathogen isolated from the disease termed "swimmer's ear" (Calderon, 1981). Numerous epidemiological studies have confirmed an association between swimming and ear infection caused by *P. aeruginosa* (Hoadely and Knight, 1975 and Seyfried and Cook, 1984).

In addition to human sources, the environment is often a source of pathogens associated with water contact. These pathogens may be indigenous to the aquatic environment or excreted by animals found in the vicinity of the recreational waters. Many *Vibrio* species are indigenous to the marine environment and are capable of causing a variety of contact diseases. These diseases include ear infections and septicemias with the majority being caused by the species, *V. parhaemolyticus, V. vulnificus,* and *V. alginolyticus. V. vulnificus* has been associated with the septicemia resulting from contact with swimming waters and has a high fatality rate (Bonner, 1983). *V. parhaemolyticus* is usually associated with food borne transmission but may also be transmitted through water contact. The mode of transmission for most of the marine vibrios is through cuts, abrasions and wounds. In addition to the naturally occurring pathogens, animal urine can be a source of water contact disease. *Leptospira* is probably the best example of a contact disease resulting from excreted urine of infected animals. A variety of animals including rats, dogs, cats, pigs and cattle may

carry *Leptospira* and excrete this pathogen in to recreational waters. *Leptospira* gain entry via skin abrasions so swimming and contact activities are two of the major means of exposure to humans. Numerous outbreaks have occurred in the United States (Table 3). Safer reported an outbreak associated with recreational waters in with an attack rate of 63% (Safer, 1961). In this study, the source of infection was a local swimming hole was fed by a stream in which dead hogs had been floating. Serological testing was positive in hogs and other animals found in the vicinity of the stream. Since *Leptospira* are not associated with fecal contamination, current water quality standards will not always predict the possible presence of this pathogen.

Table 3 Outbreaks of Leptospirosis Associated with Swimming Water

Outbreak Location	Etiologic Agent	No. of Cases	Possible Source	Reference
Geneva, Ala	*L. pomona*	50	Hogs	Cockburn et al.,1954
Jackson Hole, Wyo	*L. canicola*	24	Dogs	Schaffer et al., 1951
Standing Rock, S.D.	*L. pomona*	5	Livestock	Jellison et al., 1958
Kennewick, Wash	*L. pomona*	61	Cattle	Nelson et al., 1973
Tennessee	*L. interrigans*	7	"unkown"	CDC, 1976
Cedar Rapids, Iowa	*L. pomona*	40	Cattle	Tjalma et al.,1965

The State of Hawaii has the highest incidence of leptospirosis in the United States and accounts for 29% of the total cases reported (Sasaki et al., 1991). Leptospirosis in the is by far the most serious water-borne disease in Hawaii and has been associated with swimming, wading and working in fresh water streams (Fujioka et al., 1991). Although freshwater streams in Hawaii are considered the source of *Leptospira*, a lack of available methodology for isolating pathogenic *Leptospira* has made it very difficult to monitor recreational water for *Leptospira*.

B. Waterborne Ingestion Diseases

Unlike water contact diseases, waterborne diseases results from the ingestion of recreational waters and most result in gastroenteritis, although viral infections cause hepatitis, poliomyelitis, meningitis, and myocarditis. The pathogens involved in gastroenteritis are a large group of microorganisms which include bacteria, viruses and protozoans. For the most part these pathogens are associated with human and animal feces and the fecal\oral route of transmission. There are however, pathogens that are naturally occurring (e.g. *V. parhaemolyticus*) in the environment and can cause gastroenteritis from ingestion of contaminated waters.

1. Human and Animal Feces

Human and animal feces are the major contributors of pathogens causing water borne disease. Feces may enter recreational water by a variety of mechanisms, but the most frequent route being introduction of sewage by outfalls located near recreational waters. Although waterborne recreational water outbreaks of gastroenteritis are a rare occurrence in the U. S. and most industrialized countries, the potential health threat is always present. This risk is greatly amplified when sewage discharges occur in the proximity of recreational waters. When considering documented cases of waterborne diseases, however, the range of illness is much more limited with the majority being caused by gastroenteritis. Documented disease outbreaks of gastroenteritis are limited mainly to three types of pathogens, bacterial, viral and protozoan.

Although there are a large number of bacterial pathogens that can be transmitted by water, the incidence of recreational water outbreaks are limited to typhoid fever, salmonellosis and shigellosis. For the most part, diseases caused by these bacteria are

characterized by nausea, vomiting, diarrhea, and abdominal cramps. In the case of typhoid fever, there is also entry into the bloodstream resulting in a persistent fever and often a chronic carrier state.

Typhoid fever was one of the earliest reported diseases associated with recreational water. In 1888 Discher (Discher, 1963) reported on an outbreak involving 49 cases from swimming in the Elbe River in Germany. Another outbreak occurred ten years later in Walmer, England and was associated with marine recruits that swam in sea water-filled pools as part of their training (Reece, 1954). In this case a nearby sewage discharge was responsible for contaminating sea water that was used to fill the pool. The first documented outbreak in the United States occurred in 1923 in New Haven, Connecticut. Ciampolini (Ciampolini, 1921) reported that the cause of typhoid fever among swimmers was the contaminated water in New Haven Harbor. Unlike the previous two cases in England, this report was unable to show a direct relationship with disease and swimming. Water contact was implicated since food, milk and drinking water did not appear contaminated. Through deductive reasoning Ciampolini was able to show an association with swimming in the harbor. As is often the case with waterborne outbreaks the disease causing organism was not isolated from swimming waters and therefore only an casual relationship was demonstrated.

The second bacterial pathogen associated with gastroenteritis, salmonella, has been a rare occurrence when compared to food borne transmission of this disease. Only 3 % of water borne outbreaks between 1971 and 1978 was caused by Salmonella (Craun, 1981). The largest outbreak occurred in 1965 in Riverside, California and over 16,000 people were infected (Craun, 1986). The causative agent of this particular outbreak was determined to be *S. typhimurium* from a contaminated ground water source. Subsequent chlorination appeared to eliminate this problem.

Although the infective dose of *Shigella* is only 10 to 100 bacilli (Dupont, 1973) as compared to the infectived dose of 10^3 to 10^4 for *S. typhi* and *S. typhimurium* (Mims, 1987), there have been very few cases of outbreaks associated with swimming in contaminated waters. This may be due the fact that they do not survive long in the aquatic environment (Dunlop, 1957). The only reported outbreak caused by *Shigella* occurred in Dubuque, Iowa in 1974 when *Shigella* was isolated in swimming waters frequented by infected swimmers (Rosenberg, 1976). One strain isolated from a swimmer had an antibiotic sensitivity pattern similar to isolates from the swimming waters. An upstream sewage discharge was likely the cause of this particular outbreak. Waters down stream of this discharge point had fecal coliform levels of between 400,000 and 5,000,000 per 100ml.

The second category of microorganisms, viral pathogens, have been well documented in recreational water outbreaks. As with many bacterial pathogens, it has often been difficult to isolate viral pathogens from recreational waters at the time of the outbreak. As a result, there are very few cases of direct relationships between swimming in infected waters and disease. Three of the more common causes of viral outbreaks are those involving hepatitis A, the coxsackie viruses and norwalk viruses. In 1969 an outbreak among a Boy Scout troop was attributed to swimming exposure in a South Carolina Lake (Bryran et al., 1974). In this case an association with scouts swimming in the lake and hepatitis A was determined since a large number of swimmers contracted this illness. Although hepatitis A was never recovered, the lake was shown to have fecal contamination. Food did not appear to be a source of fecal contamination. It is important to note, however, the scouts may have obtained this disease from drinking water obtained from the same lake water. As with the majority of cases involving infectious hepatitis, contaminated drinking water is implicated most often (Craun, 1986).

In addition to hepatitis A, coxsackie viruses have been implicated in outbreaks associated with swimming in contaminated waters. In Nirot, France coxsackie A virus was isolated from stool specimens from infected swimmers as well as from a lake water sample (Denis et al., 1974). In addition, samples from the lake also contained *Escherichia coli* levels between 50 and 1000 per 100 ml. A similar outbreak occurred in Michigan in 1979 when coxsackie B virus was isolated from lake water as well as infected swimmers (Hawley et al., 1979). In both of these cases the pathogen was isolated from swimming waters used by infected swimmers.

Recently the Norwalk-like of viruses have been considered major contributors to the incidence of swimming associated gastroenteritis. Cabelli feels it is the virus responsible for most swimming associated gastroenteritis (Cabelli and Debartolomeis, 1991). Most of the symptoms associated with waterborne gastroenteritis illnesses indicate that many of these illnesses appear similar to the symptoms expressed by the Norwalk viruses.

Recreational water outbreaks of waterborne protozoan diseases associated gastroenteritis are a rare occurrence when compared to drinking water outbreaks. One such protozoan, *Giardia lamblia*, has been implicated as the most commonly identified pathogenic intestinal protozoan in waterborne outbreaks involving drinking water. The disease caused by this protozoan, Giardiasis is characterized by nausea, anorexia, and explosive diarrhea. *Giardia* cysts are capable of surviving for long periods in the environment (Rentorff and Holt, 1954) and the infective dose is as few as 10 cysts (Rentorff, 1954). These factors contribute to the health risk involved with swimming in fecal polluted waters. Giardiasis contracted by contact with water has been documented in a case involving police and fire department divers working in the Hudson River near New York City (Craun, 1986). Another case showing an

association occurred in a swimming pool in Washington (Craun, 1986). Both cases were by association since *Giardia* was not isolated from the contacted waters.

2. Environmental Sources of Gastroenteritis

In addition to the pathogens associated with feces, the environment can also be a source of pathogens capable of causing gastroenteritis. Many naturally occurring marine *Vibrio* species are capable of causing gastroenteritis. *V. cholerae V. parhaemolyticus* are human pathogens capable of causing gastroenteritis. *V. parhaemolyticus is*most often been associated with the consumption of contaminated shellfish (Colwell et al., 1973).

3. Unknown Sources of Waterborne Outbreaks

Although careful investigation is often able to determine the causes of waterborne outbreaks, they represent a fraction of the total number of outbreaks that occur. The majority of outbreaks are of an undetermined etiology. Between 1920 and 1980 Craun estimated that 77% of waterborne gastroenteritis was in this category (Craun 1986). Many factors may contribute to this group of outbreaks. Lack of methodology to isolate pathogens have been, and continues to be a major problem when isolating bacteria, protozoan and viral pathogens, although many new techniques have been developed in the last decade. Because of the difficulty in isolating pathogens and the large number of different pathogens in environmental waters, indicator systems are used to determine the sanitary quality of recreational waters.

II. Monitoring Water for Hygienic Quality: The Indicator Concept

Because of the difficulty in isolating pathogens from the environment and the wide variety of pathogens that may be transmitted through recreational waters, an indicator system for determining the hygienic quality of water was developed. Since the majority of disease causing microorganisms transmitted via water are found in sewage or human fecal material, an indicator of feces was established. While there is no ideal water quality indicator, the best indicator system is one whose densities correlate best with the risk associated with a given type of pollution (Cabelli, 1978). When considering risk analysis, the ideal indicator should possess the following characteristics:

1) The ideal indicator should always be present and occur in much greater numbers than the pathogens concerned.
2) The indicator should not be able to proliferate to a greater extent than pathogens in the aqueous environment.
3) The indicator should be more resistant to disinfects and to the aqueous environments than pathogens.
4) The indicator should be easy to isolate, identify, and enumerate.

(Dutka, 1973)

Since no microorganism fits all of these criteria, microorganisms that best fit these criteria are utilized knowing that each has positive and negative attributes. Some of the popularly accepted indicators for recreational waters will be discussed in this review. These indicators include total coliforms, fecal coliform, *Escherichia coli*, Enterococci, *Clostridium perfringens*, and the bacteriophage viruses. As seen in Table 4

each of these indicators have different sources and potential uses. It is important to note, these indicators were developed in temperate climates and thus apply to the behavior of these indicators under temperate climate conditions. These characteristics may not apply to tropical climates (Fujioka, 1985).

Table 4 Water Quality Indicators, Their Significant Sources, and Potential Uses (adapted from Cabelli. 1978)

Indicator	Significant Source[a]	Potential Use[b]
Coliforms	F S I R A	S
Escherichia coli	F S [c]	P F S A
Klebsiella sp.	S I R A	P S N
Enterobacter sp.	S I R A	S
Citrobacter sp.	F S I R A	S
Fecal coliforms	F S [c]	F S
Enterococci	F S [c]	F S A D
Clostridium perfringens	F S [c]	F S D
Candida albicans	F S	P F S
Bifidobacteria	S	F S A D
Coliphage	[d]S I R A	S
Pseudomonas aeruginosa		P S N

[a]Relative to other sources: F, feces of warm-blooded animals; S, sewage; I, industrial wastes; R, run-off from uncontaminated soils; A, fresh and marine waters.

[b]Potential use: P, pathogen;F, fecal indicators; S, sewage indicator; A, separation of human from lower animals sources; D, proximity to fecal source; N, indicator of nutrient pollution.

[c]May occur in tropical climates

[d]Questionable

Table 5 Comparison of Fecal Indicators Bacteria

	Log_{10} Density			
	Feces per gram		Sewage/100ml	
Indicator Bacteria	Human	Animal	Influent	Relative Survival
Total Coliform	6-7	4-6	6-7	++
Fecal Coliform	6-7	4-6	6-7	++
E. coli	6-7	4-6	6-7	++
Enterococci	3-4	2-3	5	+++
C. perfringens	3-4	2-3	4	++++

adapted from Cabelli, 1979

A. Coliform Bacteria: The First Indicator

In the late 19th century Escherich first proposed the concept of indicator bacteria (Escherich, 1885). This group, the "coliforms", are an ecologically heterogeneous group of bacteria some of which consistently and exclusively are associated with the feces of warm-blooded animals. *Standard Methods* defines this group as consisting of aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C (*Standard Methods*, 1989). Included in this group are the genera *Escherichia, Citrobacter, Enterobacter,* and *Klebsiella.* These bacteria are normally found in the human intestine, but also in soils on vegetation and in natural waters (Fujioka et al., 1988 and Hazen, 1988). Since these bacteria are found in sources other than sewage, there significance as water quality indicators is questionable.

According to Olivieri, there are a number of deficiencies with using total coliform bacteria as sanitary indicators (Olivieri, 1982). Coliform bacteria tend to die

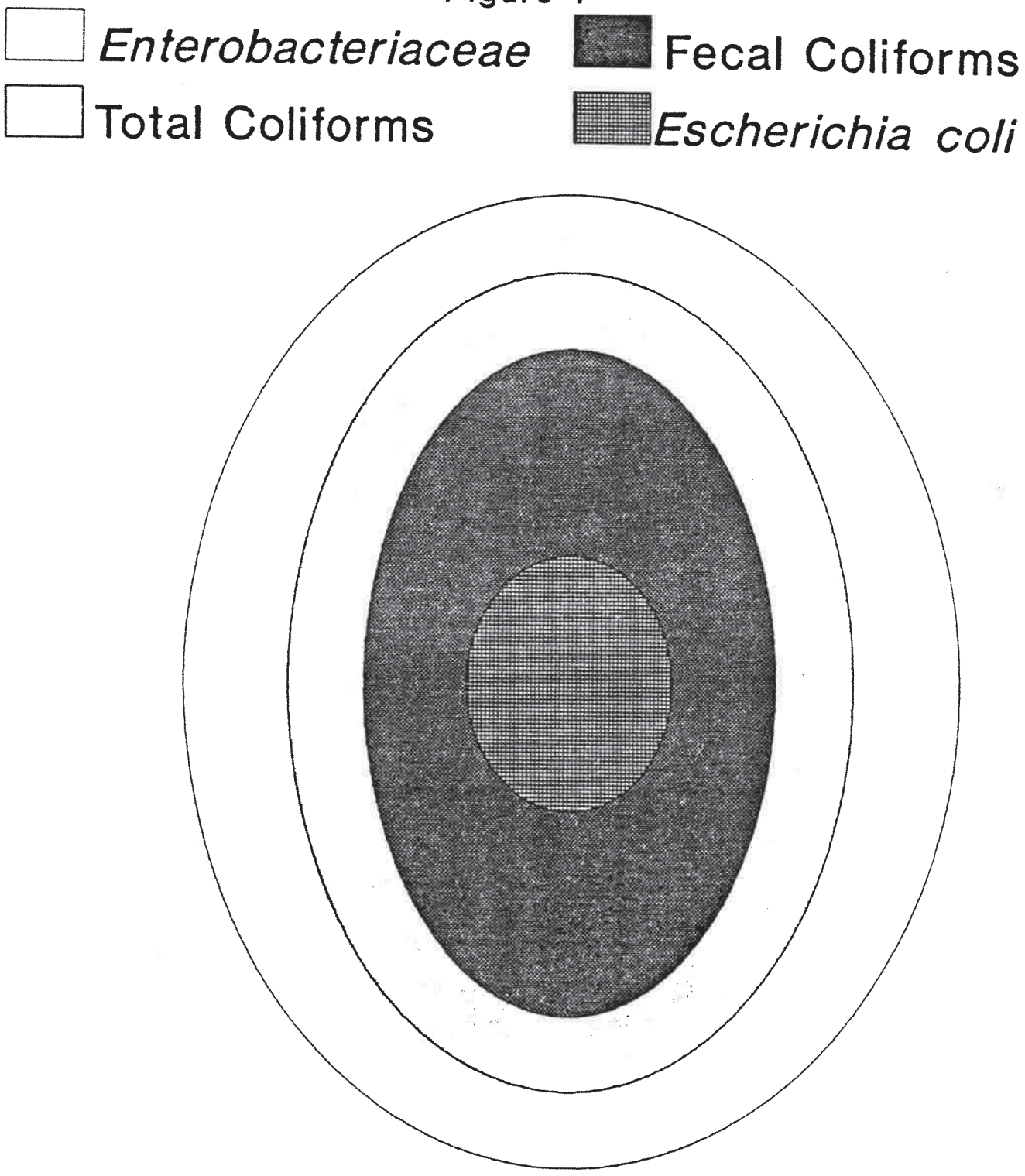
off faster than many non-bacterial pathogens like the enteric viruses and protozoans like *Giardia.* In addition, coliform bacteria are more sensitive to disinfection than are many non-bacterial pathogens. Enteric viruses and many protozoans are more resistant to disinfection. Another deficiency is their ability to survive and replicate in the environment (Hazen, 1988 and Fujioka et al.,1988) Pathogenic enteric viruses and protozoans are unable to replicate in the environment. In this case coliforms would not necessarily represent recent fecal contamination. And finally, growth of coliform bacteria may be suppressed by high populations of competing microorganisms. Although total coliform bacteria are still widely used as indicator organisms, there is a evolutionary progression toward those coliform genera that more accurately represent fecal pollution.

B. Fecal Coliform: A More Specific Indicator of Feces

As seen in Figure 1, the fecal coliform are a subgroup of the total coliform group. As mentioned earlier there has been a step wise progression toward using indicators that more accurately represent fecal pollution. Research in to the use of fecal coliforms was stimulated by findings that showed some total coliforms members included coliforms from non-fecal sources. Fecal coliform are more specifically associated with the feces of warm-blooded animals and were frequently used (1968-1986) for determining the level of fecal material in recreational waters (Geldreich et al.,1962). This group is comprised of four genera, *Escherichia, Klebsiella, Citrobacter,* and *Enterobacter* which are capable of growth at a temperature of 44.5 ^{o}C. Only a few species of *Citrobacter* and *Enterobacter* are capable of growth at 44.5^{o}C. It is the ability to grow at 44.5^{o}C that separates fecal coliform from other coliforms. Of this group, *Escherichia coli* and *Klebsiella spp.*are the species most often

Relationship of Bacteria in *Enterobacteriaceae* Family

Figure 1



isolated from human feces (Table 5) with *Escherichia coli* being the largest percentage (Dufour,1977). As seen in Table 5, *Escherichia coli* appears to best represent coliforms derived from human feces. This however does not hold true when considering the distribution of these organism in raw sewage (Table 6). Explanations for this occurrence may include inactivation of *Escherichia coli* during transport to sewage facilities and multiplication of *Enterobacter, Klebsiella,* and *Citrobacter* during transport. In addition, surface runoff and fecal coliforms from the feces of other animals may contribute to this variation.

Table 6 Percentage of Coliforms in Human Feces

Study Year	Number of samples	Number of Colonies Examined	Percent of Coliforms		
			E. coli	*Klebsiella* species	*Enterobacter/ Citrobacter*
1975	13	438	99.99	0.01	0
1976	15	285	89	5	6
Totals	28	723	96.8	1.5	1.7

(Dufour, 1977)

Table 7 Fecal Coliforms In Sewage Treatment Effluents

Indicator Bacteria	Densities at point of Sampling (CFU/100 ml)		
	Raw sewage	Primary Treated	Secondary Treated
E . coli	195	294	100
Klebsiella species	375	472	82
Citrobacter/Enterobacter species	329	242	118

(adapted from Dufour, 1977)

As with the total coliforms, the fecal coliform also have deficiencies when used as water quality indicators. Like the total coliforms, they have the same survival and sensitivity to disinfection problems. In addition regrowth of fecal coliforms and the presence of non fecal members have also contributed to the problem (Knittel, 1975).

Although this group may better represent fecal contamination they still have certain deficiencies that make them questionable as indicators.

C. *Escherichia coli:*The Major Coliform in Human Feces

As discussed earlier, *Escherichia coli* is the major coliform found in human feces. Although it would appear that this bacteria would best represent human waste, there are still the problem associated with the survivability of this organism in the environment. Enteric viruses and pathogenic protozoans are able to survive longer in the environment. This problem is partially alleviated by the high numbers of these bacteria in human feces. *Escherichia coli* can be easily distinguished from other coliforms because it lacks the enzymes necessary to degrade urea. This characteristic has made methods developed by Dufour (Dufour et al., 1981) simple and 90 % specific for isolation of *E. coli* from recreational waters. *E. coli* has shown promise in prospective epidemiological studies used to determine health risks involved in recreational waters. (Dufour,1984).

D. Enterococci: More Stable Than Coliforms

Enterococci are a subgroup of the Group D streptococci and include the genera *S. faecalis, S. faecium, and S. avium* (Standard Methods, 1989). This group are common inhabitants of the intestinal tract of warm-blooded animals and therefore are often used to detect fecal pollution. Although enterococci are more stable in marine waters than coliform bacteria, pathogens ,such as the enteric viruses and protozoans are more stable. The enterococci has, however, shown great promise in epidemiological studies relating exposure to recreational waters and the incidence of illness. Studies

performed by Cabelli show enterococci have a better correlation coefficient for total gastrointestinal symptoms than *E. coli*, fecal coliforms and *Clostridium perfringens* in marine environments (Cabelli, 1984). Enterococci have become the indicator of choice for regulatory agencies determining the water quality of marine recreational water.

E. Fecal Coliform and Fecal Streptococci Ratios

In 1976 Geldreich determined that human waste contains higher levels of fecal coliforms (FC) than fecal streptococci (FS). In contrast, animal feces are characterized by higher concentrations of fecal streptococci. Based on these results, Geldreich proposed streams with FC:FS ratios of 4 or greater indicate the presence of human feces and less than 0.7 was indicative of animal feces. In the past, this ratio has often been used to determine the source of fecal material, but this application has been recently questioned. Since FC and the FS have different survival times in the environment, and so the ratio can only be applied to fecal contamination within the past 24 hours. In addition, many members of both of these groups have been shown to replicate in the environment. This would lead to interference with this ratio. And finally, the work performed by Cabelli indicates that both of these groups have limited correlation with the occurrence of swimming associated illness (Cabelli, 1984).

F. *Clostridium perfringens*: An Alternative Indicator

Clostridium perfringens is a strict anaerobe capable of producing an environmentally stable spore. These characteristics have made *C. perfringens* an attractive indicator. Since it is a strict anaerobe it would not be able to replicate to any great extent in recreational waters. There are problems however with its ubiquity in

soils. In 932 soils samples tested by Sidorenko 85.7 % contained *C. perfringens*(Sidorenko, 1967). Although this has been the major argument against using *C. perfringens* as an indicator, there are attractive attributes. The spore of *C. perfringens* is very stable in the environment and therefore has survival pattern more similar to the pathogenic viruses and protozoans. *C. perfringens* is currently used in Europe as an indicator of sewage contamination (Cotruvo and Vogt, 1990). It has been proposed by Fujioka and Bisson as an indicator for recreational waters and water recently contaminated with sewage (Fujioka, 1985 and Bisson, 1980).

G. Bacteriophage Viruses: An Alternative Indicator

Bacterial viruses have been proposed as an alternative indicator of water quality. Two key factors help support the use of bacteriophage as indicators: 1) They are stable in the environment and survive longer than the coliform bacteria (Kott et al., 1969); 2) Bacteriophage are consistently found in sewage and (Ewert et al., 1980) ; 3) They are similar to enteric viruses in their survival in aquatic environments (Kott, 1981). Research into bacteriophage and water quality has centered around the use of coliphage and F-specific phages. Coliphage are those viruses capable of infecting members of the coliform bacteria and can be isolated from human feces. Although these bacteria have been proposed as indicators, there are problems with their association with enteric viruses. There is little data comparing the relative occurrence of coliphage and enteric viruses so their use as indicators of enteric viruses is limited. In addition, the coliphage viruses may be able to replicate in the environment (Borrego et al. , 1990). Recently the F-specific phage using Salmonella typhimurium as a host has shown promise since this host in unable to produce F-pili at temperatures below 30^{o}C. This characteristic would not allow them to replicate in the marine environment. There are problems,

however, with using this phage as an indicator. Work done by Conax indicate these phages are sporadically found in sewage contaminated waters and there densities are often much lower than coliphage (Conax, et al., 1991).

III. History of Recreational Water Quality Standards

As mentioned earlier the ideal indicator is one whose densities correlate best with health hazards associated with a given type of pollution (Cabelli, 1982). The ideal indicator will represent the health risk involved in water contact and can be associated with pathogens that may be present. In order to fill this requirement two approaches could be used. The first approach would involve determination of indicator densities in each type of pollution and at the same time, determination of the densities of all pathogens found in each type of pollution. Next the infective dose required for infection would have to be determined. At this point an association between indicator and pathogens would be able to predict the health risk associated with swimming in waters contaminated with each type of pollution. Because of the large number of pathogens that may be transmitted through water and the lack of methodology for determining each of them, this type of relationship is impossible at the current time. The second and preferable approach utilizes the densities of the candidate indicators and the illnesses contracted specifically associated with the use of a particular water. This comparison is obtained through the use of prospective epidemiological studies.

A. Epidemiological Studies

The first epidemiological study performed with recreational water was done by Stevenson in the 1950s. In this study total coliforms were used to determine the health

risk involved in swimming waters. From this study the United State Public Health Service determined that an excess of swimming associated gastroenteritis occurred in fresh water swimmers when the total coliform density exceeded 2300 per 100ml. From this study and studies conducted with the Ohio River, the 2300 per 100ml value was related to fecal coliforms. In the Ohio River study the proportion of fecal coliform to total coliform ratio was about 18%. The National Technical Advisory Committee (NTAC) then extrapolated that 400 fecal coliform represented the same health risk as the 2300 total coliform per 100 ml (National Technical Advisory Committee, 1968). An additional built in safety factor was added that then dropped this Standard to 200 fecal coliform per 100 ml. At this point the NTAC recommend the recreational water standard should not exceed a geometric mean of 200 fecal coliform per 100 ml. Prior to 1972 the recreational standards were based on total coliforms. From 1972 until 1986, the fecal coliform standard was used.

This approach at determining water quality standards was highly criticized since fecal coliform were not used directly in relating the occurrence of illness (Cabelli, 1974). In 1972 this problem was corrected by a series of studies conducted by the U.S. Environmental Protection Agency which examined the relationship between swimming-associated illness and water quality. Epidemiological studies conducted by Cabelli and Dufour were used to determine water quality standards for marine and fresh waters (Cabelli, 1983 and Dufour, 1984) One of the objective of this study was to examine several biological indicators of fecal pollution to determine which had the strongest correlation with swimming-associated health effects. These studies were the first studies dealing with risk assessment in recreational waters.

The results of the studies by Cabelli and Dufour demonstrated that fecal coliforms were not adequate indicators of water quality. Enterococci demonstrated the best correlation with swimming associated gastrointestinal illness in both fresh and

marine waters. In marine waters *E. coli* did not correlate with the incidence of gastroenteritis in swimmers. Explanations for this lack of association include a decrease in survival during sewage treatment, disinfection and instability in marine waters (Cabelli, 1983). Although *E. coli* did not correlate with the incidence of gastroenteritis in marine ,waters it did correlate equally with enterococci in fresh waters.

From these studies the EPA proposed a new set of standards based on these findings. After public comments the final revised recommendations were published in 1986 (Federal Register, 1986) For fresh water, the new criteria proposed 126 *E. coli* per 100ml or 33 enterococci per 100 ml. The proposed marine standard was 35 enterococci per 100 ml. In Hawaii the enterococci standard was modified by lowering the enterococci levels to 7 per 100 ml. These standards are applied to a series of at least five samples taken at equally spaced intervals over a thirty day period. The geometric mean of these samplings is then calculated in order to determine compliance with the before mentioned standards. The geometric mean is the antilogarithm of $\overline{\log x}$ calculated by the following equation, where X is the observation value and n is the number of observations.

$$\overline{\log x} = \frac{1}{n}\sum_{i=1}^{n} \log x_i$$

The geometric mean is utilized so that any one event (e.g. rain, sewage discharges) will not greatly influence the a series of samplings. Moreover, the epidemiological data of Cabelli and Dufour are based on the geometric mean.

B. Indicators in Tropical Climates

The majority of studies dealing with indicator organisms have been conducted in temperate climates and therefore represent the climatic behavior of these indicators. In contrast, indicator bacteria behave very differently in tropical climates and therefore many of the assumptions made about indicators in temperate climates are not applicable. One basic assumption that is not applicable to tropical climates is the ability of coliform bacteria to survive and replicate in the environment. A number of studies have shown that *E. coli* is capable of surviving and even replicating in the environment. In Puerto Rico, *E. coli* was able to survive and replicate in rain forest streams (Carrillo et al.,1985;Lopez-Torres et al., 1987; Valdes-Collazo et al.,1987). Other studies by Hardina and Fujioka indicate that fecal coliform, *E coli* and fecal streptococci are naturally occurring in Hawaii's streams and soils (Hardina and Fujioka, 1988). Once introduced, *E. coli* could remain and/or become part of the normal flora. The survival and replication would impede its ability to indicate the possible presence of pathogens.

IV. Point Source vs. Non-point Source Pollution

A. Point Source Pollution

Point source pollution is pollution derived from a known source, most often associated with practices like municipal waste discharge into marine environments. These discharges are regulated by the Clean Water Act (CWA) of 1972 and a series of permits that allow municipalities to discharge sewage in marine environments. These permits are authorized under the National Pollutants Discharge Elimination System (NPDES). The purpose of these regulations are to protect the marine environment and

recreational waters. In 1972 the Clean Water Act mandated the implementation of secondary treatment for municipal waste water facilities and as a result the City and County of Honolulu was required to implement secondary treatment at their waste facilities. NPDES permits were granted, however, that allowed for waivers for the discharges requiring secondary treatment. These waivers are called the 301 h waivers and exempted qualified treatments facilities from being required to perform secondary treatment as long as they were able to meet water quality guidelines from marine waters. The 301 h waivers are granted primary to municipalities discharging effluent into deep, cold marine waters.

B. Nonpoint Source Pollution

In addition to point source of pollution, there is also a category of pollution that is termed nonpoint sources of pollution. This type of pollution in generally the result of runoff during rainstorms. This runoff can transport pollutants via storm drains to recreational waters. The pollutants most often associated with nonpoint pollution are agricultural fertilizers, pesticides and animal feces. In the past NPDES permits have not been used to regulate the discharge of nonpoint sources of pollution. In 1987, however Congress mandated a time table for the regulation of nonpoint pollution. This required certain municipalities to obtain permits for storm drain discharges. These regulations will be implemented in October 1992 (Clean Water Act of 1987).

V. Survival of Indicators and Pathogens

When considering the health risk involved with the discharge of sewage into marine waters, it is important to consider the environmental factors that influence the

survival of both indicators and pathogens. These factors can disseminate and inactivate both indicator bacteria and enteric viruses. Work done by Loh and Fujioka indicates sea water has an antiviral activity that is capable of reducing the levels of polio viruses by as much as 90% (Fujioka, 1980 and Loh, 1979). In these same studies however, viruses were shown to survive longer than coliform bacteria, further supporting the problems associated with using coliforms as indicators.

In addition to the antiviral properties of seawater, other factors like sunlight influence the survival of indicator bacteria, research performed by Fujioka demonstrate a 90% reduction of fecal coliform and fecal streptococci in seawater within 0.5 to 2 hours (Fujioka et al. 1981). This same study however, demonstrated a resistance by these same indicator bacteria when they were placed in fresh water. Work by Barcina has indicated the same results in seawater for *E. coli* and *E. faecalis* (1990).

Chapter 2
The Proposed Study

I. Introduction to the Problem

Recent sewage discharges in the vicinity of the Kailua Beach Park have sparked public concern for the recreational water quality in Kailua Bay. These discharges have occurred at the Mokapu outfall located in Kailua Bay and it is feared that the Mokapu outfall may influence water quality along the beaches on Kailua Bay. In addition to the sewage outfall, streams such as Kaelepulu Stream, emptying into Kailua may influence the water quality of beaches located along Kailua Bay. Previous research by Fujioka has indicated that streams in Hawaii often contain high numbers of indictor organisms (Fujioka et. al., 1985 and 1988). Kaelepulu Stream may have an impact on the recreational waters along Kailua Bay since it empties into Kailua Bay at the Kailua Bay Beach Park. This beach park is the most popular and frequently used beach on Kailua Bay having many visitors daily.

II. Identification of the Problem

The Kaelepulu Stream drainage system begins in the Enchanted Lake area and proceeds toward the ocean. Under normal conditions the outlet to Kaelepulu Stream is closed by a sand bar that blocks direct contact between Kaelepulu Stream and Kailua Bay. It is frequently (approximately 30 days a year) opened, however, by abundant rain storms and artificially through the use of earth moving equipment. Kaelepulu Stream is a manmade canal that winds through residential subdivisions and is primarily influenced by storm water drainage from residential areas and a near by golf course. Kaelepulu Stream is frequented by boaters, fishermen and occasionally bathers. There is also a

City and County sewage pump station located near Enchanted Lake that has in the past, discharged sewage during high rain conditions. These reported discharges have only occurred infrequently and sporadically. However, the potential for sewage contamination of Kaelepulu Stream by this pumping station is an ongoing concern.

Sewage discharges have sparked public concern for the water quality in Enchanted Lake, Kaelepulu Stream and Kailua Bay. Residents of this community have expressed concern for the water quality and possible health effects of exposure to these waters. At the moment there is no water quality data available characterizing the microbiological water quality of Kaelepulu stream or Enchanted Lake. In addition, there is very little data determining the impact of Kaelepulu Stream's discharge on Kailua Bay.

Microbiological indicator organisms are commonly used for the assessment of recreational waters since their presence may be indicative of pathogenic microorganisms (Hazen, 1990). In addition, State and Federal recreational water standards recognize certain bacteriological indicators as representative of the sanitary quality of recreational waters. (Water Quality Standards, Hawaii and Federal Registrar) This research will utilize indicator bacteria recognized by both federal and state regulatory agencies in assessing water quality. In addition to the indicator organism recognized by the State of Hawaii and the Federal government, *Clostridium perfringens* will also be utilized as an indicator organism. Previous work by Fujioka indicate *C. perfringens* may be a better indicator organism in tropical climates. These microbiological indicator bacteria will be employed to determine the impact of Kaelepulu Stream on the Kailua Beach Park and to determine the ambient water quality characteristics of Kaelepulu Stream and Enchanted Lake. In addition, possible sources of these organisms will be determined. This will require assessment during stream

openings and closings. It will also require that samples be collected under a variety of environmental conditions and seasonal changes.

III. Goal and Objectives

The primary goal will be to determine the water quality impact of Kaelepulu Stream on the microbiological quality in Kailua Bay. In addition, the recreational water quality of Kaelepulu Stream and Enchanted Lake will be characterized and possible source of pollution determined. And finally, indicator bacteria concentration will be compared to current State and Federal recreational water standards to determine the recreational water quality within the Enchanted Lake, Kaelepulu Stream and Kailua Beach Area.

Objectives

1) Determine the impact of Kaelepulu Stream on the microbiological quality of waters in Kailua Bay

2) Characterize the bacterial (fecal coliform, *E. coli,* enterococci, and *C. perfringens*), physical (salinity, dissolved oxygen), and nutrient (phosphate) quality within Kaelepulu Stream and Enchanted Lake

3) Determine possible sources of indicator bacteria

4) Determine the impact of sewage discharge and environmental changes (e.g. rain) on indicator bacteria in Kaelepulu Stream and Enchanted Lake

5) Characterize the affect of seasonal changes on the bacterial, physical and nutrient quality of water within Kaelepulu Stream and Enchanted Lake

6) Determine the concentration of bacteriological indicators in the water of Enchanted Lake, Kaelepulu Stream and Kailua Bay and their recreational quality based on current State and Federal Standards.

Chapter 3

Experimental Design and Methodology

I. Study Site and Sampling Locations

Sample locations were chosen that best represent the entire Kaelepulu Pond and Stream system . In addition sites were chosen that represent the water entering the Kaelepulu drainage system and Enchanted Lake. Sample location codes and descriptions are given in Table I and Figure 2. Stream sample were collected at locations in the center of Kaelepulu Stream and ocean sample were collected directly off shore in approximately four feet of water.

Figure 2 Sample Locations for Kaelepulu Stream

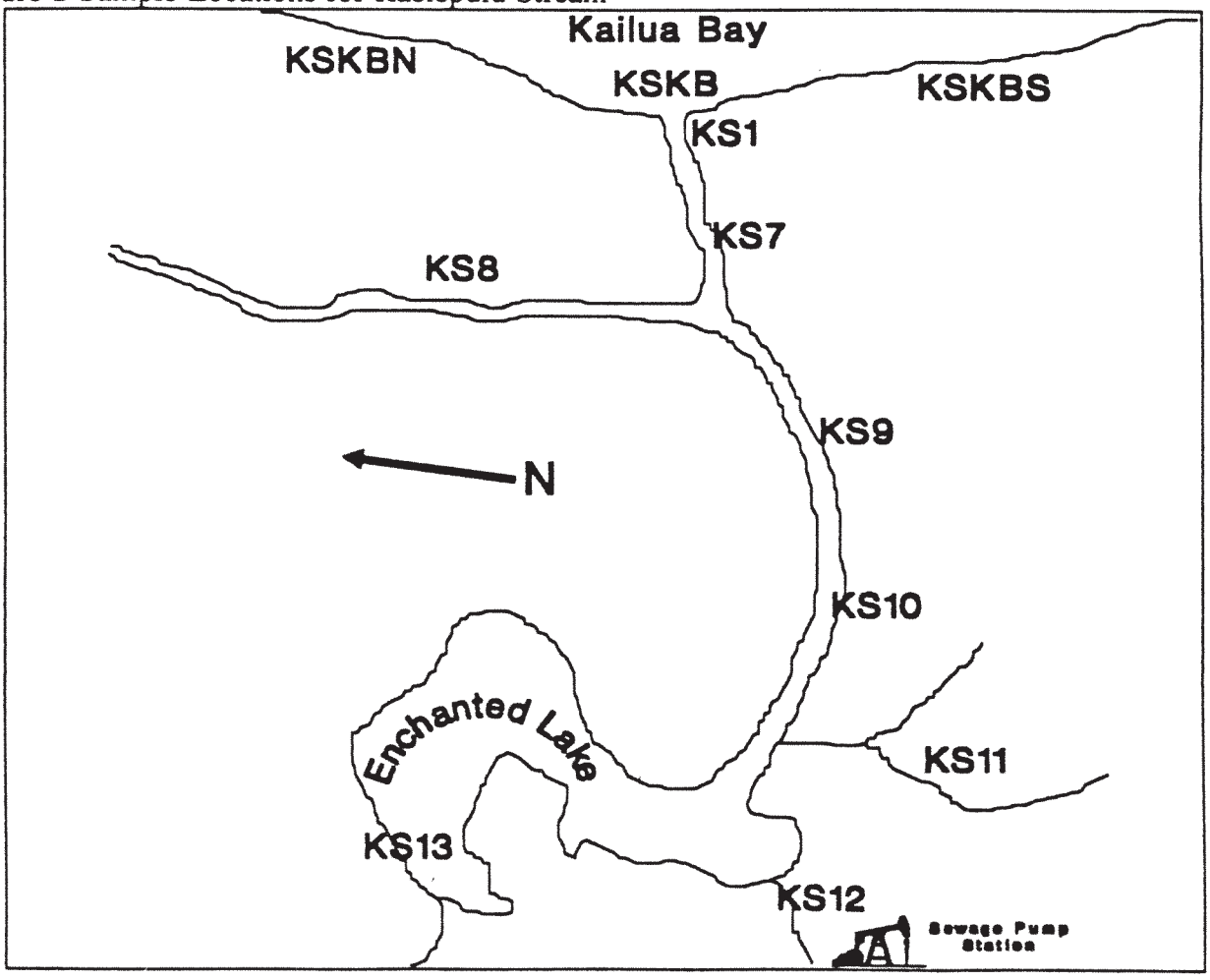


Table 8 Sample Codes and Descriptions

Location Code	Location Description
KSKB	Kailua Bay at the mouth of Kaelepulu Stream. Site is located on the ocean side of entrance to Kaelepulu Stream.
KSKBN	Kailua Bay 300 yards north of the mouth of Kaelepulu Stream along Kailua Beach Park.
KSKBS	Kailua Bay 300 yards south of the mouth of Kaelepulu Stream along Kailua Beach Park.
KS1	Kaelepulu Stream at the entrance to Kailua Bay.
KS7	Kaelepulu Stream approximately 300 yards from Kailua Bay. Site is located next to Midpac Golf Course
KS8	Kaelepulu Stream on north branch heading toward Kawainui Channel. Site is located at the Awakea Road Bridge.
KS9	Kaelepulu Stream at midpoint of Kaelepulu Stream. Sample is located next to Paopua Loop
KS10	Kaelepulu Stream at the entrance to Kaelepulu Pond at the Keolu Drive bridge.
KS11	Storm drain canal located next to St. John Vianney School near the mouth of Kaelepulu Pond, tributary to Kaelepulu Stream.
KS12	Storm drain canal located at Akumu Street Bridge next to Akumu sewage pump station, tributary to Enchanted Lake.
KS13	Enchanted Lake near headwaters of Enchanted Lake

II. Experimental Design

A. Parameters Measured

Current State and Federal bacterial water quality standards are used to determine the sanitary quality of recreational waters and the possible health risks associated with indicator bacteria densities. As mention earlier fecal coliform, *Escherichia coli* and enterococci are the indicator organisms recognized by these water quality standards. In temperate climates the density of *E. coli* and enterococci have been shown to correlate with the occurrence of gastroenteritis among people using

recreational waters. The major goal of this study was to determine the quality of water in Kaelepulu Stream and its impact on the quality of water in Kailua Bay by determining the densities of indicator bacteria and relating them to current recreational water standards. In addition, the sources of indicator bacteria entering Kaelepulu Stream and Enchanted Lake were determined. These objectives were accomplished by sampling Enchanted lake, Kaelepulu Stream and Kailua Bay at a variety of locations in this drainage system and determining the indicator bacteria densities. Sampling involved collection under a variety of environmental conditions and seasonal changes. And finally data were analyzed to determine whether these waters (Enchanted Lake, Kaelepulu Stream, and Kailua Bay) met current State of Hawaii and USEPA guidelines for recreational water quality.

In order to assess the water quality of Enchanted Lake, Kaelepulu Stream and Kailua Bay four indicator bacteria (fecal coliform, *E. coli*, enterococci and *C. perfringens* were chosen. Fecal coliform were chosen since the State of Hawaii recognizes this indicator for the assessment of water quality in inland waters like Kaelepulu Stream and Enchanted Lake. Federal standards recognize *E. coli* and enterococci as recreational water quality indicators of fresh water and the State of Hawaii will need to recognize these indicators for determining the recreational quality fresh waters. *C. perfringens* was utilized since many of Federal and State indicators may not reflect the health risks in tropical waters. In addition, salinity, dissolved oxygen and orthophosphates were utilized to determine the influence of nearby marine water and possible sources of nutrient loading.

B. Sample Collection

Skipper sea kayaks were used to collect sample from the middle of Kaelepulu Stream at each sample location. Bacteriological samples were collected 0.3 meters below the surface in sterile 500 ml nalgene containers. Samples used for chemical analysis were collected 0.3 meters below the surface in 250 ml acid washed nalgene bottles. Samples were immediately stored in the dark and transported at 4^{o}C and analyzed within 6 hours of collection. Soil samples were collected in sterile whirlpak at a depth between 3 and 6 inches below the surface at location appearing to have little human contact. Sample were transported to the laboratory at 4^{o}C and analyzed within three hours of collection. Duck feces were collected in sterile whirlpaks and transported to the laboratory at 4^{o}C and analyzed within three hour after collection. Samples were collected at sites having high duck populations and recent fecal deposition. All sample were collected in accordance with EPA guidelines for microbiological water quality monitoring (EPA Microbiological Methods, 1978)

C. Community Interaction

Community interaction was also utilized in this study to determine additional sources of pollution and assess unknown sources of pollution. Members of the Kailua community are very concerned about the quality of the water in Kaelepulu Stream and in Kailua Bay. One of these concerns was a green foam occurring in water near Kailua Bay and the community was concerned that this foam was sewage discharge. Samples of this foam were analyzed for fecal coliform, *E. coli*, enterococci and *C. perfringens* and also microscopically.

III. Methodology

A. Selective Bacteriological Growth Media

Selective growth media for the membrane filtration isolation of *Escherichia coli*, fecal coliform and enterococcus were prepared in accordance with *Standard Methods* (*Standard Methods for the Examination of Water and Waste water 17th ed.*). Selective media for *Clostridium perfringens* was as follows (in grams per 100ml of distilled water): tyrptose, 3.0; yeast extract, 2.0; sucrose, 0.5;L-cysteine, 0.1;$MgSO_4 \cdot 7H_20$, 0.1;bromocresol purple, 0.004; and agar 1.5. The ingredients were dissolved, and the pH adjusted to 7.6. After autoclaving at 121°C for 15 min, the medium was allowed to cool at 50°C, and the following were added per 100 ml: D-cycloserine, 40 mg; polymyxin B sulfate, 2.5 mg; indoxyl-β-D-glucoside, 60 mg; 2.0 ml of a filter - sterilized 0.5% phenolpthalein diphosphate solution; and 0.2 ml of a filter-sterilzed 4.5% $FeCl_3 \cdot 6H_2O$ solution (Bisson and Cabelli, 1979).

Esculin Iron Agar (EIA) agar for the the confirmation of enterococci was prepared in accordance with *Standard Methods* (*Standard Methods* 17th ed., 1989).

Dilution water was prepared in accordance with *Standard Methods* (*Standard Methods* 17th ed.,1989) for dilution blanks and membrane filtration rinse water.

Urea reagent was prepared in accordance with Standard Methods (Standard Methods 17th ed., 1989) for the conformation of *E. coli* isolates.

B. Methods

1. Isolation and Confirmation of *Escherichia coli*

Appropriate dilutions were made that would yield countable colonies ranging from 20 to 200 colonies. Dilutions ranging in volume from 25 to 100 ml were filtered through a 0.45 µm membrane filter (GN6, Gelman) under vacuum pressure and the funnel apparatus was rinsed in a clockwise and counter clockwise directions with 15 to 20 ml of buffered rinse water. Membrane filters were then placed on m-TEC agar plates and incubated at 30°C for a period of two hours and then transferred to a 44.5°C water bath for 22 h. After 22 h the membranes were transferred to a filter pad saturated with urea substrate. After 15 min., the yellow or yellow-brown colonies were counted and considered confirmed as *E. coli*. These procedures were in accordance with Standard Methods (Standard Methods 17th ed., 1989). Results were recorded in Appendix A.

2. Isolation and Confirmation of Enterococci

Appropriate dilutions were made that would yield countable colonies ranging from 5 to 500 colonies. The procedures for filtering were similar to section 1, however the membranes filters were placed on m-E agar plates and incubated at 41.0°C for a period of 48 hours. After 48 h membrane filters were transferred to EIA medium and incubated at 41.0°C for 20 min. Pink and red colonies producing a black or reddish-brown precipitate on the underside of the filter were considered confirmed for enterococci. Results were then recorded in Appendix A. These procedures where followed in accordance with Standard Methods (Standard Methods 17th ed., 1989).

3. Isolation Confirmation of Fecal Coliform

Appropriate dilutions were made and filtered as described in section 1. Membrane filters were then placed on m-FC agar plates and incubated at 44.5°C for a period of 24 h. After 24 h blue colonies were considered fecal coliform. Results were then recorded in Appendix A. These procedures where followed in accordance with *Standard Methods* (*Standard Methods 17th ed.*, 1989).

4. Isolation and Confirmation of *Clostridium perfringens*

Appropriate dilutions were made and filtered as described in section 1. Membrane filters were then placed on m-CP agar plates and incubated at 45.0°C for a period of 24 h. After 24 h plates were exposed to ammonium hydroxide for 30 sec and pink colonies were counted as C. *perfringens*. Results were then recorded in Appendix A. These procedures were followed in accordance with methods developed by Bisson and Cabelli (Bisson and Cabelli 1979).

5. Isolation of indicator Bacteria from Soil

The isolation of indicator bacteria from soil involved the collection of approximately 200 g of soil. Ten g of soil was placed in a Sorval homogenizer along with 40 ml of the following elution reagent listed in Table 8. Soil and homogenization solution were homogenized for 4 min. and 10 ml of the slurry was placed in a 90 ml dilution blank. Appropriate dilutions were made and the membrane filter techniques described in previous sections 1,2,3 and 4 were performed.

Table 9 Homogenization solution for desorption of bacteria from soil

Compound	Concentration
Zwittergent 3-12	10^{-6} M
ethyleneglycol-bis-(β amino-ethyl ether)-N-N-tetra acetic acid	10^{-3} M
Peptone	0.1%
Tris buffer	10^{-2} M

Mixture was homogenized (16,000 rpm) for 4 min at 4oC. Adapted form Camper et al 1985.

Because of the adherence of bacteria to soil, this elution technique will not result in the recovery of the maximum number of bacteria actually found in the soil. Actual numbers will be higher than those observed using this technique.

6. Isolation of Indicator Bacteria From Duck Feces

Three g of duck feces were placed in a sterile Sorval mixing chamber along with 50 ml of phosphate buffer and homogenized for 3 min. at 16,000 rpm. After homogenization, 10 ml of the slurry were placed in a 90 ml dilution blank and appropriate dilutions were made. Water samples were then submitted to the membrane filtration technique described earlier.

7. Phosphates

Reactive phosphate was determined using an ascorbic acid method and a Hach DR/3000 spectrophotometer. Procedures were used following the Hach Methods Manual (procedure P.4), Hach Campany, Loveland CO

8. Salinity

Salinity was determined using a YSI Model 33 SCT salinity meter. Readings were determined as parts per thousand (ppt) with sea water reading at 33-35 ppt.

Chapter 4

Results and Discussion

I. Physical and Chemical Characteristics of Sample Sites

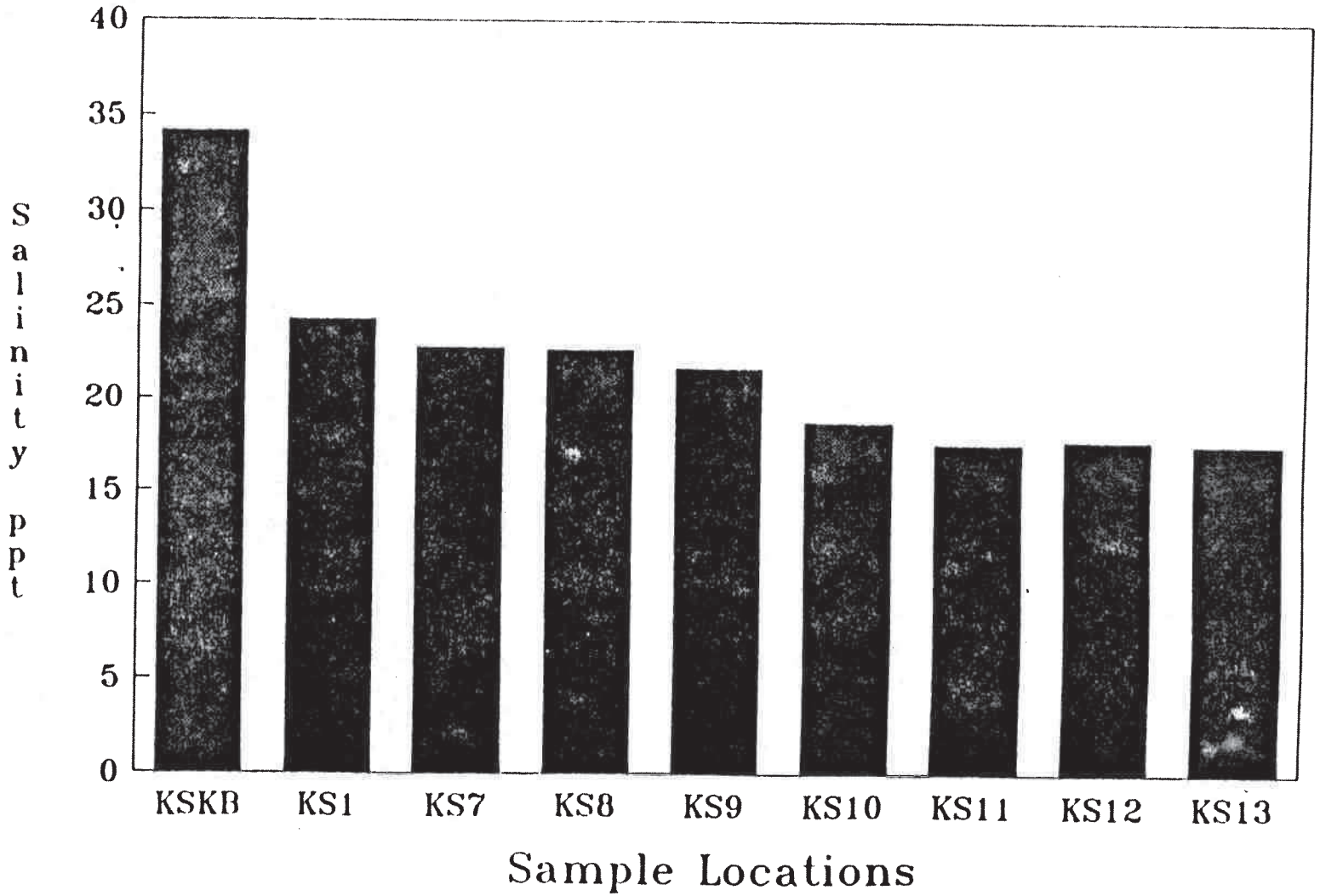
In order to better understand the water quality characteristics of Kaelepulu Stream, salinity, Ortho-phosphate and dissolved oxygen levels were determined and recorded for each sampling location. Salinity was utilized to determine the influence of ocean water from Kailua Bay. Ortho phosphates and dissolved oxygen were used to help determine possible nutrient loading.

A. Salinity

Although the Department of Health considers Kaelepulu fresh recreational water, salinity readings taken through out the sampling period indicate these waters are actually more marine-like than fresh. The average salinity (n=31) for all sites are plotted in Figure 3. These results show that the salinity is above 17 ppt at several locations. Monthly average salinity for KS13 are represented in Figure 4. This location was the furthest from the ocean and represents water at the beginning of this drainage system. Figure 4 represents what appears to be seasonal changes in salinity. The salinity increases during the drier months of the year (June, July August and September). During these months the tributaries entering Enchanted Lake and Kaelepulu Stream have very little or no flow, therefore the increase in salinity may be due to evaporation. Other factors that may influence salinity are salt water intrusion from the ocean and rain runoff. In addition, ground water and springs may contribute to these variations in salinity.

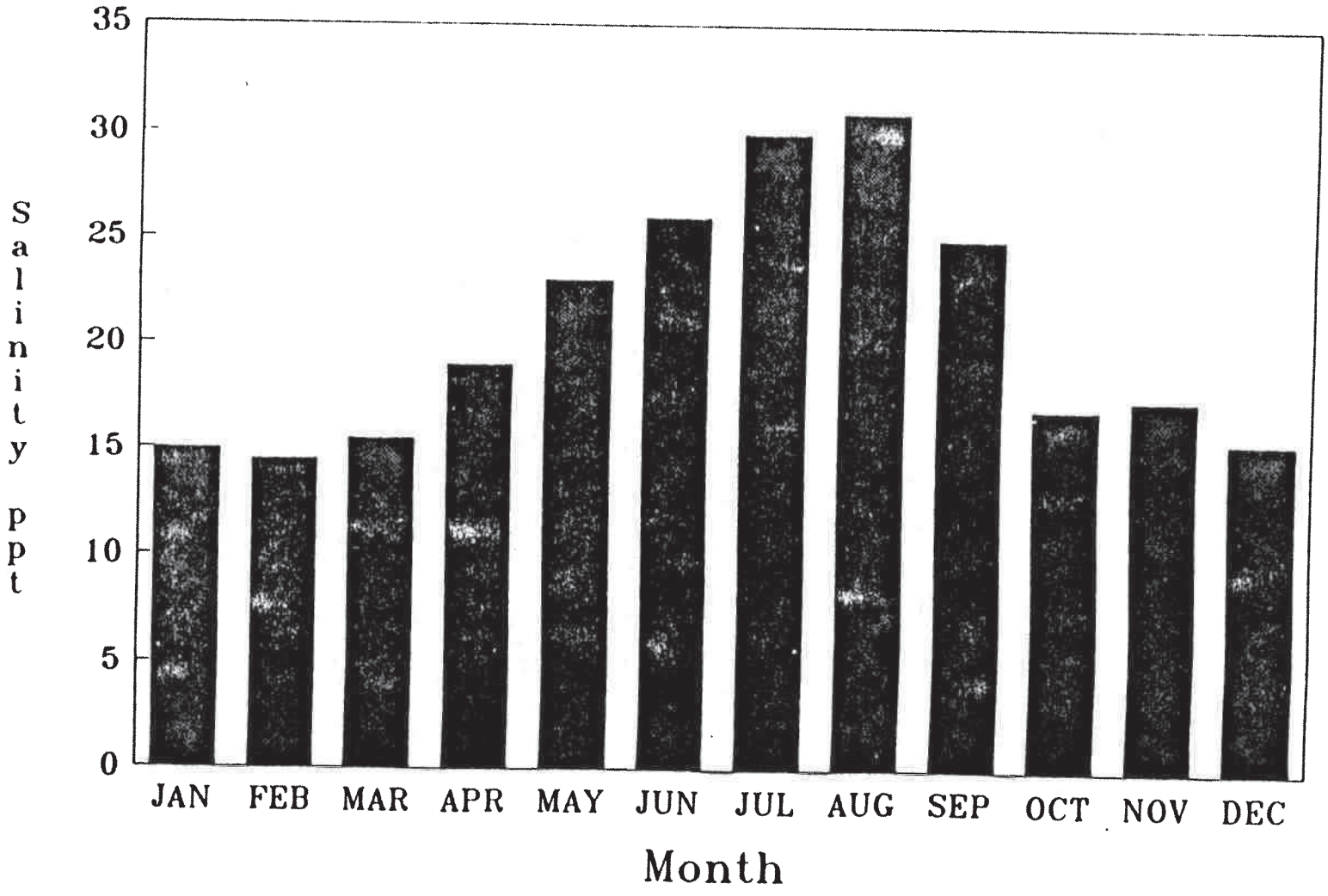
Average Salinity of Sampling Sites

Figure 3



Salinity Monthly Average
Location KS13

Figure 4



B. Ortho Phosphates

Phosphates are often the limiting growth factor in environmental waters and as a result the input of phosphates can lead to oxygen depletion and eutrophication. Sample locations KS8, KS11, and KS12, appear to have the highest levels of reactive phosphate (0.416 to 0.810 ppm) found for all locations (Appendix A). KS8 is located on the north branch of Kaelepulu Stream (Figure 2) and appears to be influenced by storm water from urban areas. These storm water may contribute the high levels seen at location KS8. KS11 and KS12 are located on two of the major tributaries feeding Enchanted Lake and Kaelepulu Stream. As with KS8 these locations are primarily influenced by storm drains. In addition sewage discharges at KS12 appeared to contribute to high levels of phosphates. A sample taken soon after sewage discharge showed a level of 0.804 ppm and was the highest level of ortho phosphate detected at this location.

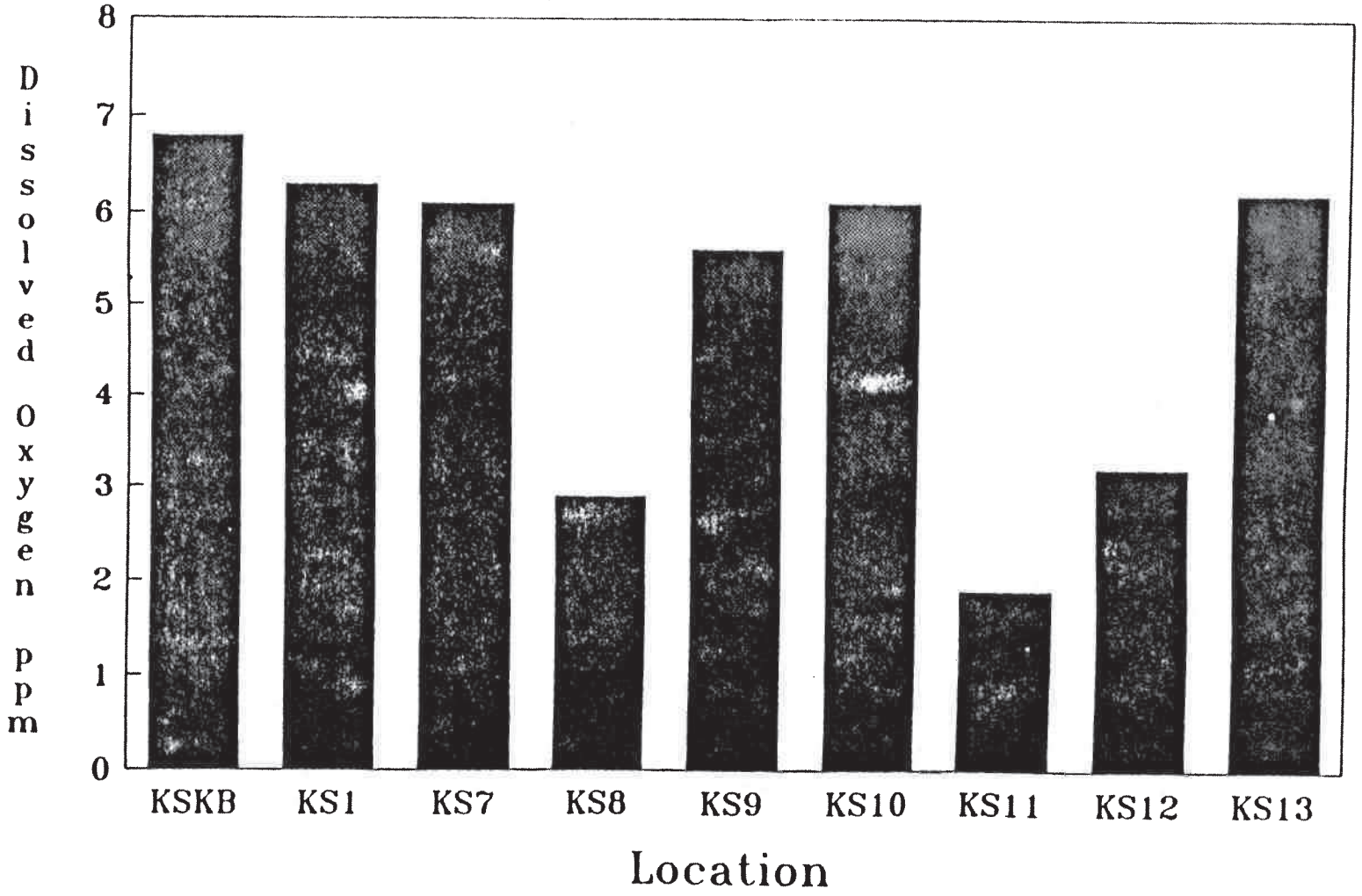
Although these results indicate the possible sources of nutrient loading, additional research would be needed to determine the sources of reactive phosphate and to determine the impact of nutrients on aquatic life. In addition to reactive phosphate, total phosphate and the various forms of nitrogen (e.g. nitrate, nitrite, ammonia and total nitrogen) would be needed to accurately determine the impact of nutrients in Kaelepulu Stream.

C. Dissolved Oxygen

Dissolved oxygen was taken on a quarterly intervals and the results are recorded in Table 9. Sea water saturated with oxygen at 26^{o}C has a concentration of approximately 6.6 ppm. This value would represent levels consistently found at

Dissolved Oxygen Levels
Kaelepulu Stream

Figure 5



site KSKB. As would be expected at the shore break in Kailua Bay, dissolved oxygen levels signify almost complete saturation. EPA water quality criterion for ambient dissolved oxygen states a thirty day mean of 5.5 ppm. At these levels and above, aquatic life most susceptible to oxygen depletion are able to survive and reproduce. Locations KS1, KS7, KS9, KS10, and KS13 all have levels meeting these guidelines (Table 9 and Figure 5). Locations KS8, KS11, and KS12 have levels (1.4 to 2.1 ppm) below these standards and therefore may not support aquatic life susceptible to oxygen depletion (Table 9 and Figure 5). It is important to note however, additional readings at different times of the day would be needed to determine the actual impact on aquatic life. These results indicate that areas which appear to have unusually low dissolved oxygen levels (KS8, KS11 and KS12). This could be due to nutrient loading at these locations. In addition, oyster populations were not seen at these locations and this may be the result of the low dissolved oxygen levels seen at these locations.

Table 10 Quarterly Dissolved Oxygen Levels

	Dissolved Oxygen (ppm)				
Location	1st Quarter[a]	2nd Quarter[b]	3rd Quarter[c]	4th Quarter[d]	Average
KSKB	6.8	6.9	6.9	6.5	6.8
KS1	6.1	6.4	6.0	6.7	6.3
KS7	5.9	6.4	6.8	5.1	6.1
KS8	2.1	4.8	1.9	2.9	2.9
KS9	5.4	6.3	5.2	5.4	5.6
KS10	6.9	6.2	5.1	6.0	6.1
KS11	1.6	2.7	1.4	1.7	1.9
KS12	2.9	4.1	2.1	3.8	3.2
KS13	5.7	6.8	7.1	5.1	6.2

[a] Sampling during September, October, November, 1990
[b] Sampling during December, 1990, and January, February, 1991
[c] Sampling during March, April, May, 1991
[d] Sampling during June, July, August, 1991

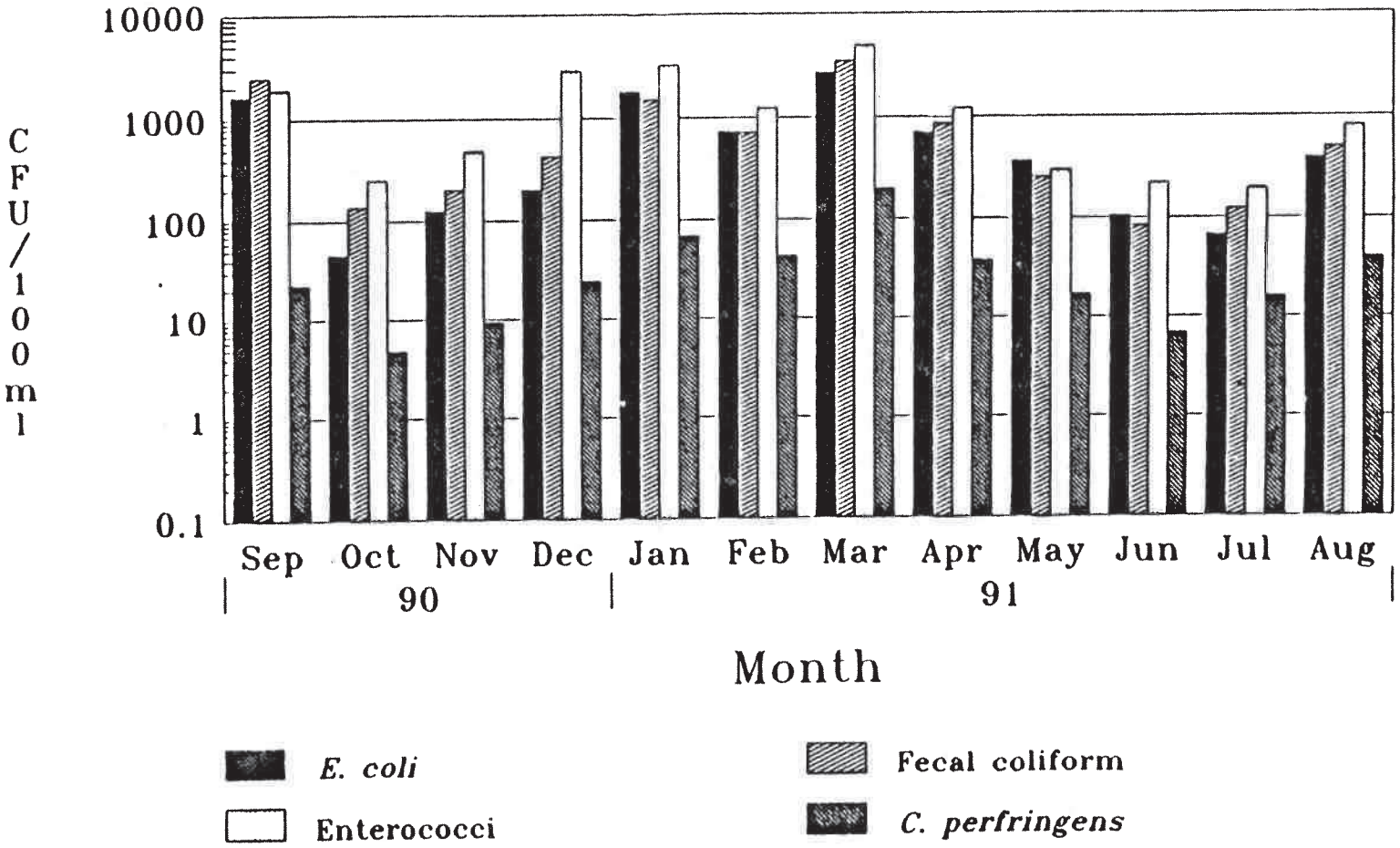
II. Microbial Water Quality at Sample Locations

A. Microbial Water Quality of Source Waters

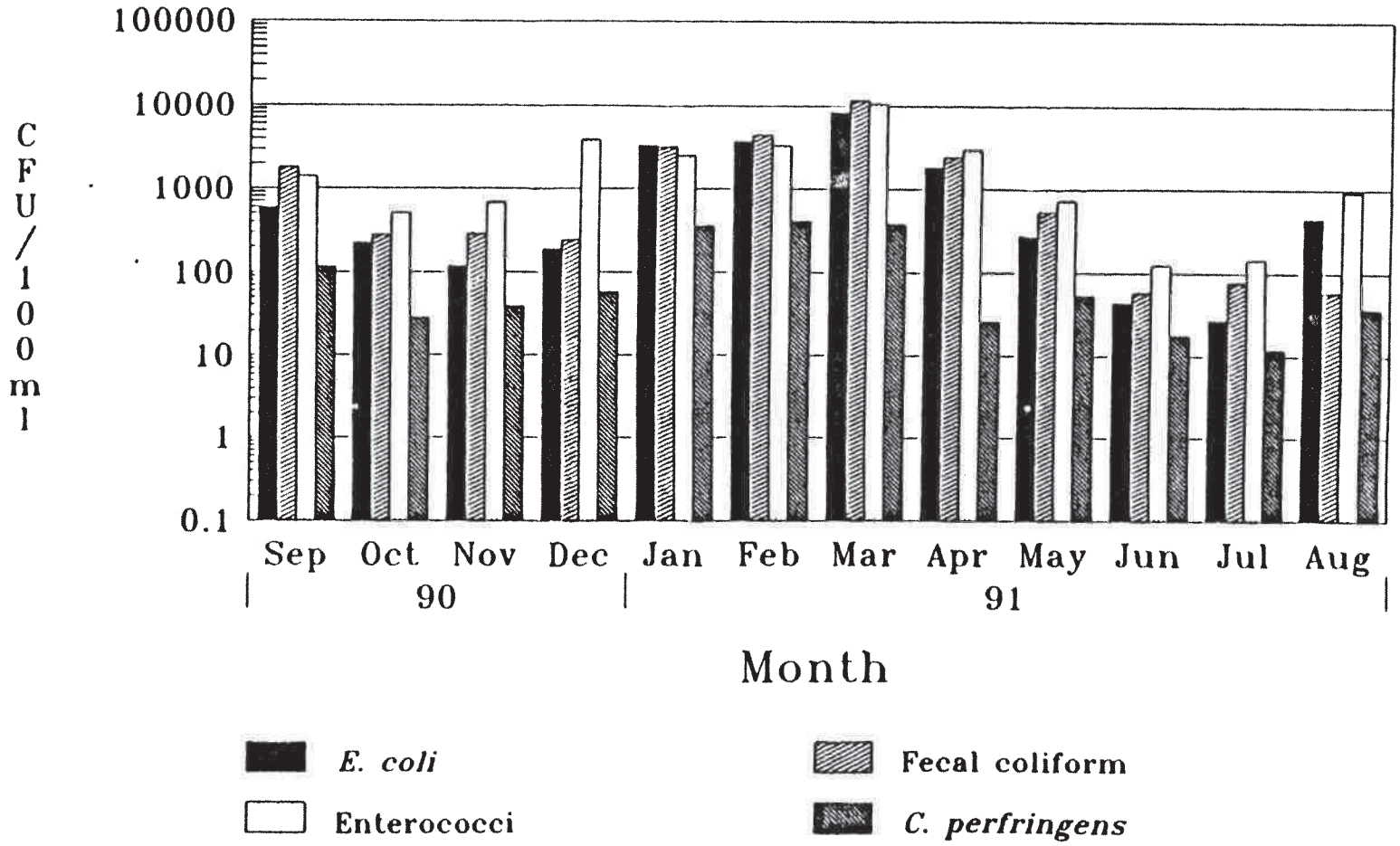
Sample locations were chosen which represented the water entering Kaelepulu Stream, Enchanted Lake and Kailua Bay. As seen in Figure 2, sample locations KS13, KS12 and KS11 were located on the major tributaries emptying into Enchanted Lake and Kaelepulu Stream. These locations represent the major sources of water entering this system. The densities of *E. coli,* fecal coliform, enterococci and *C. perfringens* were the highest at these locations. As seen in Figure 6, 7, and 8 the levels of fecal coliforms ranged from 57 to 11,638 (CFU/100ml) while *E. coli* levels ranged from 28 to 8434 (CFU/100ml). Enterococci levels were also high with a range from 92 to 10646.8 (CFU/100ml). *C. perfringens* was found to have lower levels ranging from 4 to 402 (CFU/100ml). It would appear that these locations are major contributors of indicator bacteria entering Kaelepulu Stream and Enchanted Lake. The ranges of these indicator bacteria appear to fluctuate with seasonal changes. As seen in Figures 6, 7, and 8, levels of indicator bacteria appear to be the highest during the months of December, January, February, March and April. These are the months when the flow of water from these tributaries is the greatest. In contrast the levels seen during the summer months of May, June, July and August are the lowest seen during the year. During these months there was very little flow of water from these tributaries. Explanation for this occurrence may be the result of a series of factors: 1) High flow from these tributaries may be introducing high levels of these indicator bacteria; 2) The higher salinity seen during the summer months may results in a decline in the numbers of indicator

Monthly Geometric Means at KS13

Figure 6

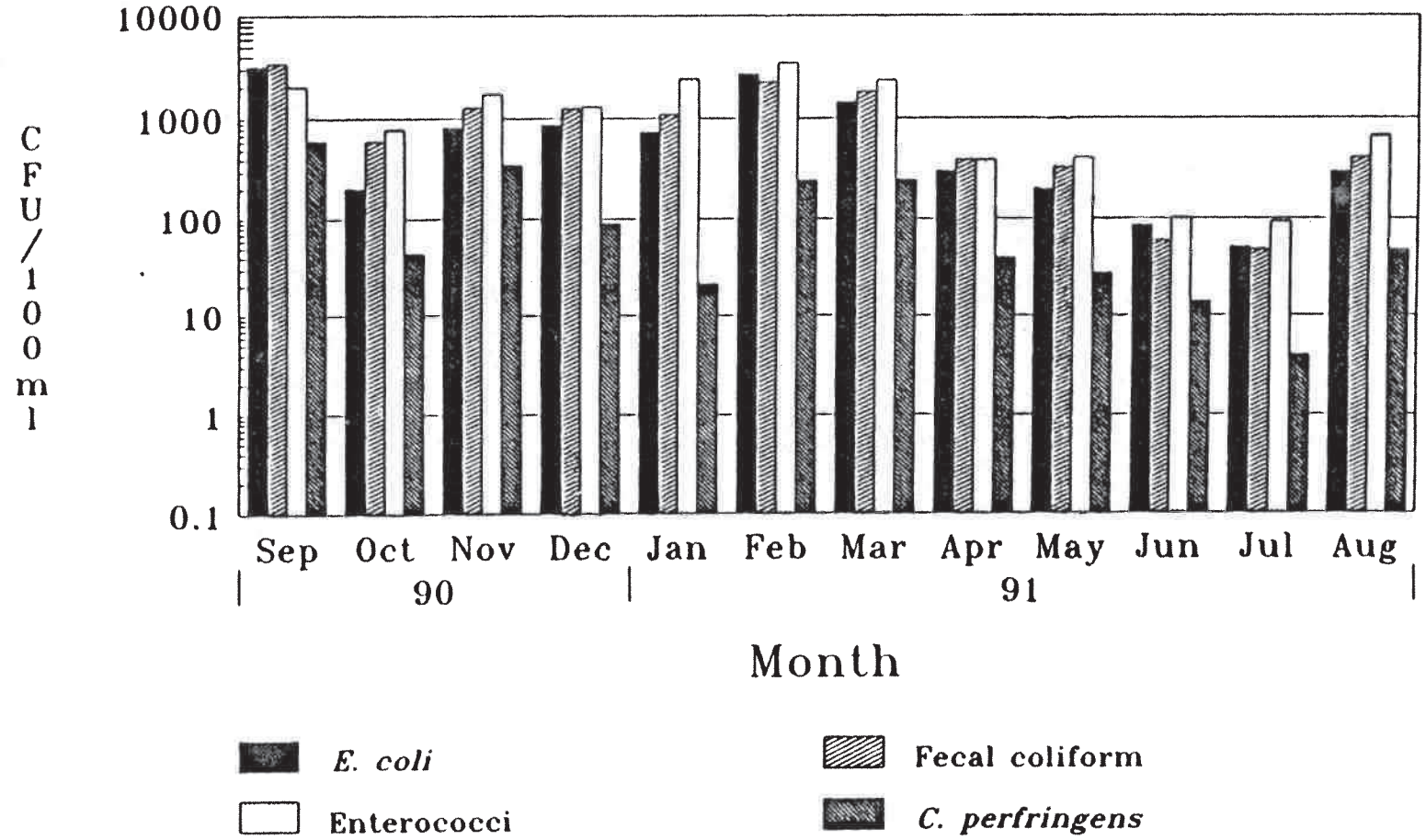


Monthly Geometric Means at KS12
Figure 7



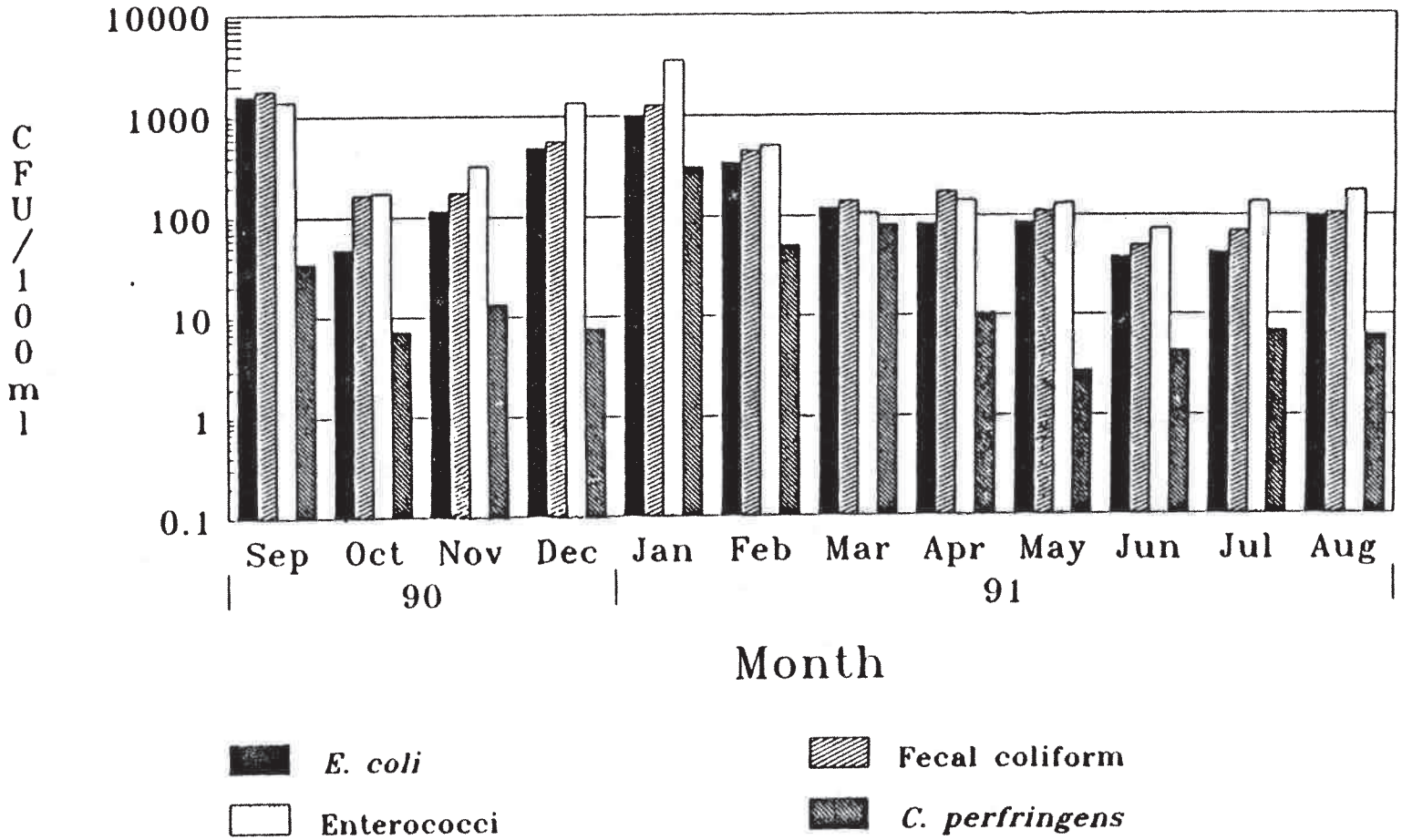
Monthly Geometric Means at KS11

Figure 8



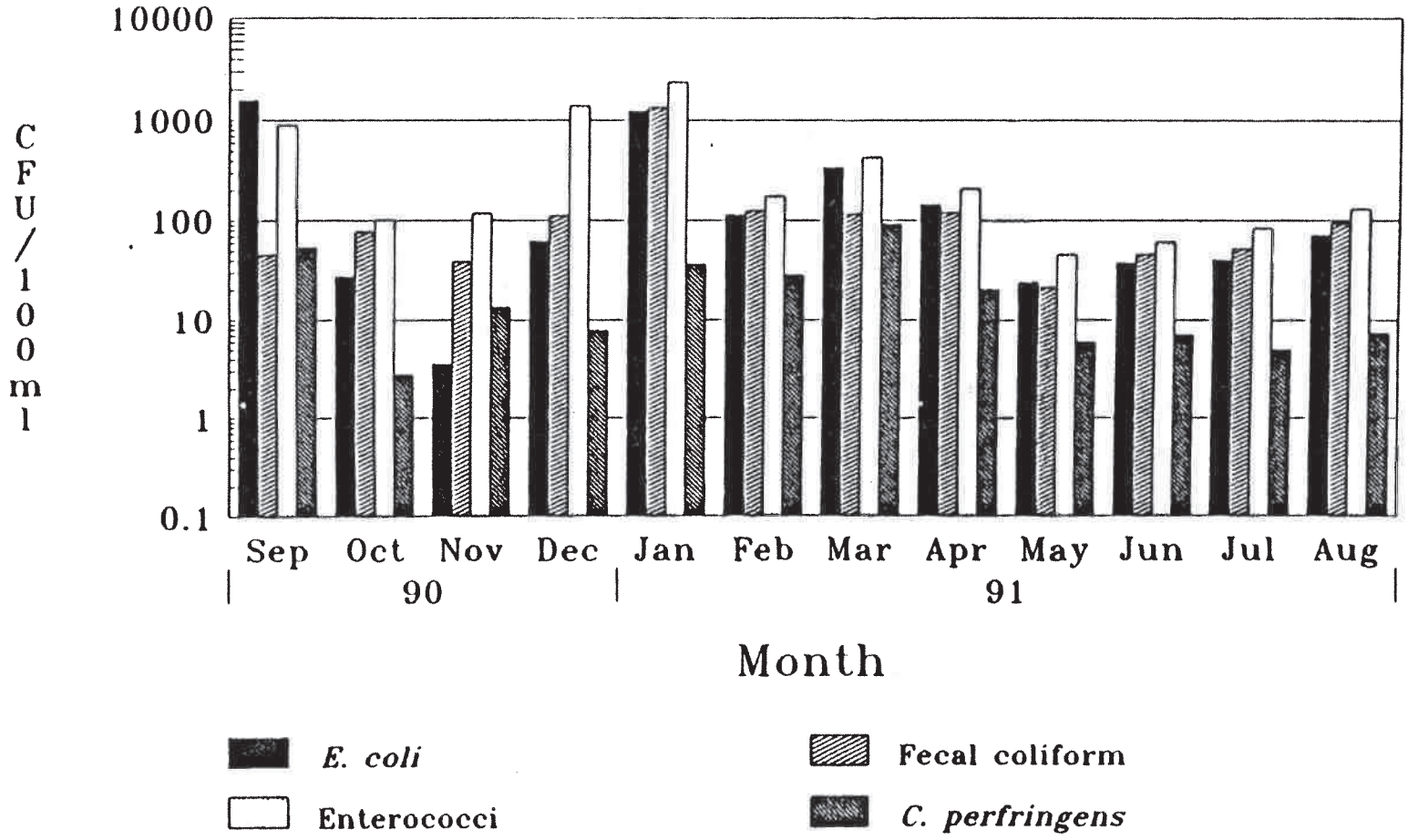
Monthly Geometric Means at KS10

Figure 9

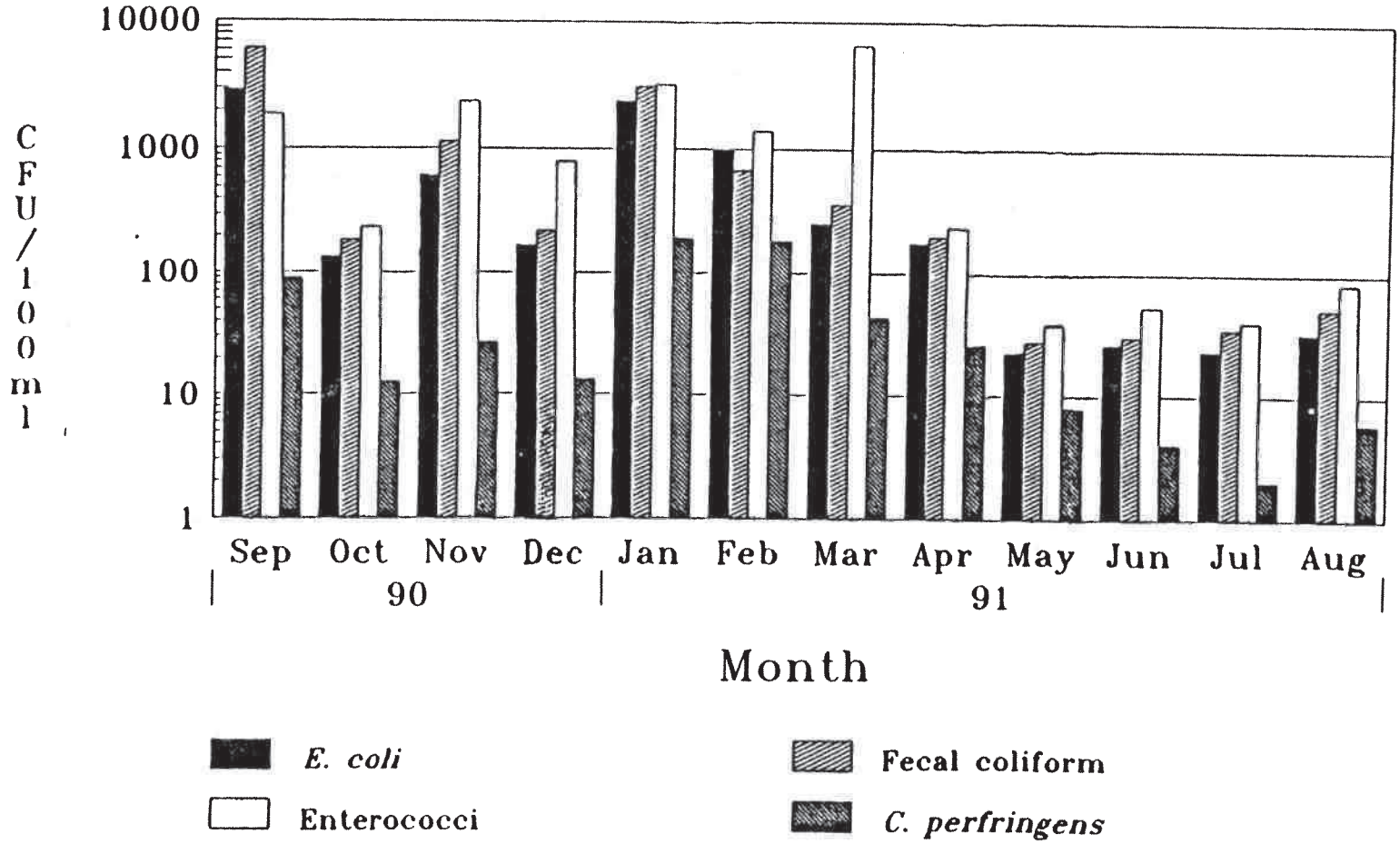


Monthly Geometric Means at KS9

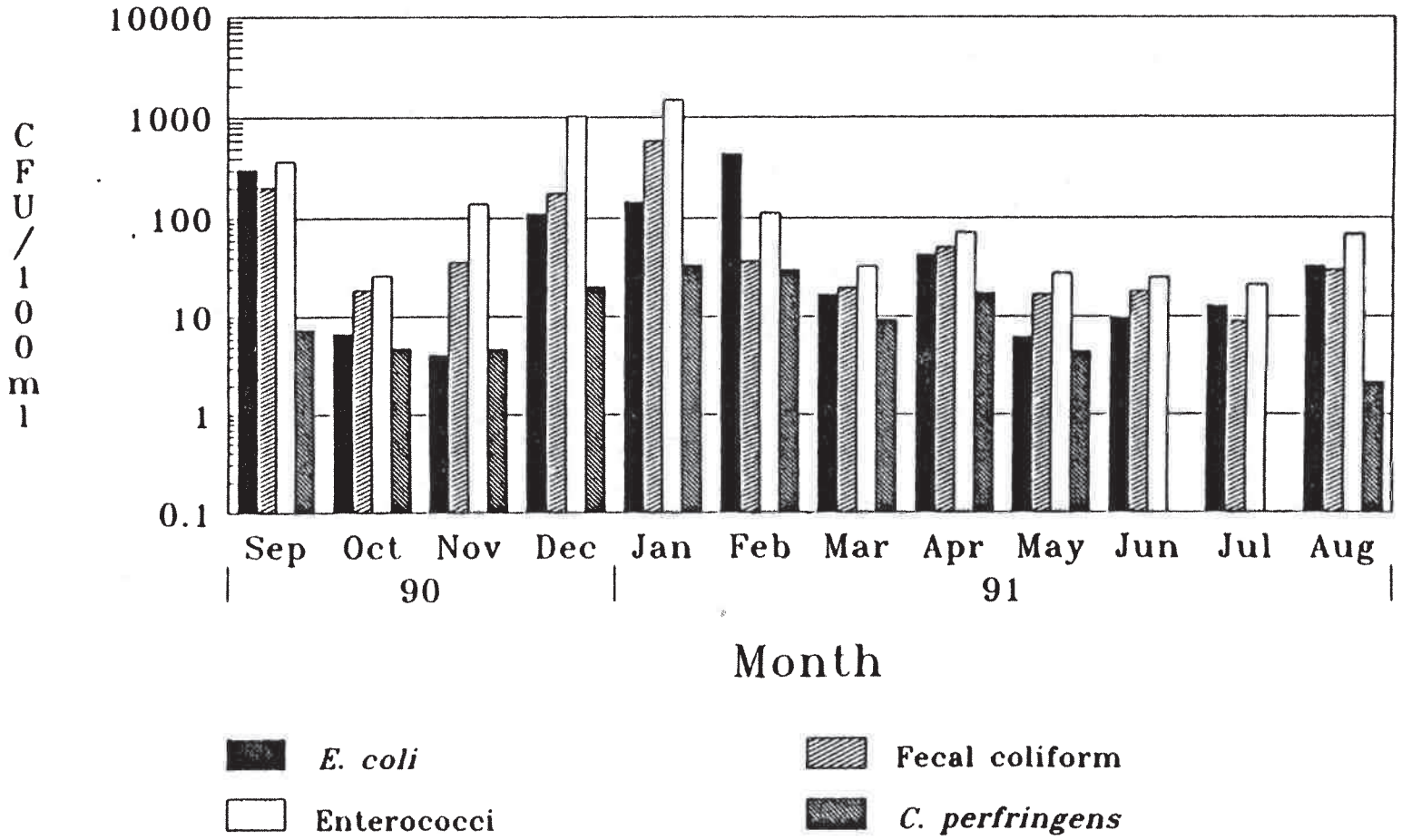
Figure 10



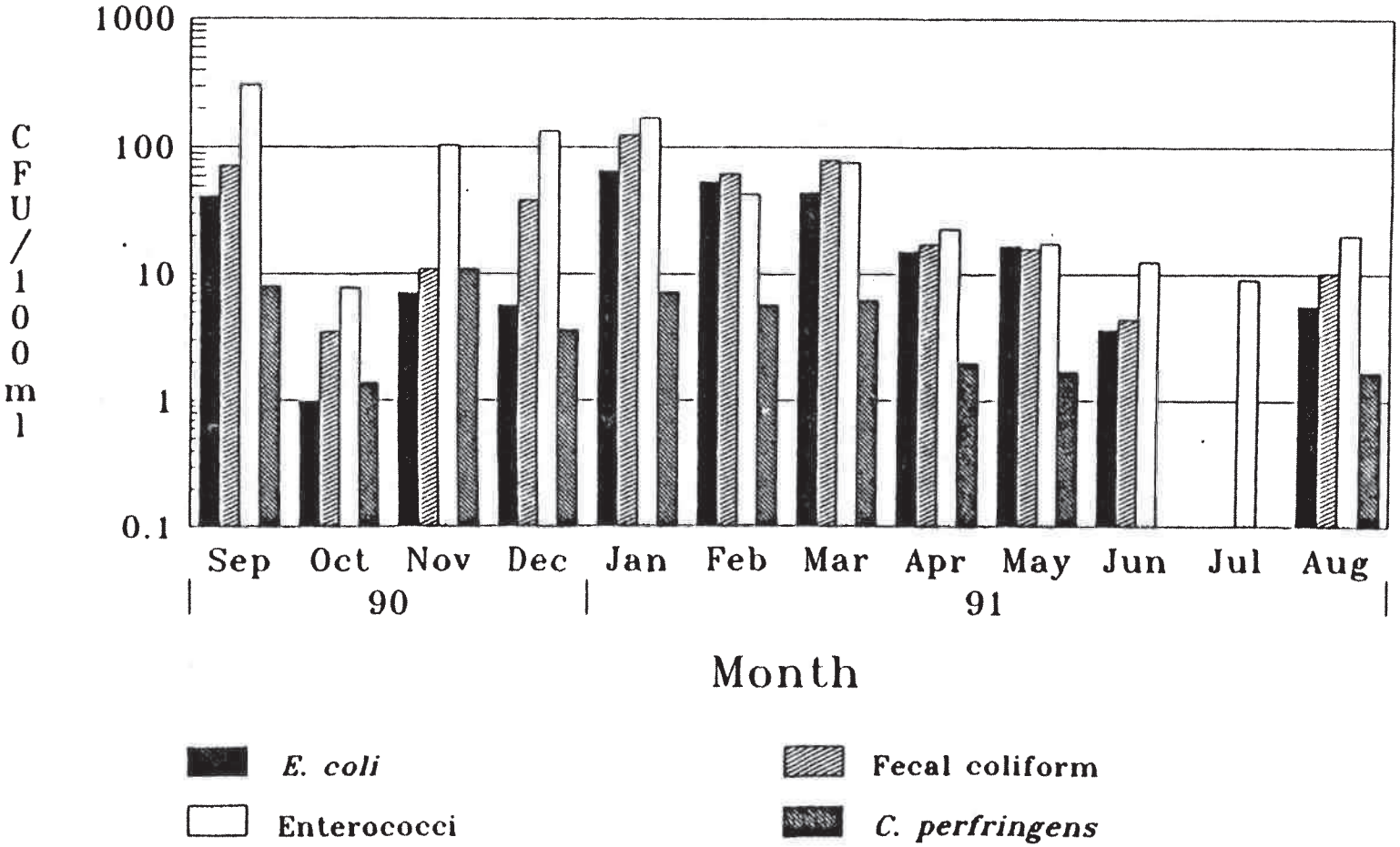
Monthly Geometric Means at KS8
Figure 11



Monthly Geometric Means at KS7
Figure 12

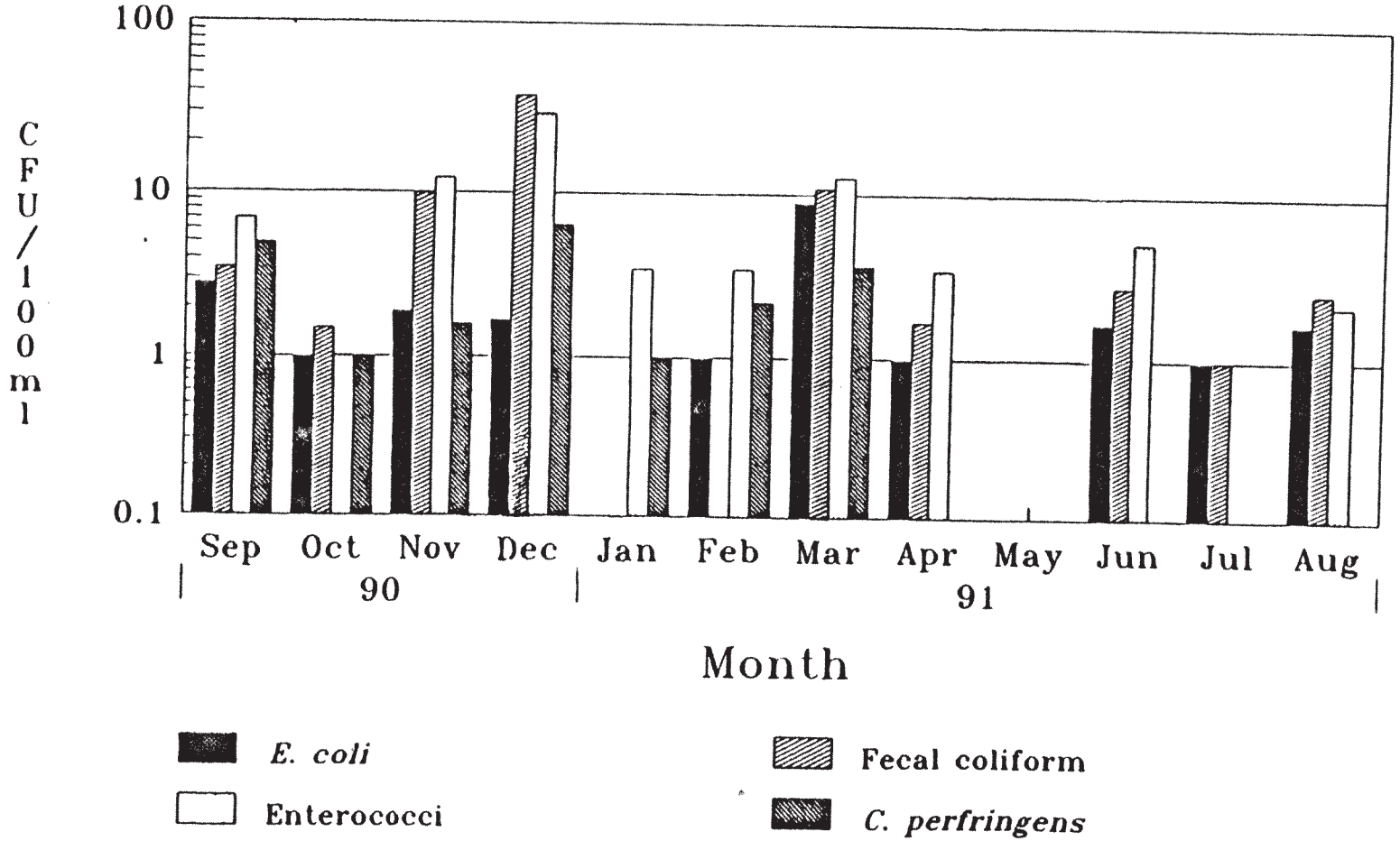


Monthly Geometric Means at KS1
Figure 13



Monthly Geometric Means at KSKB

Figure 14



bacteria and; 3) The increase exposure to sunlight during the summer months may result in a decrease in indicator bacteria levels.

B. Microbial Water Quality of Kaelepulu Stream

Sample location KS10, KS9, KS8, KS7 and KS1 are representative of the water found in Kaelepulu Stream as it moves toward the ocean. As seen in Figure 9, 10, 11, 12, and 13 sample location KS8 has the highest levels of indicator bacteria among these sites. As seen in Figure 2, KS8 is located on the north branch of Kaelepulu Stream. This branch proceeds north until it deadends at approximately 1.5 miles. The major waters contributing to this north branch appear to be storm water drainage. The levels of indicator bacteria drop dramatically as water proceeds toward Kailua Bay. As demonstrated in Figures 7, 8, 9, 10 and 11 the levels of fecal coliform, *E. coli*, enterococci and *C. perfringens* are the highest at locations furthest from the ocean. By the time waters reach the mouth of Kaelepulu Stream these levels have dropped to the lowest levels seen at any site in Kaelepulu Stream.

Sample locations located in Kaelepulu Stream appeared to have the same seasonal variation seen in samples collected from source waters. The summers months have the lowest levels of indicator bacteria. As with the source water sites, the same factors may apply to the variation seen throughout the year. It is interesting to note, enterococci levels were higher than any of the other indicators during each month. This would be consistent to with studies indicating their stability in marine environments.

The majority of samples collected in Kaelepulu Stream were collected during daylight hours. There were samples, however, that were collected during the night. The levels of indicator bacteria were 10 to 100 times higher at night than samples

collected during the day (Tables 15 to 22, Appendix A). This may be a reflection of the bactericidal effects of sunlight (Fujioka et al., 1981). Further sampling would be required to determine the causes for this increase.

III. Thirty Day Geometric Means

Four indicator bacteria, fecal coliform, *Escherichia coli*, enterococci and *C. perfringens*, were utilized to determine the bacteriological water quality of Enchanted Lake, Kaelepulu Stream and Kailua Bay. Current State and Federal regulations require a geometric mean of a series of at least five samples over a thirty day period, in order to determine compliance. The geometric mean was calculated for sampling that occurred in October 1990. From a regulatory standpoint, sampling did not occur with the same frequency during every month so the geometric means calculated over other months did not meet these requirements but they were still useful in assessing the quality of water.

Table 11 Recreational Fresh Water Standards (CFU/100ml)

Indicator	State of Hawaii	USEPA
Enterococci	Not Recognized	33
E. coli	Not Recognized	126
Fecal Coliform	200	Old Standard

Table 12 Recreational Marine Water Standards (CFU/100ml)

Indicator	State of Hawaii	USEPA
Enterococci	7	35

As seen in a Tables 10 and 11, Hawaii and the USEPA recognize different standards. The results for the geometric mean are displayed in Tables, 24, 25, 26, 27, 28, 29, 30, 31, and 32 (Appendix B). The State of Hawaii recognizes 200 fecal coliform per 100 ml as their fresh water standard which is also applied to Kaelepulu Stream. Figure 15 shows two locations KS12 and KS11 that exceed State standards. These locations are located on major tributaries emptying into Enchanted Lake and Kaelepulu Stream.

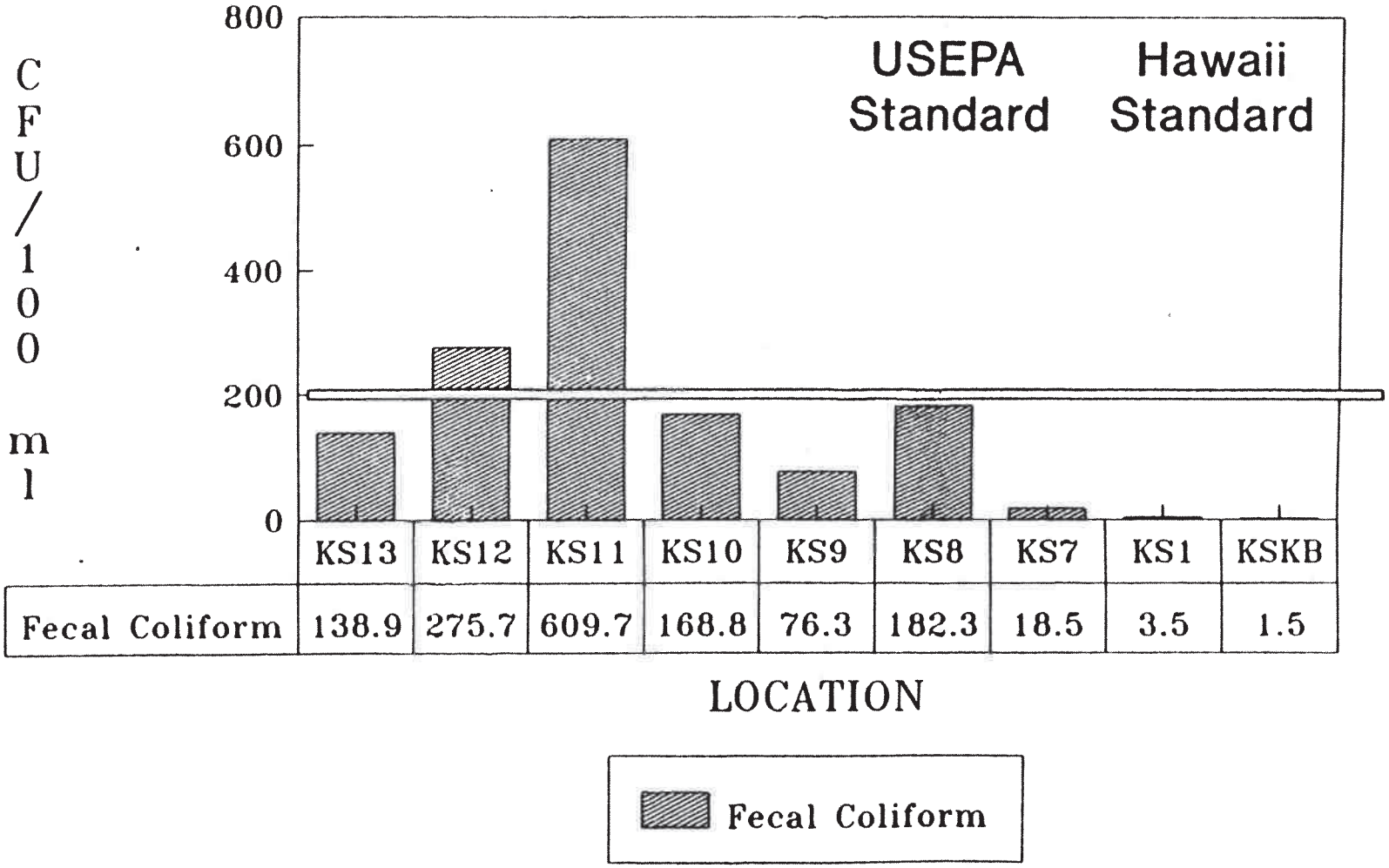
Since the State will eventually adopt the Federal standards, a geometric mean for enterococci and *E. coli* were also computed. *E. coli* standards are exceed at three location, KS12, KS11 and KS8 (Figure 16). KS8 is located on the north branch of Kaelepulu Stream. As would be expected, E. coli levels are below those seen for fecal coliforms since they are a subgroup of the fecal coliforms.

The geometric mean for enterococci are displayed in Figure 17. The USEPA Standard was exceeded at sites KS13, KS12, KS11, KS9, and KS8 while the State standard was exceed at all location except KSKB. Enterococci levels are by far the highest for these indicator organism. This may be a reflection of its stability in the a marine environment (Cabelli, 1983).

As mentioned earlier, *C. perfringens* has been proposed as an indicator for tropical climates. Studies done by Fujioka proposed a standard of 50 *C. perfringens* per 100 ml for stream waters (Fujioka, 1985) As Seen in Figure 18 the levels were well below this proposed standard at all locations. Location KS11 had a density of 44.7 and was the only location with levels near 50. Although these levels are low when compared to the other indicators tested, *C. perfringens* is normally found in lower concentrations in feces and sewage (Hoadley and Dutka, 1977).

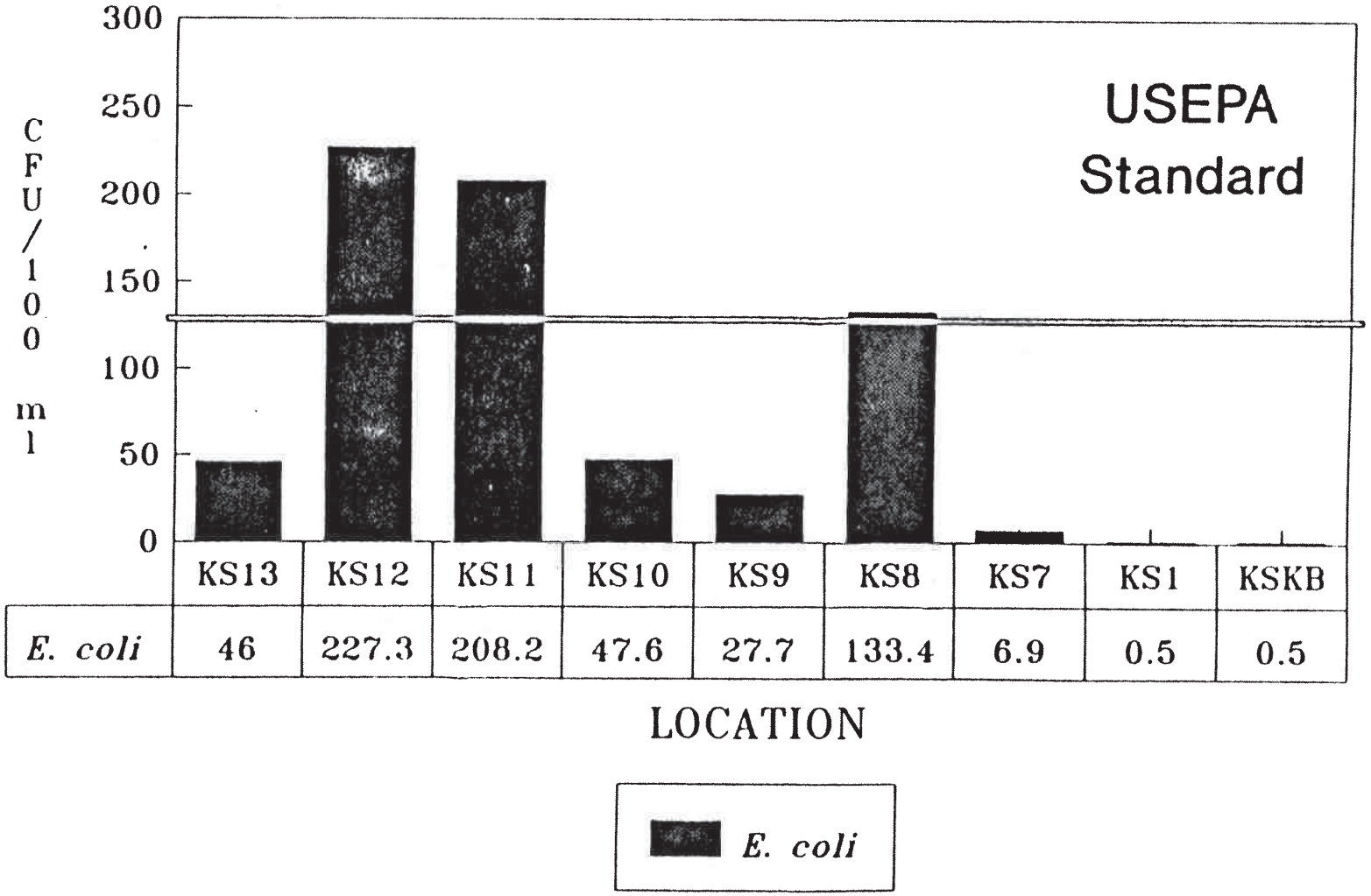
Geometric Mean Fecal Coliform
5 Consecutive Samples in 30 Day Period

Figure 15



Geometric Mean *E. Coli*
5 Consecutive Samples in 30 Day Period

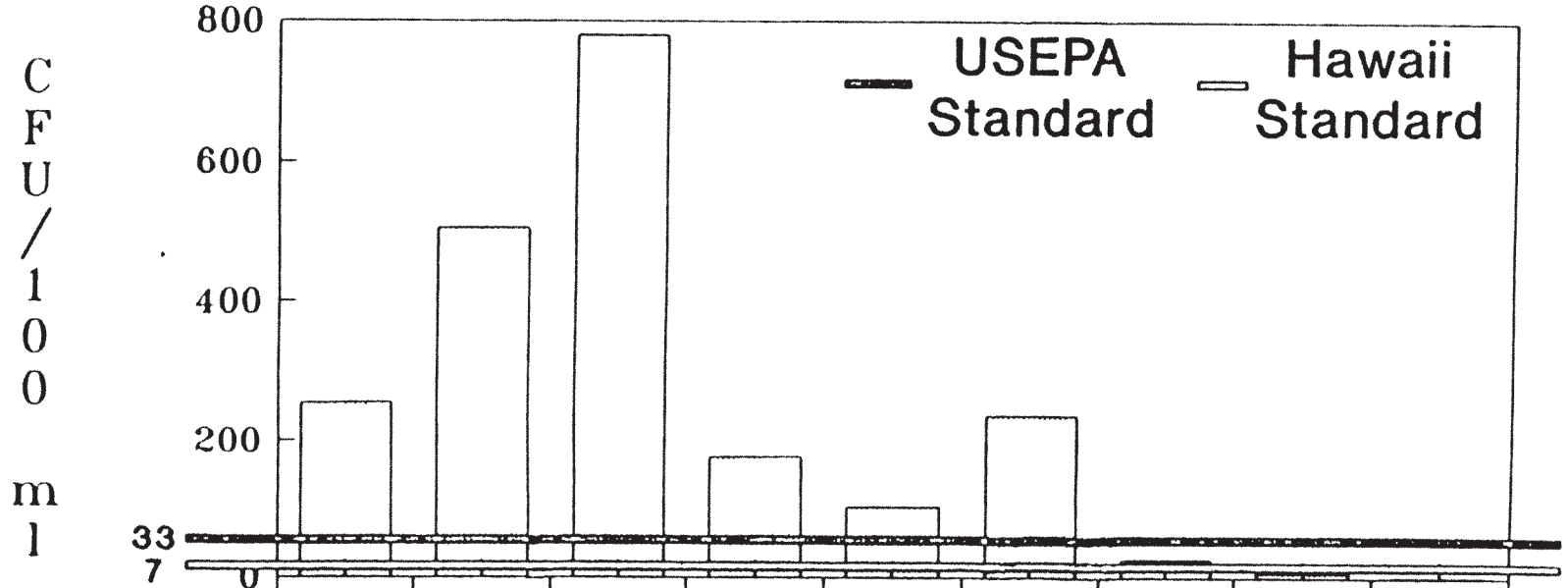
Figure 16



Geometric Mean For Enterococci

5 Consecutive Samples in 30 Day Period

Figure 17



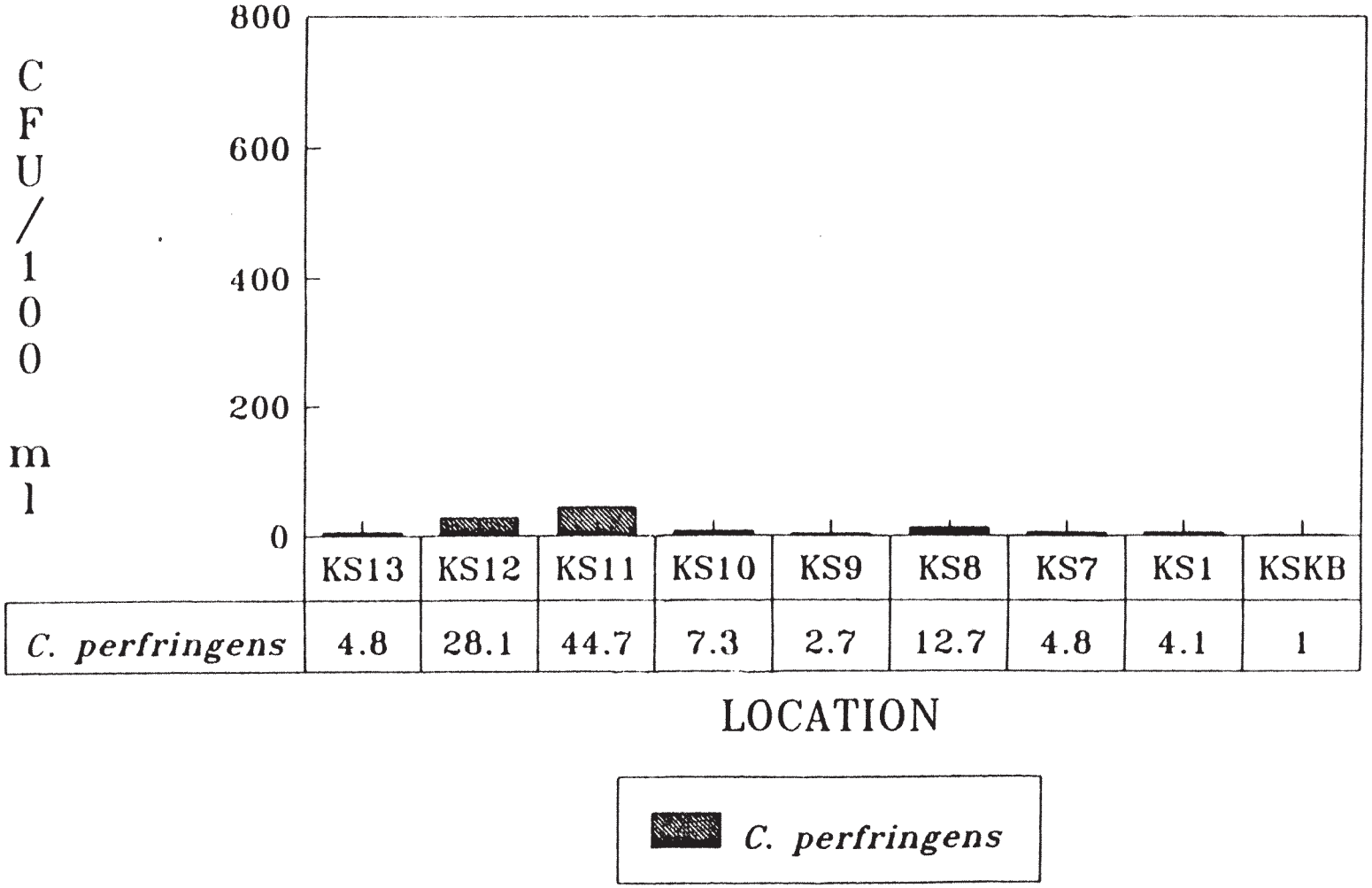
	KS13	KS12	KS11	KS10	KS9	KS8	KS7	KS1	KSKB
Enterococci	254.9	504	780.4	175.3	101.1	236	26	7.7	1

LOCATION

Enterococci

Geometric Mean *C. perfringens*
5 Consecutive Samples in 30 Day Period

Figure 18



IV. Sources of Fecal Bacteria

A. Waters Entering System

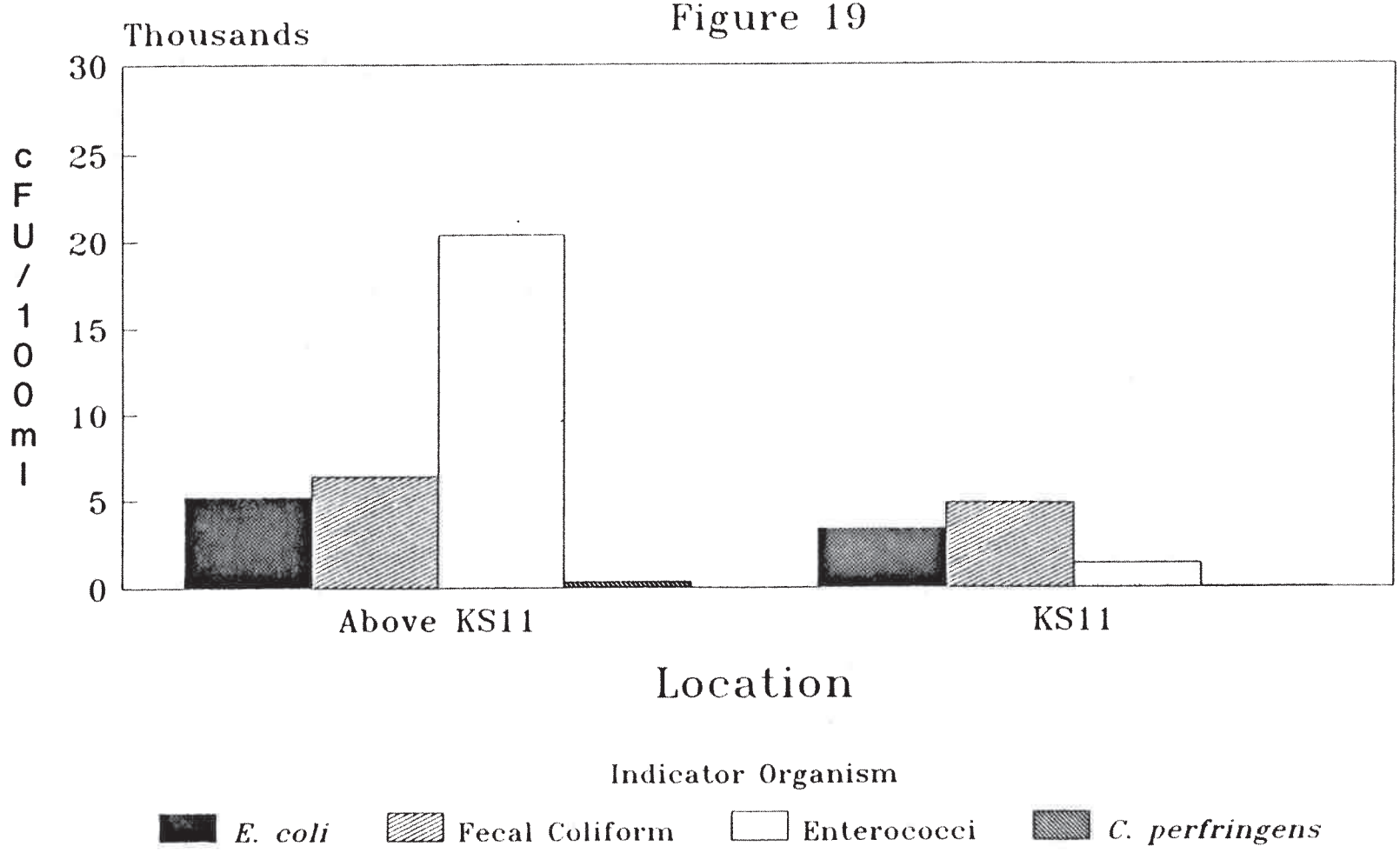
As seen by the Figures 12, 13 and 14, geometric means for the indicator bacteria were the highest at locations KS11, KS12 and KS13. Since KS11, 12 and 13 were located on the major tributaries feeding into Enchanted Lake and Kaelepulu Stream the water flowing in to these locations was analyzed for indicator densities. As seen in Figures 19, 20 and 21 the levels of indicator bacteria coming into this drainage system were between two and ten times greater than levels in Kaelepulu Stream and Enchanted Lake. Explanations for decreased levels in Kalelpulu Stream and Enchanted Lake include dilution and die off associated with sea water. It appears the water entering at these locations are contributing to the high indicator counts seen at these locations. This would be consistent with reports by Fujioka indicating high levels of indicator bacteria in fresh water streams in Hawaii (Fujioka, 1983). Natural waters in Puerto Rico appear to also have high numbers of indicator bacteria (Hazen, 1988).

B. Soil as a Source of Indicator Bacteria

Although no known source of sewage contamination was determined in tributaries entering Kaelepulu Stream, the indictor bacteria levels were very high. Previous work by Hardina indicated high levels of *E. coli* and enterococci can be found in the soils of Hawaii (Hardina and Fujioka, 1991). Indicator organisms may be washed from soil into streams during rainstorms and thus contribute to high

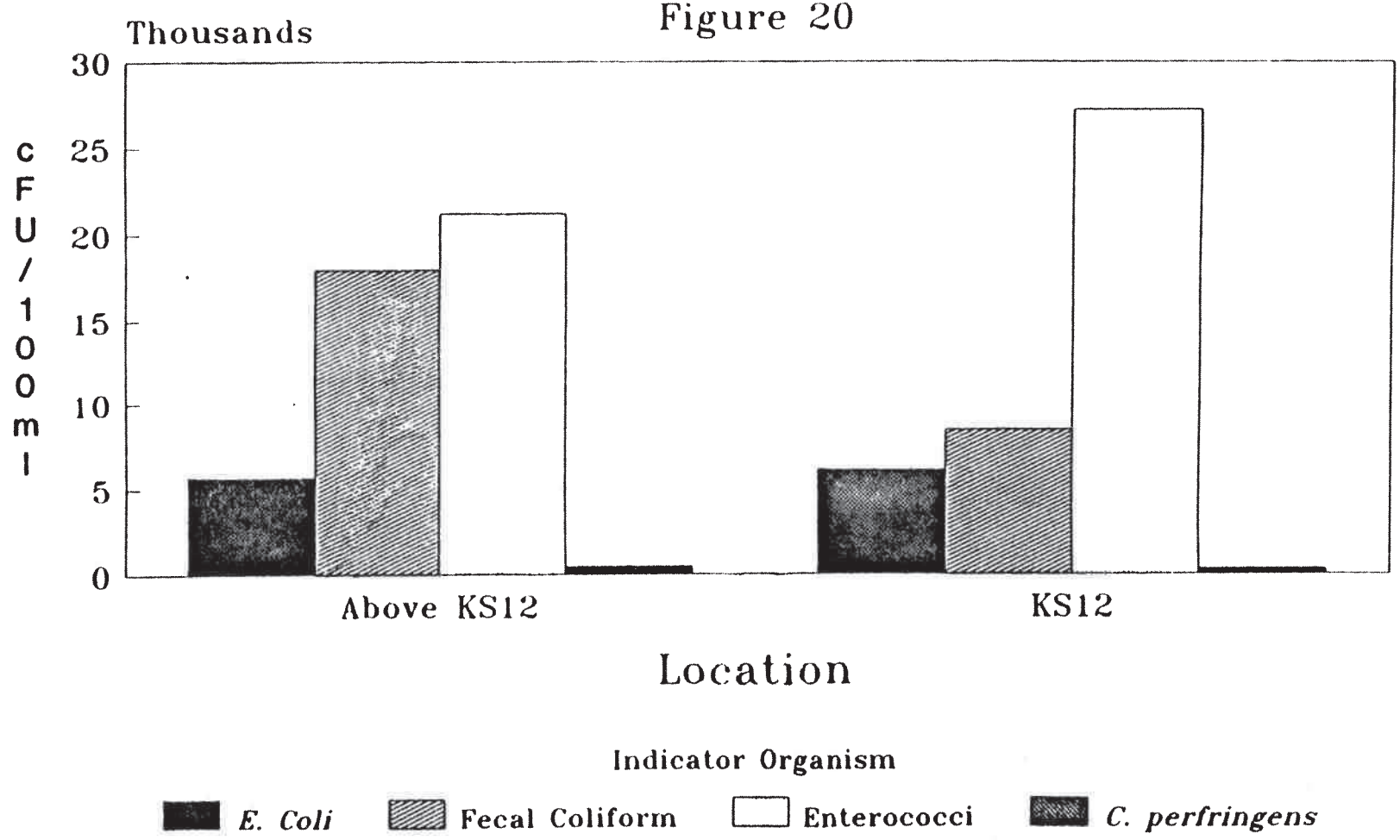
Source Water Sampling Comparison
Sampling Site KS11

Figure 19



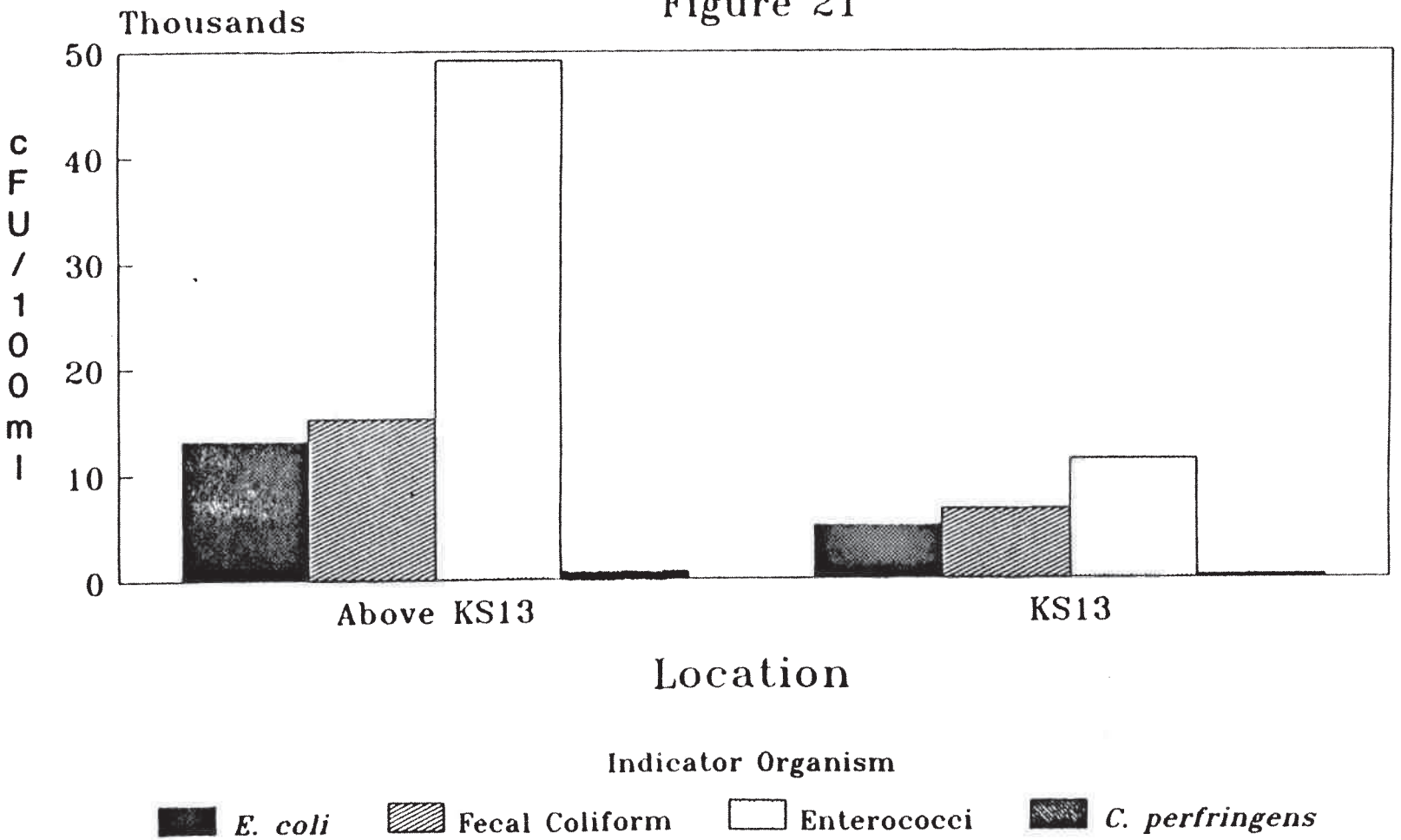
Source Water Sampling Comparision
Sampling Site KS12

Figure 20



Source Water Sampling Comparison
Sampling Site KS13

Figure 21



numbers seen in the fresh water streams. As seen in Figure 22 and Table 12 soils located in the vicinity of KS13, KS12 and KS11 demonstrate high levels of *E. coli*, fecal coliform, and enterococci. All of these sites contained similar levels of inidcator bacteria ranging between 104 and 106 CFU/ gram of soil (Table 12). Fecal coliforms averaged 58,000 per gram of soil while *E. coli* averaged 46,000. Enterococci were isolated in densities of 39,000 per gram of soil. Although the levels of these indictors were very high, *C. perfringens* maintained levels similar to those found in water samples. This may indicate a natural low population of *C. perfringens* in soil. The high levels of fecal coliform, *E coli* and enterococci indicates either soil contamination by feces or possibly naturally occurring populations of these indicators.

Table 13 Indicator Bacteria in Soil at Sites KS11, KS12 & KS13(CFU/g. of soil)

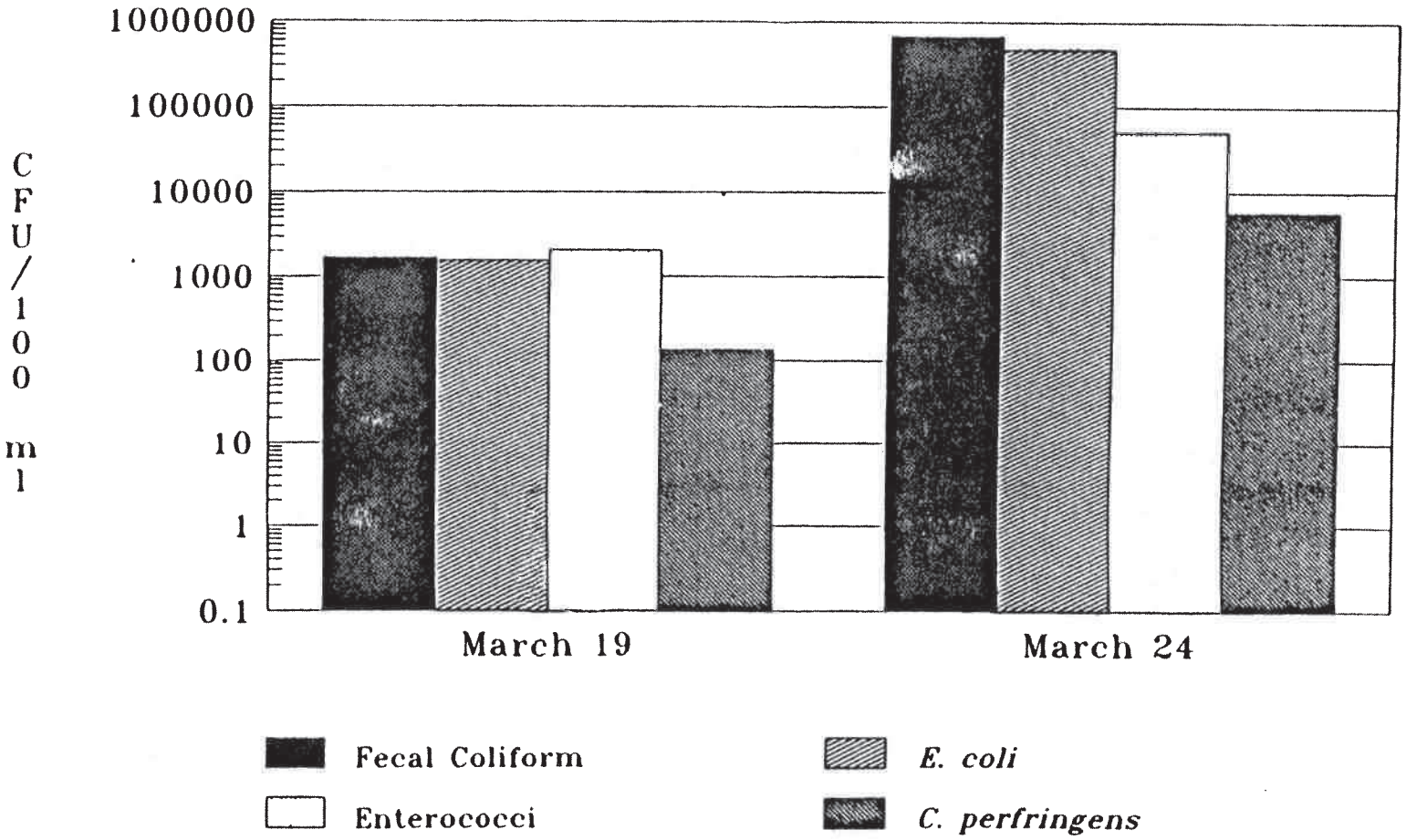
Indicator Bacteria	Average CFU/g*	KS13	KS12	KS11
E. coli	4.6×10^5	9.0×10^4	4.1×10^5	9.6×10^5
Fecal Coliform	5.8×10^5	6.0×10^4	8.6×10^5	8.2×10^5
Enterococci	3.9×10^5	1.5×10^4	1.0×10^6	1.3×10^5
C. perfringens	130	120	180	90

*average of samples taken at locations KS13, KS12 and KS11

C. Sewage Discharges

Sewage discharges from the Akumu Street pumping station are a major concern for the residents of Kailua. During abundant rain, sewage is occasionally discharged into Enchanted Lake. This occurs at a location next to KS12. As seen in Table 13, there were two days when sewage was discharge into Enchanted Lake.

Sewage Discharge at Akumu Street
Figure 22



These sewage discharges occurred when sewage was forced through a manhole cover in Akumu Street. To stop sewage from entering the street, pumping occurred into Enchanted Lake.

Table 14 Sewage Discharge in Enchanted Lake

Date of Discharge	Duration (hours)	Gallons Discharged
March 19-21	29 hours	8,700
March 23, 1991	7.67	23,000

Evidence of the impact of these discharges can be seen in samples collected on March 19 before discharged occurred and on March 24 after sewage discharges. Figure ~~13~~ 22 displays indicator densities during these times. The levels detected at KS12 on March 24 were the highest recorded levels during the entire study. These levels however, decreases dramatically soon after the event. Sample collected on April 8, displayed levels three log units lower. Apparently there was a die off associated with introduction into this saline environment.

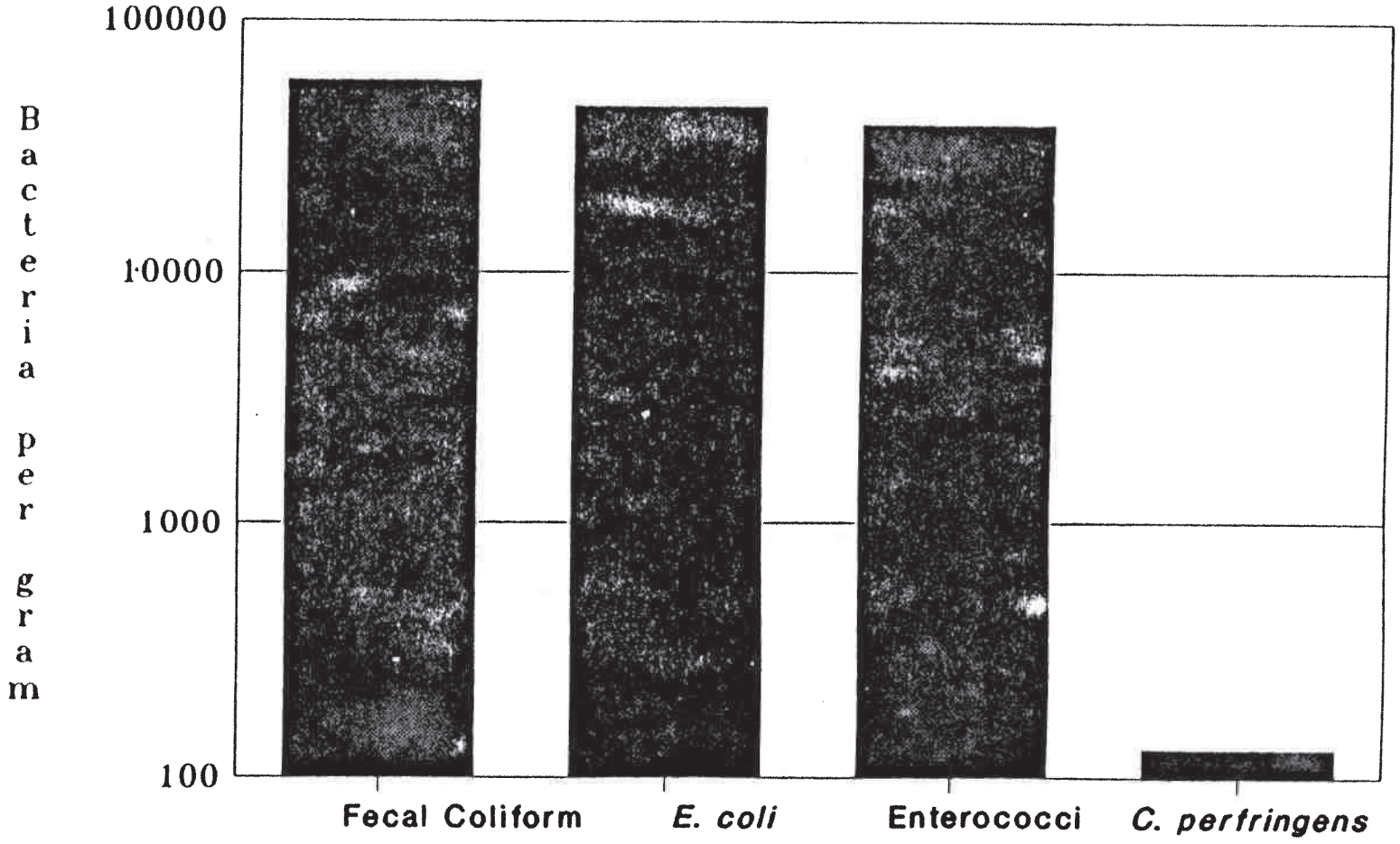
D. Storm Water As A Source Of Indicator Bacteria

As evident by the sewage spills, rain events impact the water quality in Enchanted Lake and Kaelepulu Stream. Previous studies by Gannon have indicated high levels of indicator bacteria in storm waters (Gannon and Busse, 1989). In order to determine if storm water (non-sewage related) was impacting Enchanted Lake and Kaelepulu Stream, samples where collected from locations KS13, KS12 and KS9 during rainstorms. These levels were then compared during dry sampling periods.

Indicator Bacteria in Soil Samples

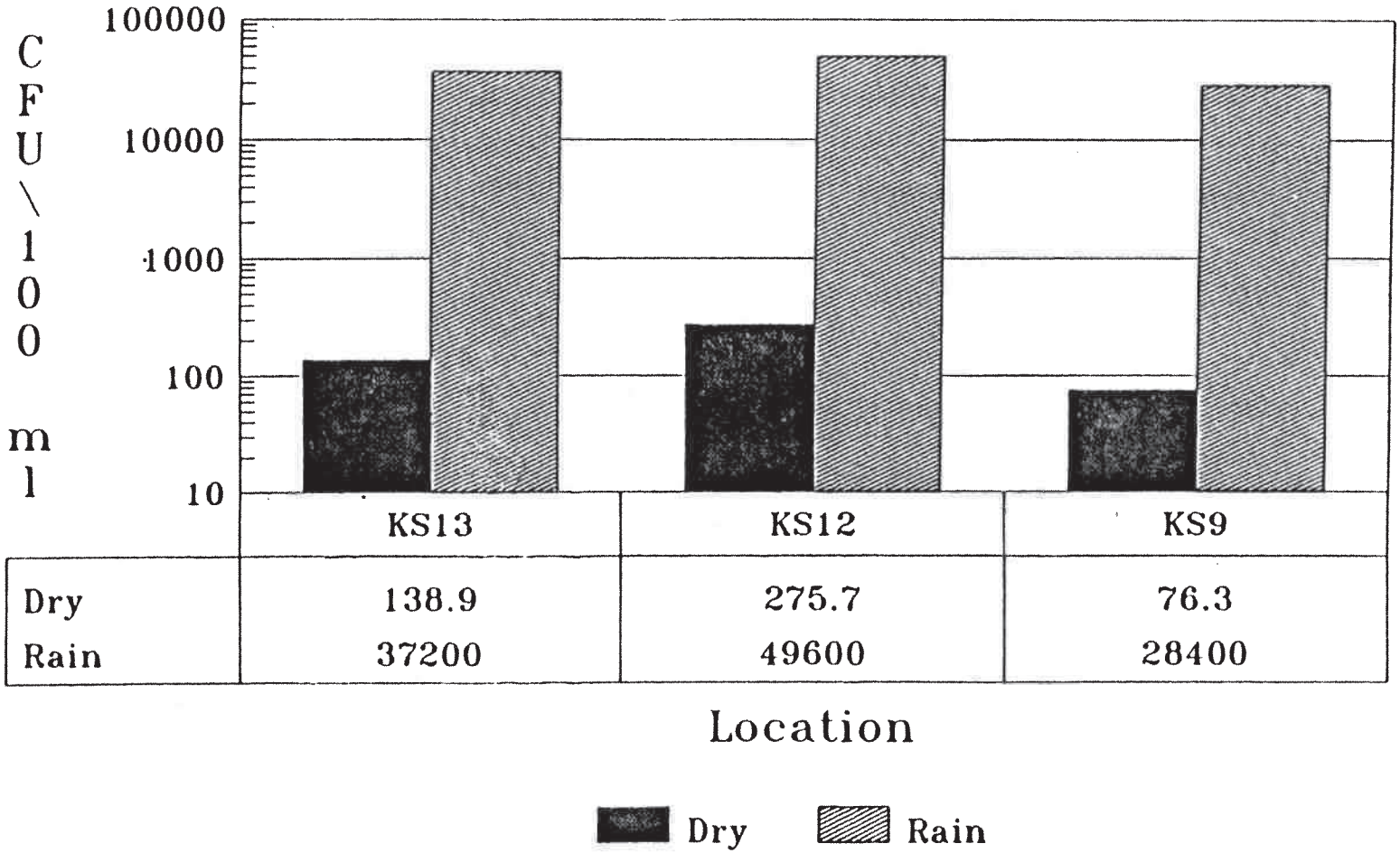
Bacteria Per Gram of Soil

Figure 23



Kaelepulu Under Rain Conditions
Fecal Coliform per 100 ml

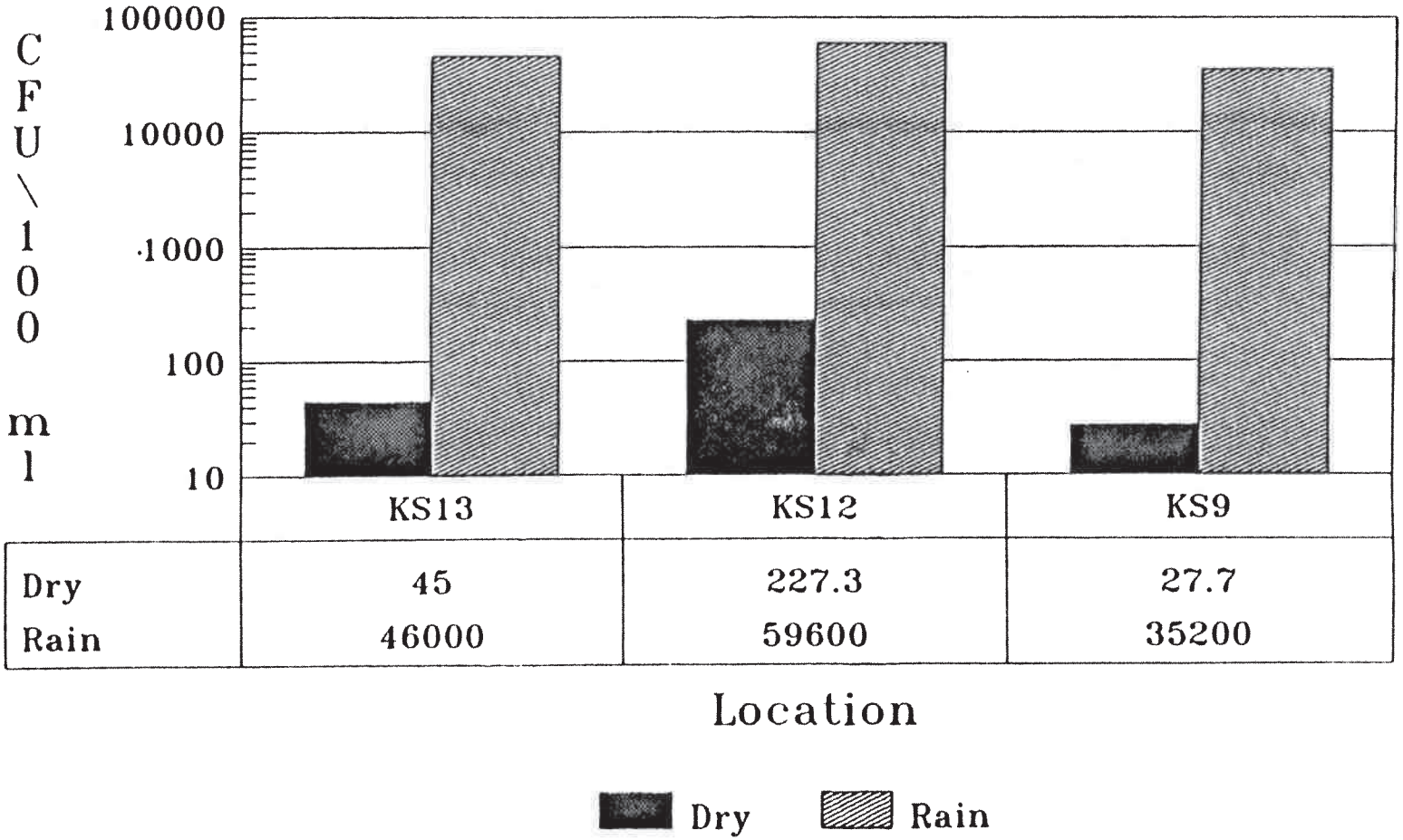
Figure 24



	KS13	KS12	KS9
Dry	138.9	275.7	76.3
Rain	37200	49600	28400

Kaelepulu Under Rain Conditions
Escherichia coli per 100 ml

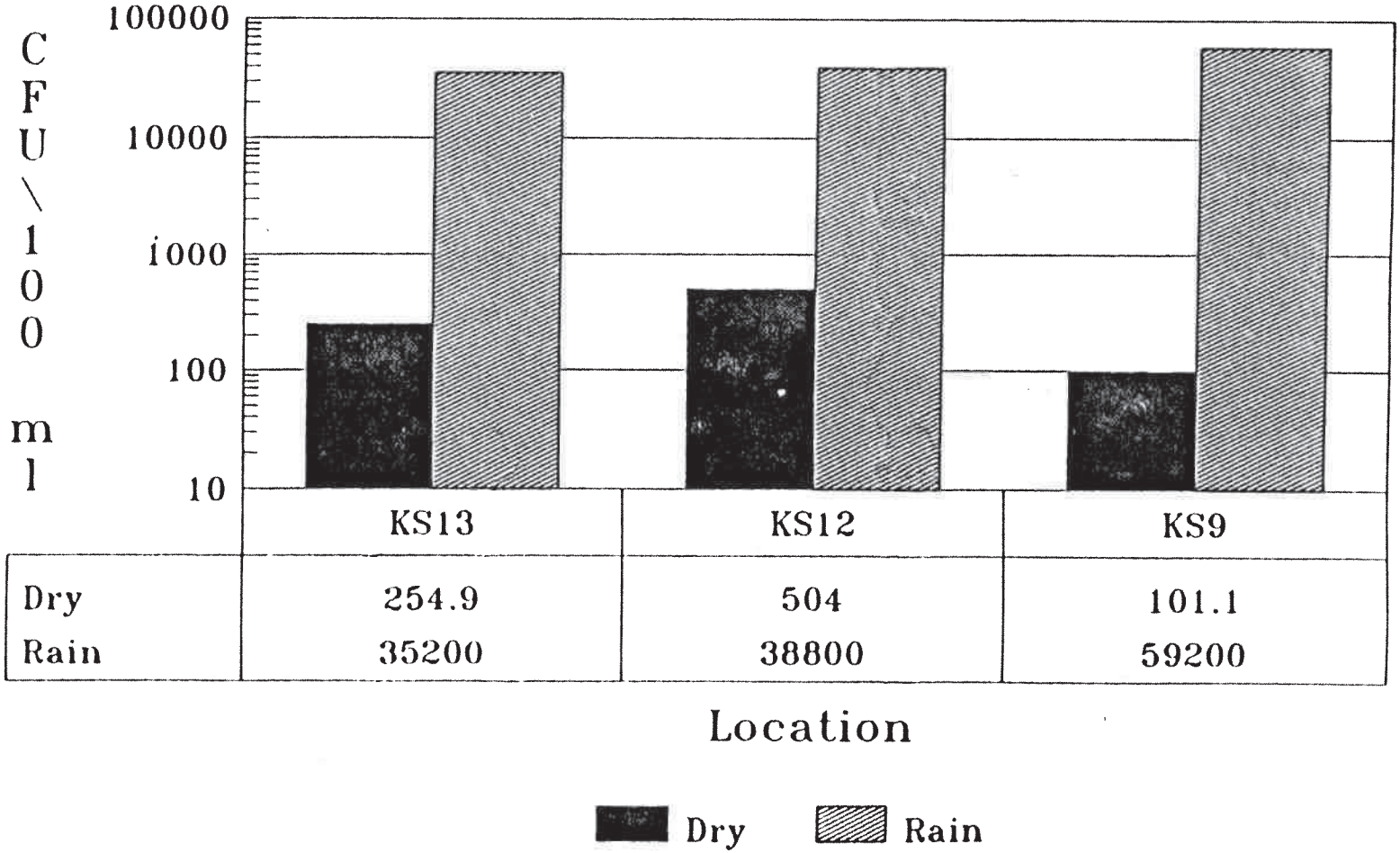
Figure 25



	KS13	KS12	KS9
Dry	45	227.3	27.7
Rain	46000	59600	35200

Kaelepulu Under Rain Conditions
Enterococci per 100 ml

Figure 26

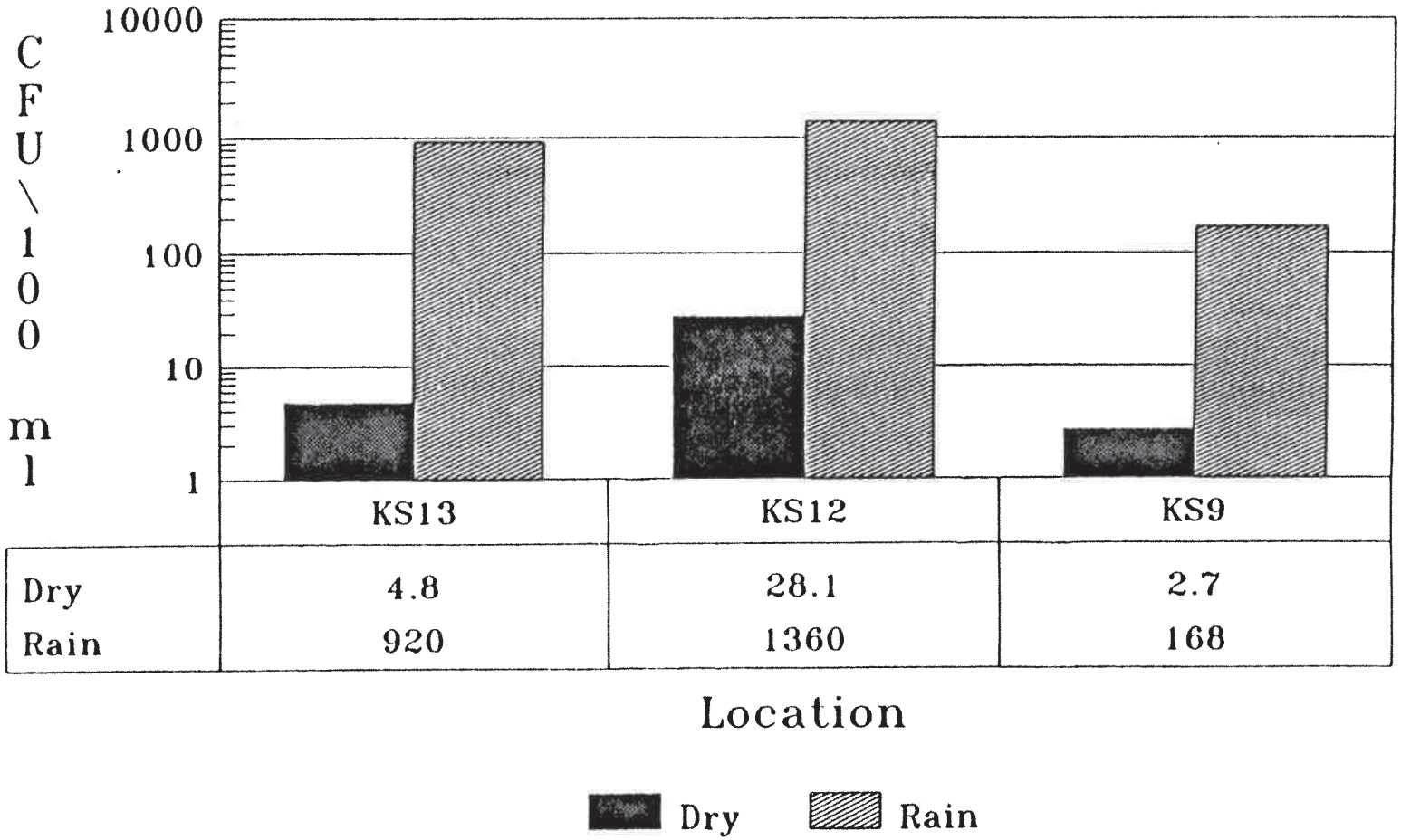


	KS13	KS12	KS9
Dry	254.9	504	101.1
Rain	35200	38800	59200

Kaelepulu Under Rain Conditions

Clostridium perfringens per 100 ml

Figure 27



and displayed in Figures 23, 24, 25 and 26. In almost all cases the levels of indicator bacteria increased at least 10 fold during rain storms. This would indicate storm water is a major contributor of indictor bacteria.

E. Duck Feces As A Source Of Indicator Bacteria

The Kaelepulu Stream and Enchanted Lake are home to a large duck population (*Anas platyrhynchos*) . Previous work by Geldriech indicated that ducks may be a source of indicator bacteria. Geldreich was able to isolate fecal coliform at level of 3.3 x 10^7 and fecal streptococci at levels of 5.4 x 10^7 per gram of feces (Geldreich, 1962). Four samples were collected near Kaelepulu Stream and the averages of these samples are displayed in Table 15 and Figure 28. As seen in Figure 28 ducks are a major source of indicator bacteria.

Table 15 Indicator Bacteria in Duck Feces[a]

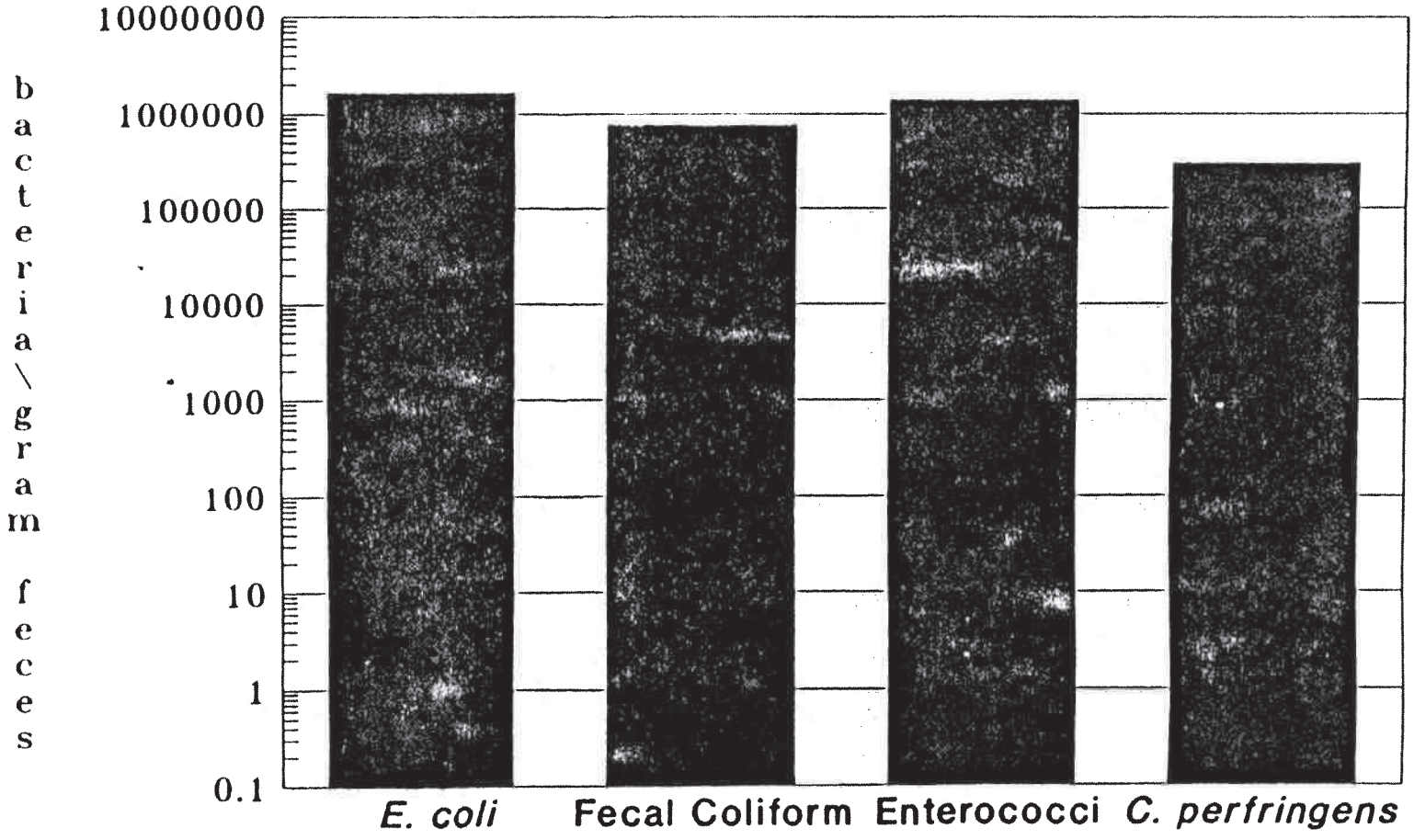
Indicator Bacteria	CFU/gram feces
E. coli	1.6×10^6
Fecal Coliform	7.6×10^5
Enterococci	1.4×10^6
C. perfringens	2.9×10^5

[a] Average for 4 samples

Indicator Bacteria in Duck Feces

Bacteria per gram of Feces

Figure 28



F. Water Quality at Kailua Beach

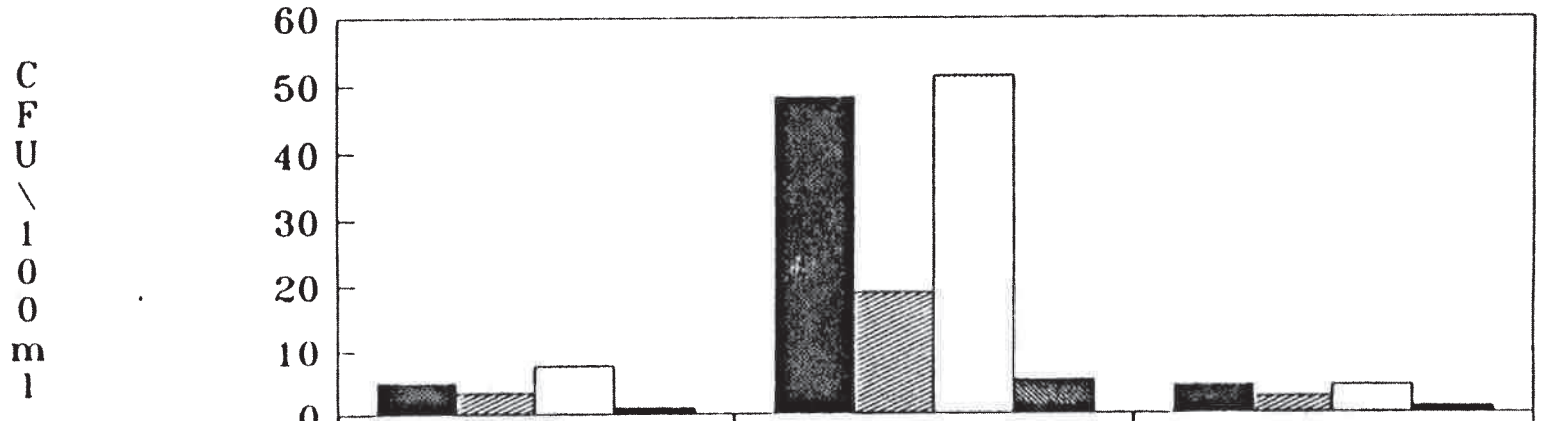
One of the major goals of this study was to determine the impact of Kaelepulu Stream on Kailua Bay. The Hawaii State standard was rarely exceeded when Kaelepulu was closed from direct contact with Kailua Bay. It did however, often exceed State standards when it was opened either through mechanical means (bulldozer) or from abundant rains. As seen in Figure 29 levels of indicator bacteria at Kailua Beach sites increased dramatically when Kaelepulu was opened. During these times samples were taken at the mouth of Kaelepulu Stream (KSKB) and at locations located on either side of the mouth. The State standard was exceeded at the mouth and the location located north of the mouth of Kaelepulu Stream.

G. Algal Growth In Kalua Bay

Green foam was collected in Kailua Bay and analyzed for fecal coliform, *E. coli*, Enterococci and *C. perfringens*. None of these indicators were detected in these samples. In addition, samples were collected and analyzed for the presence of algae and the green foam appeared to consist primarily of one genus of the green algae, *Pyramimonas*. This would indicate that the green foam seen in Kailua Bay is not sewage but rather an algal bloom.

Concentration of Indicator Bacteria at Kailua Beach With Kaelepulu Open

Figure 29



	KSKBN	KSKB	KSKSS
Fecal Coliform	5.1	48.3	4.5
E. coli	3.4	18.9	2.6
Enterococci	7.7	51.4	4.3
C. perfringens	1	5.3	1

Chapter 5

Summary and Conclusions

I. Summary

The waters of Enchanted Lake and Kaelepulu Stream have bacterial indicator counts exceeding the current State and Federal standards. The highest counts appear at locations KS11, KS12, KS13 and KS8 which are locations representative of the waters entering this drainage system. During this study there was a 2 day period when sewage was discharged at the Akumu Street pumping station. KS12 is located next to this pumping station and recorded the highest single day levels of indicator bacteria during the entire study. Although the impact resulted in very high levels of indicator bacteria, its effect appeared to be limited to a duration of about one week and did not appear to dramatically increase the levels of bacteria at other location along Kaelepulu Stream. Sampling that occurred the following week did not reflect the high levels seen during the discharge.

In addition to the sewage discharge there are a number of additional sources that contribute to the high levels of indicator bacteria. Unlike the sporadic sewage discharges, these sources appear to be a constant source of indicator bacteria and would be contributing to the high levels year round. One of theses sources is ducks and can be found throughout the drainage system and their feces appeared to contribute to the high levels of indicator bacteria. In addition to duck feces, soil and waters entering Enchanted Lake and Kaelepulu Stream contribute to the high levels of indicator bacteria. Storm drains appear to be a major constant contributor of indicator bacteria and would be responsible for increased levels each time there was a rain storm. Combined these sources (soil, storm drains, duck feces and source waters) are the major contributors of indicator bacteria in Kaelepulu Stream and

Enchanted Lake. Sewage discharge is a sporadic source and this source of indicator bacteria did not persist in the stream.

A primary goal of this study was to determine the impact of Kaelepulu Stream on Kailua Bay. Its effect is dependent on whether the mouth of Kaelepulu Stream is opened or closed. When the mouth of Kaelepulu Stream was closed, samples taken in Kailua Bay (KSKB) were always below State and Federal Water Quality standards. When Kaelepulu stream was open it exceeded the State standard 80 % of the time and the Federal standard 60 % of the time. These results would indicate Kaelepulu stream does effect the water quality of Kailua Bay.

II. Conclusions

Based on the data collected the following conclusions were drawn:

1) The salinity of Kaelepulu Stream is similar to marine environments, and therefore should be considered a marine or brackish water system. The fluctuation in salinity is under tidal influence and is further influenced by rainfall.
2) Dissolved oxygen levels and Ortho phosphate levels indicate possible nutrient loading at sites KS8, KS11 and KS12. Low levels of dissolved oxygen and high levels of phosphate occur at sites representative of the Kaelepulu drainage system.
3) Bacterial indicator levels were the highest at locations representative of water entering the Kaelepulu drainage system. These levels appear to have seasonal variation with higher levels occurring during the rainy winter months and lowest counts occurring during the dry summer months.

4) Recreational water standards were exceeded at almost all locations in Kaelepulu stream. Standards were never exceeded in Kailua Bay when the mouth of Kaelepulu Stream is closed. Levels of indicator bacteria decline as water move toward the ocean.

5) The major sources of indicator bacteria in Kaelepulu Stream are sewage discharges, duck feces, source waters, soil and storm drain run-off. Sewage discharges and duck feces have the highest concentrations of indicator bacteria.

6) When the mouth of Kailua Bay is open, recreational standards are exceeded in Kailua Bay and therefore Kaelepulu Stream does impact the water quality of Kailua Bay.

7) Green foam seen in Kailua Bay appears to be caused by an algal bloom and is not sewage discharge.

8) Of all the indicator bacteria, Clostridium perfringens is the best indicator of fecal contamination.

III. Speculation and Recommendations

Some people in the community of Kailua have expressed a strong concern for the possible health risk associated with the discharge of sewage into nearby recreational waters. These concerns are amplified by media coverage, environmental groups and EPA fines which question the integrity of the sewage systems on Oahu. In order to determine the health risks involved with sewage discharge, scientific data must be collected to determine if these concerns are justified. This data must reflect the regulatory guidelines for determining the sanitary quality of water and health risks involved with swimming in Kailua Bay. Currently EPA and the State of

Hawaii recognize *E. coli* and enterococci as determinants of recreational water quality. These standards were established to reflect sewage or feces. This study indicates there are a variety of other sources that contribute to indicator levels and therefore current USEPA standards are not applicable to recreational waters found in Hawaii. *C. perfringens* was found in high concentrations in sewage and duck fesecs but in low numbers in the enviroment (e.g. soil). Moreover since it is unable to to replicate in the presence of oxygen this indicator appears to be the best indicator of fecal pollution. Further work, however, would be needed in order to determine the sources of the high levels of *C. perfringens* in storm water. This leads to the recommendation that the Hawaii Department of Health recognize that indicator bacteria are present in soil and streams and therefore may not be good indicators of fecal contamination. It appears that *C. perfringens* is a better indicator of fecal contamination for the recreational waters in Hawaii.

When considering the impact of Kaelepulu Stream on Kailua Bay, location KSKB never appeared to be influenced by any other sources than Kaelepulu Stream since levels were never exceeded when Kaelepulu was closed. This would lead to the recommendation that beach areas in the vicinity of Kaelepulu Steam be closed when the mouth of Kaelepulu Stream was open. This closing would be based on the high levels of indicator bacteria contributed by Kaelepulu Stream.

Since the waters of Enchanted Lake and Kaelepulu Stream have a high salinity, the State of Hawaii should recognize these waters as marine waters and therefore utilize enterococci to determine water quality.

Appendix A
Kaelepulu Stream Site KS13
Bacteriological,Salinity and Phosphate Results
Table 16

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS13	9-17-90	480	1640	1530	6	24	0.074
	9-21-90	5800	3900	2400	84	26	0.116
	10-1-90	920	1520	2080	7	15	0.042
	10-8-90	1560	1960	3760	38	19	0.001
	10-15-90	0	12	236	2	17	0.006
	10-22-90	4	24	8	0	18	0.014
	10-29-90	36	60	72	5	19	0.009
	11-5-90	0	4	32	1	17	0.048
night	11-5-90	1360	720	284	0	17	0.035
submerge	11-5-90	0	4	20	0	17	0.160
	11-19-90	44	64	116	3	17	0.019
	11-26-90	5120	6600	11200	272	16	0.076
KS13 A	11-26-90	13200	15200	49200	736	0	0.041
	12-3-90	1160	1920	3840	39	14	0.033
	12-10-90	36	96	2120	15	16	0.103
	1-21-91	72	60	192	5	14	0.164
	1-28-91	46000	37200	55200	920	16	0.214
	2-3-91	1040	584	1240	33	14	0.049
	2-20-91	520	880	1240	56	15	0.016
	3-6-91	960	680	1360	20	16	0.008
	3-19-91	1080	1280	760	56	15.5	0.041
	3-25-91	20000	50000	110000	7000	12	0.084
	4-8-91	920	640	1240	52	20	0.416
	4-24-91	560	1160	840	28	18	0.108
	5-8-91	68	56	104	9	24	0.047
	5-23-91	2040	1240	840	32	22.5	0.100
	6-5-91	32	16	52	4	27	0.068
	6-26-91	348	440	960	12	25	0.047
	7-17-91	68	124	192	16	30	0.061
	8-7-91	72	92	196	12	31	0.009
	8-19-91	2120	2680	3240	136	31	0.012

1 Sample collected at night
2 Sample exposed to sunlight
3 sample collected above site

Appendix A
Kaelepulu Stream Site KS12
Bacteriological,Salinity and Phosphate Results
Table 17

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
	9-17-90	>2000	>2000	>2000	>800	24	0.053
	9-21-90	600	1800	1400	116	25	0.173
	10-1-90	4480	4920	6320	272	15.5	0.094
	10-8-90	2240	2440	2840	89	22	0.112
	10-15-90	36	112	212	14	19.5	0.146
	10-22-90	140	296	356	13	17.5	0.094
	10-29-90	12	4	24	4	14.5	0.147
	11-5-90	0	28	52	14	17	0.115
night	11-5-90	244	312	536	34	17	0.077
submerge	11-5-90	16	8	68	22	17	0.084
	11-19-90	20	44	84	2	17	0.048
	11-26-90	6120	8440	27200	296	17	0.094
KS12 A	11-26-90	5720	18000	21200	484	0	0.416
	12-3-90	760	600	2520	61	15	0.109
	12-10-90	48	96	6080	53	16	0.233
	1-21-91	196	212	164	96	14	0.245
	1-28-91	59600	49600	38800	1360	15	0.124
	2-3-91	10440	11560	4840	604	13	0.610
	2-20-91	1360	1720	2280	268	15	0.042
	3-6-91	840	1360	1160	68	16	0.047
	3-19-91	1520	1680	2040	136	16	0.084
	3-25-91	470000	690000	510000	5700	13	0.804
	4-8-91	1160	1440	1840	12	21	0.440
	4-24-91	3040	4080	4920	56	19	0.315
	5-8-91	64	124	192	21	24	0.112
	5-23-91	1240	2320	2760	136	22	0.095
	6-5-91	36	48	124	16	27	0.087
	6-26-91	56	68	124	20	25	0.042
	7-17-91	28	76	144	12	30	0.119
	8-7-91	112	136	236	16	30	0.184
	8-19-91	1880	2120	3680	84	31	0.149

1 Sample collected at night
2 Sample exposed to sunlight
3 sample collected above site

Appendix A
Kaelepulu Stream Site KS11
Bacteriological,Salinity and Phosphate Results
Table 18

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS11	9-17-90	>2000	>2000	>2000	>800	23	0.324
	9-21-90	3240	3400	2000	612	25	0.125
	10-1-90	6280	7560	8560	196	15	0.221
	10-8-90	4160	>8000	7280	232	22.5	0.548
	10-15-90	4	52	140	8	20	0.113
	10-22-90	72	216	244	41	15.5	0.048
	10-29-90	52	124	136	12	14	0.075
	11-5-90	600	1320	2240	572	15	0.049
night	11-5-90	1360	2040	2760	552	15	0.146
submerge	11-5-90	524	756	1160	500	15	0.112
	11-19-90	44	64	136	26	16	0.119
	11-26-90	3360	4840	1360	348	16	0.280
KS11 A	11-26-90	5200	6400	20400	1320	0	0.065
	12-3-90	1240	2120	1520	41	15	0.143
	12-10-90	612	744	1080	192	15	0.510
	1-21-91	28	44	196	7	13	0.321
	1-28-91	20400	26800	30000	64	15	0.148
	2-3-91	>8000	>8000	>8000	508	14	0.421
	2-20-91	920	640	1560	212	15	0.090
	3-6-91	440	880	560	156	16	0.084
	3-19-91	1080	720	1480	116	15	0.119
	3-25-91	6400	9200	15600	840	13	0.086
	4-8-91	760	960	560	44	21	0.076
	4-24-91	128	164	276	36	19	0.084
	5-8-91	56	92	156	9	24	0.184
	5-23-91	760	1240	1120	84	22.5	0.214
	6-5-91	116	68	124	12	27	0.475
	6-26-91	64	52	84	16	25	0.094
	7-17-91	52	48	92	4	29	0.087
	8-7-91	76	104	144	32	26	0.047
	8-19-91	1240	1680	3120	72	25	0.090

1 Sample collected at night
2 Sample exposed to sunlight
3 sample collected above site

Appendix A
Kaelepulu Stream Site KS10
Bacteriological,Salinity and Phosphate Results
Table 19

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS10	9-17-90	2210	>2000	>2000	295	17	0.117
	9-21-90	1200	1800	1400	4	20	0.214
	10-1-90	1640	1880	2520	74	15.5	0.159
	10-8-90	208	240	356	13	22.5	0.095
	10-15-90	36	96	412	22	20	0.182
	10-22-90	20	72	28	0	17.5	0.092
	10-29-90	0	44	16	0	18.5	0.080
	11-5-90	52	92	164	11	17	0.113
night	11-5-90	140	184	276	18	17	0.145
	11-19-90	292	376	448	5	20	0.086
	11-26-90	92	152	516	32	22	0.048
	12-3-90	516	632	2360	3	17	0.015
	12-10-90	488	512	792	19	18	0.048
	1-21-91	36	68	316	24	25	0.148
	1-28-91	29200	24400	40400	4000	15	0.740
	2-3-91	1880	1720	1360	57	20	0.460
	2-20-91	64	120	196	46	17	0.074
	3-6-91	88	140	56	68	19	0.049
	3-19-91	168	144	204	96	15	0.058
	3-25-91	nd	nd	nd	nd	nd	nd
	4-8-91	68	104	144	4	22	0.116
	4-24-91	104	296	148	28	24	0.031
	5-8-91	23	17	35	1	25	0.178
	5-23-91	320	760	520	8	23	0.159
	6-5-91	19	27	43	1	26	0.009
	6-26-91	76	92	124	19	27	0.008
	7-17-91	43	69	136	7	27	0.061
	8-7-91	41	38	69	3	25	0.074
	8-19-91	248	284	448	13	29	0.094

1 Sample collected at night
2 Sample exposed to sunlight
3 sample collected above site

Appendix A
Kaelepulu Stream Site KS9
Bacteriological,Salinity and Phosphate Results
Table 20

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS9	9-17-90	1920	>2000	>2000	35	18	0.149
	9-21-90	1320	2120	900	84	21	0.135
	10-1-90	44	96	156	13	18	0.148
	10-8-90	24	60	204	4	26.5	0.079
	10-15-90	20	76	84	3	24.5	0.087
	10-22-90	48	92	76	1	18	0.119
	10-29-90	16	64	52	0	21.5	0.142
	11-5-90	0	28	104	48	17	0.078
night	11-5-90	0	8	84	17	17	0.015
	11-19-90	0	32	48	3	20	0.094
	11-26-90	168	312	468	14	21	0.081
	12-3-90	84	144	600	15	16	0.147
	12-10-90	48	88	3280	4	19	0.048
	1-21-91	44	64	96	8	24	0.148
	1-28-91	35200	28400	59200	168	19	0.610
	2-3-91	104	84	120	26	20	0.016
	2-20-91	128	188	252	31	17	0.094
	3-6-91	68	92	128	84	19	0.056
	3-19-91	72	148	196	17	15	0.041
	3-25-91	8400	5600	3240	520	20	0.310
	4-8-91	92	56	144	12	22	0.116
	4-24-91	232	256	296	36	25	0.125
	5-8-91	19	29	31	3	25	0.010
	5-23-91	32	16	68	12	23	0.019
	6-5-91	23	32	39	3	27	0.009
	6-26-91	52	68	96	17	27	0.012
	7-17-91	41	53	84	5	29	0.047
	8-7-91	32	41	59	4	28	0.071
	8-19-91	164	212	292	14	29	0.091

Appendix A
Kaelepulu Stream Site KS8
Bacteriological,Salinity and Phosphate Results
Table 21

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS8	9-17-90	1640	4800	1520	63	21	0.214
	9-21-90	5120	7800	2320	124	34	0.091
	10-1-90	612	680	1080	23	18.5	0.074
	10-8-90	36	44	64	14	25	0.034
	10-15-90	252	376	336	23	24.5	0.049
	10-22-90	112	172	272	15	19	0.119
	10-29-90	68	104	116	3	26.5	0.247
	11-5-90	676	2720	5040	10	17	0.310
night	11-5-90	4080	4960	9240	105	17	0.094
	11-19-90	236	268	464	12	20	0.091
	11-26-90	232	452	1440	41	21	0.412
	12-3-90	124	236	720	16	16	0.251
	12-10-90	224	204	884	11	19	0.048
	1-21-91	204	288	284	76	24	0.148
	1-28-91	28000	33200	37200	480	19	0.610
	2-3-91	1000	680	1400	180	20	0.008
	2-20-91	nd	nd	nd	nd	nd	0.078
	3-6-91	76	136	156	17	19	0.094
	3-19-91	144	156	116	34	15	0.047
	3-25-91	1560	2240	2440	144	20	0.810
	4-8-91	164	188	212	52	22	0.116
	4-24-91	188	212	276	13	25	0.125
	5-8-91	8	5	16	4	25	0.010
	5-23-91	68	156	92	16	23	0.019
	6-5-91	23	37	59	2	27	0.009
	6-26-91	34	26	49	8	27	0.012
	7-17-91	24	36	41	2	29	0.047
	8-7-91	9	17	31	4	28	0.071
	8-19-91	128	164	228	9	29	0.086

Appendix A
Kaelepulu Stream Site KS7
Bacteriological,Salinity and Phosphate Results
Table 22

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS7	9-17-90	248	196	276	14	21	0.120
	9-21-90	400	212	500	4	23	0.090
	10-1-90	72	156	168	21	19	0.085
	10-8-90	28	12	92	0	25	0.061
	10-15-90	0	4	4	3	26	0.050
	10-22-90	8	24	24	43	19	0.300
	10-29-90	0	12	8	0	25	0.008
	11-5-90	0	4	76	8	17	0.007
night	11-5-90	0	64	128	15	17	0.004
	11-19-90	0	12	52	1	20	0.091
	11-26-90	316	524	760	4	21	0.410
	12-3-90	404	532	3280	24	16	0.301
	12-10-90	32	60	328	17	19	0.129
	1-21-91	20	45	164	9	24	0.011
	1-28-91	10000	7600	13600	124	19	0.114
	2-3-91	16	9	64	43	20	0.005
	2-20-91	136	148	204	20	17	0.009
	3-6-91	15	17	31	4	19	0.042
	3-19-91	19	22	34	21	15	0.051
	3-25-91	nd	nd	nd	nd	nd	nd
	4-8-91	15	19	31	16	22	0.116
	4-24-91	124	136	164	19	25	0.125
	5-8-91	5	14	12	1	25	0.010
	5-23-91	8	20	64	20	23	0.016
	6-5-91	12	23	32	0	27	0.005
	6-26-91	8	14	19	0	27	0.009
	7-17-91	13	9	21	0	29	0.048
	8-7-91	19	14	34	1	28	0.073
	8-19-91	56	64	136	5	29	0.041

Appendix A
Kaelepulu Stream Site KS1
Bacteriological,Salinity and Phosphate Results
Table 23

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KS1	9-17-90	48	52	312	8	24	0.021
	9-21-90	36	100	300	8	26	0.007
	10-1-90	0	32	116	0	19	0.009
	10-8-90	0	4	20	0	27	0.029
	10-15-90	0	4	12	5	28	0.007
	10-22-90	0	0	0	1	21.5	0.070
	10-29-90	0	0	0	0	26	0.080
	11-5-90	0	0	20	16	17	0.008
night	11-5-90	0	0	44	23	17	0.007
	11-19-90	16	68	104	5	20	0.019
	11-26-90	160	204	1280	8	21	0.150
	12-3-90	4	28	116	0	16	0.251
	12-10-90	8	52	156	13	19	0.089
	1-21-91	1	3	8	0	24	0.011
	1-28-91	4400	5200	3600	52	19	0.018
	2-3-91	32	60	32	8	20	0.021
	2-20-91	92	64	56	4	17	0.045
	3-6-91	5	19	14	0	19	0.070
	3-19-91	11	9	15	3	15	0.040
	3-25-91	1600	2900	2100	80	20	0.113
	4-8-91	11	21	16	4	22	0.004
	4-24-91	21	14	32	1	25	0.009
	5-8-91	0	1	0	0	25	0.002
	5-23-91	276	244	296	3	23	0.024
	6-5-91	0	0	5	0	27	0.049
	6-26-91	14	19	31	0	27	0.115
	7-17-91	0	0	9	0	29	0.400
	8-7-91	8	13	19	3	28	0.005
	8-19-91	4	8	21	1	29	0.001

Appendix A
Kailua Bay At Kaelepulu Stream
Bacteriological,Salinity and Phosphate Results
Table 24

		CFU/100ml					
		mTEC	mFC	mE	mCP		
Location	Date	*E.coli*	Fecal Coliform	Enterococci	*C. perfringens*	Salinity ppt	Phosphate mg/l
KSKB	9-17-90	8	0	48	3	34	0.021
	9-21-90	0	12	0	8	33	0.007
	10-1-90	0	0	0	0	35	0.009
	10-8-90	0	1	0	0	31	0.029
	10-15-90	0	8	0	0	35	0.007
	10-22-90	0	0	0	0	35	0.003
	10-29-90	0	0	0	0	35	0.004
	11-5-90	0	64	12	3	29	0.006
night	11-5-90	0	0	0	0	34	0.007
	11-19-90	0	0	8	0	33	0.013
	11-26-90	12	152	232	2	28	0.008
	12-3-90	12	40	72	2	23	0.078
	12-10-90	24	36	12	21	30	0.129
	1-21-91	0	0	4	0	34	0.011
	1-28-91	0	0	3	0	33	0.007
	2-3-91	1	0	3	5	35	0.004
	2-20-91	0	0	4	0	34	0.009
	3-6-91	0	0	0	0	34	0.008
	3-19-91	0	0	0	0	33	0.010
	3-25-91	700	1200	1800	50	29	0.081
	4-8-91	0	0	0	0	34	0.004
	4-24-91	1	3	12	0	33	0.009
	5-8-91	0	0	0	0	34	0.010
	5-23-91	0	0	0	2	34	0.016
	6-5-91	0	0	3	0	34	0.005
	6-26-91	3	8	9	0	34	0.003
	7-17-91	0	1	0	0	33	0.010
	8-7-91	3	7	5	0	33	0.005
	8-19-91	0	0	0	0	34	0.001

Table 25
Monthly Geometric Means For Kaelepulu Stream Location KSKB

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	2.8	3.5	6.9	4.9
Oct 90	5	1.0	1.5	1.0	1.0
Nov 90	3	1.9	9.9	12.2	1.6
Dec 90	2	1.7	37.9	29.4	6.5
Jan 91	2	<1	<1	3.5	<1
Feb 91	2	1	<1	3.5	2.2
Mar 91	3	8.9	10.6	12.7	3.7
Apr 91	2	1	1.7	3.5	<1
May 91	2	<1	<1	<1	<1
Jun 91	2	1.7	2.8	5.2	<1
Jul 91	1	<1	1	<1	<1
Aug 91	2	1.7	2.6	2.2	<1

Table 26
Monthly Geometric Means For Kaelepulu Stream Location KS1

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	41.6	72.1	305.0	8.0
Oct 90	5	1.0	3.5	7.7	1.4
Nov 90	3	7.1	10.9	104.0	11.0
Dec 90	2	5.7	38.2	134.5	3.6
Jan 91	2	66.3	124.9	169.7	7.2
Feb 91	2	54.3	62.0	42.3	5.7
Mar 91	2	44.5	79.2	76.1	6.2
Apr 91	2	15.2	17.1	22.6	2.0
May 91	2	16.6	15.6	17.2	1.7
Jun 91	2	3:7	4.4	12.4	<1
Jul 91	1	<1	<1	9.0	<1
Aug 91	2	5.7	10.2	20.0	1.7

Appendix B

Table 27

Monthly Geometric Means For Kaelepulu Stream Location KS7

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	315.0	203.9	371.5	7.5
Oct 90	5	6.9	18.5	26.0	4.8
Nov 90	3	4.2	35.6	140.0	4.7
Dec 90	2	113.7	178.7	1037.2	20.2
Jan 91	2	147.2	584.8	1493.5	33.4
Feb 91	2	447.2	36.5	114.3	29.3
Mar 91	2	16.9	19.3	32.5	9.2
Apr 91	2	43.1	50.8	71.3	17.4
May 91	2	6.3	16.7	27.2	4.5
Jun 91	2	9.8	17.9	24.7	<1
Jul 91	1	13.0	9.0	21.0	<1
Aug 91	2	32.6	29.9	68.0	2.2

Table 28

Monthly Geometric Means For Kaelepulu Stream Location KS8

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	2897.7	6118.8	1877.9	88.4
Oct 90	5	133.4	182.3	236.0	12.7
Nov 90	3	623.4	1130.7	2361.8	26.8
Dec 90	2	166.7	219.4	797.8	13.3
Jan 91	1	2389.9	3092.2	3250.4	191.0
Feb 91	2	1000.0	680.0	1400.0	180.0
Mar 91	2	257.5	362.2	6644.9	43.7
Apr 91	2	175.6	199.6	241.9	26
May 91	2	23.3	27.9	38.4	8.0
Jun 91	2	27.0	31.0	53.8	4.0
Jul 91	1	24.0	36.0	41.0	2.0
Aug 91	2	33.9	52.8	84.1	6.0

Table 29

Monthly Geometric Means For Kaelepulu Stream Location KS9

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	1592.0	46.1	900.0	54.2
Oct 90	5	27.7	76.3	101.1	2.7
Nov 90	3	3.6	38.7	118.4	13.6
Dec 90	2	63.5	112.6	1402.9	7.8
Jan 91	2	1251.6	1348.2	2383.9	36.7
Feb 91	3	115.7	125.7	173.9	28.4
Mar 91	2	345.2	116.7	433.2	90.6
Apr 91	2	146.1	119.7	206.5	20.8
May 91	2	24.7	21.5	45.9	6.0
Jun 91	2	38.4	46.6	61.2	7.1
Jul 91	1	41.0	53.0	84.0	5.0
Aug 91	2	72.4	93.2	131.3	7.5

Table 30

Monthly Geometric Means For Kaelepulu Stream Location KS10

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	1628.5	1800.0	1400.0	34.4
Oct 90	5	47.6	168.8	175.3	7.3
Nov 90	3	118.63	176.4	319.8	13.4
Dec 90	2	501.8	568.9	1367.2	7.6
Jan 91	2	1025.3	1288.1	3573.0	309.8
Feb 91	2	346.9	454.3	516.3	51.2
Mar 91	2	121.6	142.0	106.9	80.8
Apr 91	2	84.1	175.5	146.0	10.6
May 91	2	85.8	113.7	134.9	2.8
Jun 91	2	38.0	49.8	73.0	4.4
Jul 91	1	43.0	69.0	136.0	7.0
Aug 91	2	100.8	103.9	175.8	6.2

Appendix B

Table 31

Monthly Geometric Means For Kaelepulu Stream Location KS11

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	3240.0	3400.0	2000.0	612.0
Oct 90	5	208.2	609.7	780.4	44.7
Nov 90	3	830.7	1261.8	1732.7	351.5
Dec 90	2	871.1	1235.9	1281.2	88.7
Jan 91	2	755.8	1085.9	2424.9	21.2
Feb 91	2	2712.9	2262.7	3532.7	247.7
Mar 91	3	1448.8	1799.7	2347.1	247.7
Apr 91	2	311.9	396.8	393.1	39.8
May 91	2	206.3	337.8	418.0	27.5
Jun 91	2	86.2	59.5	102.1	13.9
Jul 91	1	52.0	48.0	92.0	4.0
Aug 91	2	307.0	418.0	670.3	48.0

Table 32

Monthly Geometric Means For Kaelepulu Stream Location KS12

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	600.0	1800.0	1400.0	116.0
Oct 90	5	227.3	275.7	504.0	28.1
Nov 90	3	118.2	278.6	671.7	38.0
Dec 90	2	191.0	240.0	3914.3	56.9
Jan 91	2	3417.8	3242.7	2522.5	361.3
Feb 91	2	3768.1	4459.1	3321.9	402.3
Mar 91	3	8434.8	11638.6	10646.8	375.0
Apr 91	2	1877.9	2423.9	3008.8	25.9
May 91	2	281.7	536.4	727.0	53.4
Jun 91	2	44.9	57.1	124.0	17.9
Jul 91	1	28.0	76.0	144.0	12.0
Aug 91	2	458.9	58.2	931.9	36.7

Appendix B

Table 33

Monthly Geometric Means For Kaelepulu Stream Location KS13

Month	#of samples	*E. coli*	Fecal Coliform	Enterococci	*C. perfringens*
Sep 90	2	1668.5	2529.0	1916.2	22.4
Oct 90	5	46.0	138.9	254.9	4.8
Nov 90	3	126.2	204.9	475.9	9.2
Dec 90	2	204.4	429.3	2853.2	24.2
Jan 91	2	1819.9	1494.0	3255.5	67.8
Feb 91	2	735.4	716.9	1240	43
Mar 91	3	2747.3	3517.4	4844.5	198.7
Apr 91	2	717.7	861.6	1220.6	38.2
May 91	2	372.5	254.9	295.6	17.0
Jun 91	2	105.5	83.9	223.4	6.9
Jul 91	1	68.0	124.0	192.0	16.0
Aug 91	8	390.7	496.5	796.9	40.4

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