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[12]Abstract (Purpose, method, results, conclusions)

Fecal streptococcus (FS) is the most often used alternative to fecal coliforms to assess the quality of recreational water. Because the reliability of KF agar to recover FS bacteria has been reported to approach 100%, this medium was used to test the water quality of Hanauma Bay which was suspected as the source of disease transmission to a group of swimmers using this beach park. Marine water samples from Hanauma Bay, Oahu, were characterized by low concentrations of fecal coliforms and Clostridium perfringens but unusually high concentrations of presumptive FS when KF agar was used. Most of the presumptive FS colonies on KF agar could not be verified and was therefore concluded to be "false positive". At least two types of catalase-positive bacteria were determined to be responsible for the formation of false-positive colonies on KF agar: one, a gram-positive coccus; the other, a gram-negative, NaCl-requiring bacillus. These false-positive, FS-like bacteria were present in marine recreational waters obtained from 15 other sites, although at lower concentrations than in Hanauma Bay. The presumptive FS counts on KF agar ranged from 53 to 1205/100 ml for the 15 marine sites. In contrast, less than 20 presumptive enterococcus (EC) colonies were recovered and readily confirmed as true enterococci when mEnterococcus agar was used to assay these samples. Thus in tropical climates such as Hawaii, KF agar and its recommended technique should not be used to assay marine waters for FS. Three modifications in the KF agar technique could however prevent the bacteria present in marine waters from producing false-positive FS colonies on KF agar: delete NaCl from KF agar, increase sodium azide concentrations in KF agar, or incubate KF agar anaerobically rather than aerobically.

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RECOVERY OF FALSE POSITIVE FECAL STREPTOCOCCUS ON KF AGAR FROM MARINE RECREATIONAL WATERS

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ABSTRACT

Fecal streptococcus (FS) is the most often used alternative to fecal coliforms to assess the quality of recreational water. Because the reliability of KF agar to recover FS bacteria has been reported to approach 100%, this medium was used to test the water quality of Hanauma Bay which was suspected as the source of disease transmission to a group of swimmers using this beach park.

Marine water samples from Hanauma Bay, O'ahu, were characterized by low concentrations of fecal coliforms and Clostridium perfringens but unusually high concentrations of presumptive FS when KF agar was used. Most of the presumptive FS colonies on KF agar could not be verified and was therefore concluded to be "false positive". At least two types of catalase-positive bacteria were determined to be responsible for the formation of false-positive colonies on KF agar: one, a gram-positive coccus; the other, a gram-negative, NaCl-requiring bacillus. These false-positive, FS-like bacteria were present in marine recreational waters obtained from 15 other sites, although at lower concentrations than in Hanauma Bay. The presumptive FS counts on KF agar ranged from 53 to 1205/100 ml for the 15 marine sites. In contrast, less than 20 presumptive enterococcus colonies were recovered and readily confirmed as true enterococci when mEnterococcus agar was used to assay these samples.

Thus in tropical climates such as Hawai'i, KF agar and its recommended technique should not be used to assay marine waters for FS. Three modifications in the KF agar technique could however prevent the bacteria present in marine waters from producing false-positive FS colonies on KF agar: delete NaCl from KF agar, increase sodium azide concentrations in KF agar, or incubate KF agar anaerobically rather than aerobically.

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INTRODUCTION

Water Quality Standards for Recreational Waters

The prevention, control, and abatement of water pollution in the state of Hawai'i are regulated by water quality standards specified in the Hawaii Revised Statutes (State of Hawaii 1968, §§342-31, 342-32) and in the Hawaii State Department of Health Public Health Regulations (rev. 1982, chap. 54), as well as statutes of the Federal Water Pollution Control Act, as amended. Water quality standards are established for specific uses. Thus, the monitoring of the quality of marine waters used for recreation (swimming, surfing, skin diving) is for the protection of users who could contract diseases after ingesting microbial pathogens that might be present in the water. Microbial pathogens originate in the feces of man and warm-blooded animals and can be transmitted in water. Thus, these bacteria are called fecal-borne pathogens and the illness they cause are called waterborne diseases. Table 1 lists these microbial pathogens and the disease symptoms observed in man. Two things should be noted in this table: (1) the pathogens include three major groups of microorganisms (bacteria, viruses, protozoans); and (2) the diseases observed in man are not restricted to the intestinal tract but may also affect the heart muscle, liver, nervous system, eyes, and respiratory tract.

The logical approach in determining whether recreational waters are suitable (hygienically safe) for aquatic activities is to detect whether pathogens are present in the water. This approach was quickly determined to be unfeasible with the discovery of many different types of pathogens that required specific methods suited for their detection. Many of the techniques were too time consuming, too complicated, and inadequately sensitive. Also, for some pathogens, such as hepatitis A virus, no laboratory methodology is as yet available for their detection.

A practical approach was taken to determine whether the water was contaminated with feces, rather than any of the specific pathogens, based on the principle that any water contaminated with feces may contain pathogens. Also, the greater the fecal contamination, the greater the potential for the presence of fecal-borne pathogens. The detection of fecal bacteria was subsequently determined to be much more sensitive than any chemical means for detecting feces diluted in water. Thus, fecal bacteria served as indicators for the presence of feces in water and were used to assess the

TABLE 1. ENTERIC PATHOGENS IMPLICATED IN TRANSMITTING WATERBORNE DISEASES

Pathogen	Disease or Symptom
BACTERIAL	
Salmonella typhi	Typhoid fever
Salmonella spp.	Gastroenteritis, enteric fever
Shigella dysenteriae	Bacterial dysentery
Shigella spp.	Gastroenteritis
Vibrio cholera	Cholera
Yersinia enterocolitica	Gastroenteritis
Campglobacter fetus	Enteritis
VIRAL	
Hepatitis A	Infectious hepatitis
Poliovirus	Poliomyelitis, meningitis; paralysis
Rotavirus	Infantile gastroenteritis
Adenovirus	Respiratory disease, eye infection
Coxsackie, Echo- and Reoviruses	Aseptic meningitis, myocarditis, respiratory disease; fever, rash, paralysis
PROTOZOAN	
Entamoeba histolytica	Amoebic dysentery
Giardia lamblia	Giardiasis
Balantidium coli	Dysentery, intestinal ulcers

hygienic quality of recreational marine waters. Four criteria established to select the fecal microorganism to be used as an indicator of water quality included the following characteristics:

1. Should be present and occur in much greater numbers than the pathogens concerned
2. Should not proliferate to any greater extent than enteric pathogens in the aquatic environment
3. Should be more resistant to disinfectants and to the aquatic environment than pathogens
4. Should yield characteristic and simple reactions enabling, as much as possible, an unambiguous identification of the microorganism group.

Based on these criteria and on the literature available since 1880,

the coliform group of bacteria was selected as the indicator of feces in water and was used to assess the hygienic quality of water for drinking and, consequently, for marine (and inland) recreational waters. In the early 1900s water quality standards for recreation were under the jurisdiction of the individual states and therefore varied from state to state. In 1944 U.S. bathing water quality standards were based on total coliform counts ranging from 50 to 2400/100 ml. In subsequent years it was apparent that many of the total coliforms recovered in environmental waters were not of fecal origin, but from soils and plants (Geldreich 1976; Hoadly and Dutka 1977). In 1968 the Federal Water Pollution Control Administration (FWPCA) recommended that fecal coliforms, which are more specifically related to feces from warm-blooded animals, should be used instead of total coliforms to assess recreational water quality (National Technical Advisory Committee 1968). Based on the total coliform to fecal coliform ratio of 5:1, the FWPCA recommendation for recreational waters stated:

> The fecal coliform content of primary contact recreational waters shall not exceed a log mean of 200/100 ml nor shall more than 10 percent of total samples during any 30-day period exceed 400/100 ml.

Since that time, this recommendation has been adopted by federal and state regulations. Thus, the recreational water quality standards for the state of Hawaii (Department of Health 1982) are as follows:

> §11-54-08 Specific criteria for recreational areas. In inland recreational waters and marine recreational waters within 1,000 feet of the shoreline, including natural public bathing areas:
>
> (a) Fecal coliform content shall not exceed a geometric mean of 200 per 100 ml in 10 or more samples collected during any 30-day period and not more than 10% of the samples shall exceed 400 per ml in the same period.
>
> (b) Raw or inadequately treated sewage or other pollutants of public health significance, as determined by the director of health, shall not be present in natural public bathing or wading area. [Eff. Nov. 12, 1982](Auth: HRS §§342-3, 342-32)(Imp: HRS §§342-3, 342-32)

Acknowledged Problems with Coliform Standards

Despite years of using coliforms to assess the quality of recreational waters, evidence has not been obtained demonstrating that high concentrations of coliforms in recreational waters will result in increased inci-

dences of diseases to swimmers, whereas low concentrations of coliforms will result in lower incidences of diseases. Also, epidemics of waterborne diseases have been reported in which the suspect water contained acceptably low concentrations of coliforms (Craun 1978). In other studies conducted in Hawai'i (Loh, Fujioka, and Lau 1979), and elsewhere (Goyal, Gerba, and Melnick 1978), pathogens such as human enteric viruses have been recovered from marine recreational waters deemed safe based on low fecal coliform recoveries. These results have led many prominent scientists to question the reliability of using coliforms as indicators of water quality and to reassess recent data on coliforms based on the four criteria by which fecal coliforms were initially selected as water quality indicators. Of the four criteria, listed earlier, fecal coliform fulfills criteria one and four, but fails to fulfill criteria two and three under all conditions. Specifically, fecal coliforms are known to multiply under some nutrient-rich environmental conditions and, as a result, may indicate more fecal contamination than is actually present. A more serious limitation of coliform bacteria is their relative instability to disinfectants, such as chlorine, or to natural environmental conditions, such as marine waters, as compared with pathogens such as viruses and protozoans. Fujioka and Loh (1978) verified this limitation of coliform bacteria by recovering infectious human enteric viruses from sewage effluent which had been chlorinated to reduce the levels of coliforms well below 100/100 ml. In another study Fujioka et al. (1981) showed that high concentrations of coliforms suspended in marine waters and exposed to sunlight are reduced to negligible levels in less than 1 hr, whereas pathogenic viruses will persist under these same conditions. Based on these results, we concluded that coliforms are unreliable indicators of water quality, especially for marine recreational waters.

Alternative Indicators of Water Quality

The accumulated evidence that coliforms may not be reliable indicators of water quality has initiated an active search to find a more reliable, alternative indicator. A conference to address this problem was sponsored by the American Society for Testing and Materials and the proceedings published in Bacterial Indicators/Health Hazard Associated with Water (Hoadley and Dutka 1977). Another workshop addressing this problem

was sponsored by the National Academy of Sciences and the proceedings published in Bacterial Indicators of Pollution (Pipes 1982). Based on the studies reported in these two books, the list of viable alternative indicators of fecal pollution include Escherichia coli, fecal streptococcus, enterococcus, Clostridium perfringens, bifidobacteria, bacteriophage, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Of these microorganisms, S. aureus, P. aeruginosa, and C. albicans are not true enteric microorganisms since they do not normally multiply in the intestinal tract of man. Thus, these microorganisms fail in the most basic criteria for indicator bacteria: their presence does not necessarily indicate the presence of fecal matter. Bacteriophages or viruses of E. coli bacteria are direct indicators of fecal pollution. However, these viruses can multiply in E. coli cells under environmental conditions. Thus, their concentrations may not be related to the concentrations of fecal matter in the water environment.

The group of bacteria which are the true normal inhabitants of the intestinal tract of man and animals include: total coliforms; fecal coliforms, of which E. coli is the primary member; fecal streptococcus or enterococcus, of which Streptococcus faecalis is the primary member; bifidobacteria; and Clostridium perfringens. The density, source, and relative stability of these fecal bacteria are summarized in Table 2. As shown in this table, bifidobacteria are found in the highest concentration and do not appear to have an extrafecal source. However, these bacteria are strict anaerobes and are unstable in environmental waters. Also, no reliable method exists to recover and identify bifidobacteria from sewage and natural waters containing many types of bacteria. The usefulness of bifidobacteria appears to be its unambiguous relationship to fecal matter. However, because it is very unstable, the concentrations of bifidobacteria cannot be related to the presence of enteric pathogens which are much more stable in environmental waters.

Of the many proposed alternative indicators of water quality, more studies have been conducted using fecal streptococcus (FS) than any other microorganism. Fecal streptococcus is broadly used to include all species of the genus Streptococcus which is normally present in animal feces. Similar to coliforms, the concentrations of fecal streptococcus in feces are consistently high and methods for their recovery and enumeration have

TABLE 2. FECAL INDICATOR BACTERIA

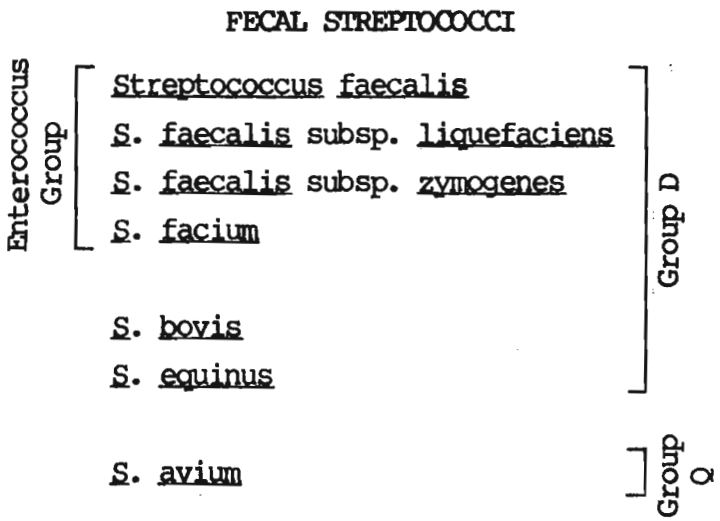
INDICATOR BACTERIA	LOG_{10} DENSITY				RELATIVE SURVIVAL	EXTRA-FECAL SOURCES
	Human	Animal	Influent	Cl*		
	---(Feces/g)---		(Sewage/100 ml)			
Bifidobacteria	7-8	1	7	<1	+	None
Total coliform	6-7	4-6	6-7	<1	++	Enterobacter and Citrobacter from uncontaminated soil; Klebsiella from vegetation and industrial effluent
Fecal coliform	6-7	4-6	6-7	<1	++	Klebsiella from vegetation and industrial effluent
E. coli	6-7	4-6	6-7	<1	++	None
Enterococci (S. faecalis, S. faecium)	3-4	2-3	5	1	+++	S. faecalis biotypes from insects and vegetation
C. perfringens (spores)	3-4	2-3	4	4	++++	Spore collection in soil and sediment with possible multiplication

SOURCE: Adapted from Cabelli (1979).
*Chlorinated.

been reported to be reliable and feasible. Thus, water contaminated with FS would also indicate that the water was contaminated with fecal matter. Geldreich (1976) determined that human fecal waste contains higher concentrations of FC than FS, whereas animal fecal waste is characterized by higher concentrations of FS and FC. Based on these results, Geldreich proposed that streams with FC:FS ratios of 4 or greater indicate the presence of human fecal waste, whereas streams with FC:FS ratios of less than 0.7 indicate contamination with animal fecal waste. The determination that a body of water was contaminated with human or animal wastes is critical for two reasons. First, it may be used to identify the source of contamination, such as an improperly operating sewage treatment plant, a leaking septic tank, or runoff from domestic animal operations, such as cattle, dairy or piggery. Second, natural waters contaminated with human feces are potentially more dangerous to humans than waters contaminated with animal feces because many human diseases, such as all enteric virus infections and most parasitic infections as well as many bacterial infections (shigellosis, cholera), are diseases transmitted only from man to man.

The term FS was originally established to identify those species of the genus Streptococcus which normally multiply in the intestinal tract of man and warm-blooded animals. The streptococci which typically fulfill this definition are Streptococcus faecalis, S. faecalis subsp. liquefaciens, S. faecalis subsp. zymogenes, S. faecium, S. bovis, S. equinus, and S. avium (see Fig. 1). It is significant that all the fecal streptococci contain a specific group D antigen which can be easily detected by an immunological precipitation test. This is currently one of the basic tests for the identification of a fecal streptococcus bacterium. S. avium should be noted as also containing the group D antigen; however, because it also contains another group Q antigen, S. avium is also classified as a group Q streptococcus. The biochemical definition of fecal streptococci are "gram positive cocci", usually in chains or in pairs that are catalase negative, facultative anaerobes which can grow at 37° and up to 45°C.

Enterococcus is a subgroup of fecal streptococci and includes only those fecal streptococci that are more commonly found in human feces (e.g., S. faecalis, S. faecium) and that are much more stable in natural water environments than are the other fecal streptococci. Enterococci, which also contain group D antigens, are defined as those fecal streptococci



SOURCE: APHA, AWWA, WPCF (1980).

Figure 1. Diagram of subgroups within fecal streptococcus group

which can grow at 10°C in 6.5% NaCl and at pH 9.5. Because of their greater specificity to human feces, their greater stability in environmental waters and better correlation to disease incidences among users of recreational waters, Cabelli (1983) and Dufour (1982) have recently proposed the use of enterococci as indicators of recreational water quality.

Clostridium perfringens is an anaerobic spore-forming bacterium which normally inhabits the intestinal tract of warm-blooded animals and man. Thus, the recovery of C. perfringens from natural waters, indicates that the environment has been contaminated with fecal matter. In Europe C. perfringens is used as an indicator of water quality. However, in the U.S., C. perfringens has not been accepted as an indicator of water quality for two major reasons. First, the spores of C. perfringens are known to be very stable, and, thus, the recovery of this organism does not differentiate between a past or recent source of fecal pollution. Second, the methods used to recover this anaerobic bacteria have been neither reliable enough nor simple enough for the capabilities of most laboratories. However, a relatively simple membrane filtration method for the recovery of C. perfringens from wastewaters was recently reported by Bisson and Cabelli (1979). In an independent WRRC study, Fujioka and Shizumura (1983) determined that the reported methodology for the recovery of C. perfringens is

not only reliable and practical, but that concentrations of C. perfringens in stream water can be better correlated with the presence of sewage effluent than FC or FS. On a theoretical basis, the use of C. perfringens can be especially helpful in situations where the suspected water environment is not normally polluted but becomes contaminated only sporadically with fecal material.

Closing of Hanauma Bay Beach Park

On 16 May 1982 a group of ten people picnicked at Hanauma Bay Beach Park. One member of this group developed diarrhea and was diagnosed by a Honolulu physician who suspected that the patient contracted the disease when swimming at Hanauma Bay. It was subsequently reported but not confirmed that six of the ten members in the group suffered diarrheal symptoms. The physician informed the Hawaii State Department of Health that Hanauma Bay waters may have been contaminated with sewage-borne pathogens. The Hawaii State Department of Health and the City and County of Honolulu, Division of Wastewater Management responded by immediately conducting a sanitary survey of the area and analyzing samples of beach water for the presence of fecal coliforms as prescribed by current public health regulations. The initial water samples contained very low levels of fecal coliforms, indicating that the beach water was not contaminated with sewage. However, the sanitary survey revealed that one of the cesspools in the park had overflowed and may have contaminated the swimming area. Mayor Anderson closed the beach park to the public on 1 June 1982. During the time the park was closed, more beach water samples were tested and the suspected cesspool was pumped out and disconnected for further use. All beach water samples tested contained consistently low concentrations of fecal coliforms, indicating that the beach water was not contaminated with sewage. Hanauma Bay was reopened to the public on 10 June 1982. The contaminant source which apparently affected 6 of 10 members who picnicked at Hanauma Bay was never determined. It should be noted that the possibility that contamination of the food the picnickers brought was never ruled out and may have been a more likely source since there were no other reports of illnesses among other swimmers who used the beach on the same day.

Identification of a Problem in Assessing Hygienic Quality of Hanauma Bay Beach Water

Based on current federal and state regulations, the hygienic quality of recreational waters is determined by the concentrations of fecal coliform in that water. Using this criteria, the Hawaii State Department of Health (DOH) and the City and County of Honolulu Division of Wastewater Management (DWM) concluded that the beach water at Hanauma Bay was not contaminated with fecal matter. However, based on earlier recommendations by the Water Resources Research Center (WRRC), the DWM also analyzed beach water samples for fecal streptococcus, an alternative and more stable indicator of fecal contamination. The results of these analyses demonstrated that although the concentrations of FC in these water samples were consistently low (0-3 CFU/100 ml), the concentrations of FS in these same water samples were sporadic (0-30,000 CFU/100 ml) and often exceeded 1000 CFU/100 ml. Thus, the low FC concentrations indicated that the beach water was not contaminated with sewage, whereas the high concentrations of FS indicated that the beach water was contaminated with sewage. WRRC was consulted to resolve this dilemma.

Carefully following the procedures outlined in Standard Methods (APHA, AWWA, and WPCF 1980), the WRRC laboratory obtained split samples of Hanauma Bay beach water from the City and County of Honolulu and analyzed these samples for FC and FS. The results confirmed that Hanauma Bay beach water samples contained low concentrations of FC but relatively high concentrations of FS. Standard Methods (APHA, AWWA, and WPCF 1980, sec. 910-B) prescribes the use and reliability of KF agar to recover FS from water samples:

> In examining samples from sources other than swimming pools, results reported to date indicate that practically 100% of the red and pink colonies growing on filters placed on KF agar are fecal streptococci.

However, the characteristic red colonies observed on KF agar must be considered presumptively positive and additional tests prescribed in Standard Methods must be completed to confirm that the growth on the KF agar is true fecal streptococcus bacteria. In confirmation tests run by the University of Hawaii at Manoa, Water Resources Research Center, most of the characteristic red colonies observed on KF agar could not be confirmed as true fecal streptococcus bacteria. A literature review revealed no other reports of

recovering high concentrations of false-positive FS when marine waters are analyzed using KF agar. These results indicated that the method recommended in Standard Methods was not reliable in recovering FS from Hanauma Bay water samples.

GOALS AND OBJECTIVES

The overall goal was to evaluate the reliability of the KF method prescribed in Standard Methods to recover fecal streptococci from Hawai'i marine waters as a means of assessing its water quality for recreation.

Some of the specific objectives are as follows:

1. Confirm our original observation that most of the presumptive FS colonies recovered on KF agar from beach waters taken from Hanauma Bay are not true FS bacteria but, instead, false presumptive FS, "false FS"
2. Determine the expected levels of these false-FS bacteria in other recreational beaches, open ocean, harbor waters, estuarine waters, and fresh stream waters
3. Correlate the concentrations of false-FS bacteria in marine waters with that of other water quality indicators, such as fecal coliforms, true fecal streptococcus, enterococcus, and Clostridium perfringens
4. Determine the physical, chemical, and biological properties of false-FS bacteria so they can be identified and their role in water evaluated
5. Assess the public health significance of false-FS bacteria and their role in evaluating the quality of recreational marine waters
6. Determine the best method to differentiate true-FS from false-FS bacteria in marine waters.

MATERIALS AND METHODS

Methodology

The membrane filtration technique as described in Standard Methods (APHA, AWWA, and WPCF 1980) for the recovery of FC with MFC agar, FS with

KF streptococcus agar, and EC bacteria with M-Ent agar was used to enumerate the presumptive concentrations of fecal bacteria in selected marine water samples. The focus of this study was to explain the high presumptive counts obtained on KF agar as compared with M-Ent agar and to determine the basis for this observed difference. The chemical composition of the two media is significantly different as shown in Table 3. Other more recently devised media to recover enterococcus bacteria, such as Pfizer selective enterococcus (PSE) agar (Daoust and Litsky 1975) and Gentamycin-Thallous carbonate agar (Donnelly and Hartman 1978), were also used. The presumptive positive colonies observed on KF and M-Ent agar were confirmed using the procedures described in Standard Methods. In addition, the group D antigen associated with all true FS and enterococcus bacteria were analyzed using the Phadebact (Pharmacia Diagnostics, Piscataway, New Jersey) streptococcus coaggulutination test. Water samples were also analyzed using the MCP medium of Bisson and Cabelli (1979) for concentrations of Clostridium perfringens, a fecal-borne bacterium that was selected because of its unusual stability in environmental waters. Thus, samples of beach water or sediments which are negative for this bacterium are strongly indicative

TABLE 3. KF STREPTOCOCCUS AND M-ENTEROCOCCUS AGAR COMPOSITION PER LITER OF WATER

MEDIA COMPOSITION*	AGAR	
	KF	M-Ent
	(g)	
Proteose Peptone No. 3	10.0	...
Yeast Extract	10.0	5.0
Sodium Chloride	5.0	...
Sodium Glycerophosphate	10.0	...
Maltose	20.0	...
Lactose	1.0	...
Sodium Azide	0.4	0.4
Tetrazolium Chloride	0.1	0.1
Agar	20.0	10.0
Tryptose	....	20.0
Dextrose	....	2.0
Dipotassium Phosphate	....	4.0

*Per liter of water.

that this beach is not and has not been polluted with fecal matter. The API 20E or 20S identification kits (Analylab Products, Plainview, New York) were used to identify isolates of bacteria.

Sampling Sites

Most of the study was conducted in Hanauma Bay Beach Park since the problem of high presumptive FS was first detected in this area and the water quality of this beach has a great impact on public health. For this study, Hanauma Bay was divided into east, middle, and west sectors (Fig. 2). The seawater circulation pattern at Hanauma Bay is generally from east to west; that is, most of the open seawater entering the bay is through the eastern sector and most of the water leaving the bay is through the western sector. Water circulation in the middle sector, inside the reef where most of the public swimming takes place, is poorest and this water tends to move toward the western sector. Emphasis was placed on analyzing waters from the middle sector since most of the swimming as well as land runoff was greatest in this sector. Figure 2 shows the location of the concession stand, the restrooms, cesspools, ditch leading to the beach, and the three sites where sand samples were taken for analysis. Analysis of water samples from east and west sectors were compared with that taken from the middle sector to enable conclusions regarding the entire bay. For comparative purposes, water samples were also taken from all major coastal areas (beaches, harbors, bays) of O'ahu, Hawai'i, including open ocean sites (see Fig. 3).

RESULTS AND DISCUSSION

Analysis of Hanauma Bay Waters

Because of a local physician's allegations that Hanauma Bay Beach Park may have been contaminated, the entire park was closed to the public on 1 June 1982 (Fig. 4). On 3 June, the Water Resources Research Center obtained and analyzed its first sample of Hanauma Bay beach water for microbial indicators of fecal pollution. This sampling program was continued through 31 January 1984. For this study, Hanauma Bay was divided into three sectors (Fig. 2). The middle sector was considered the most

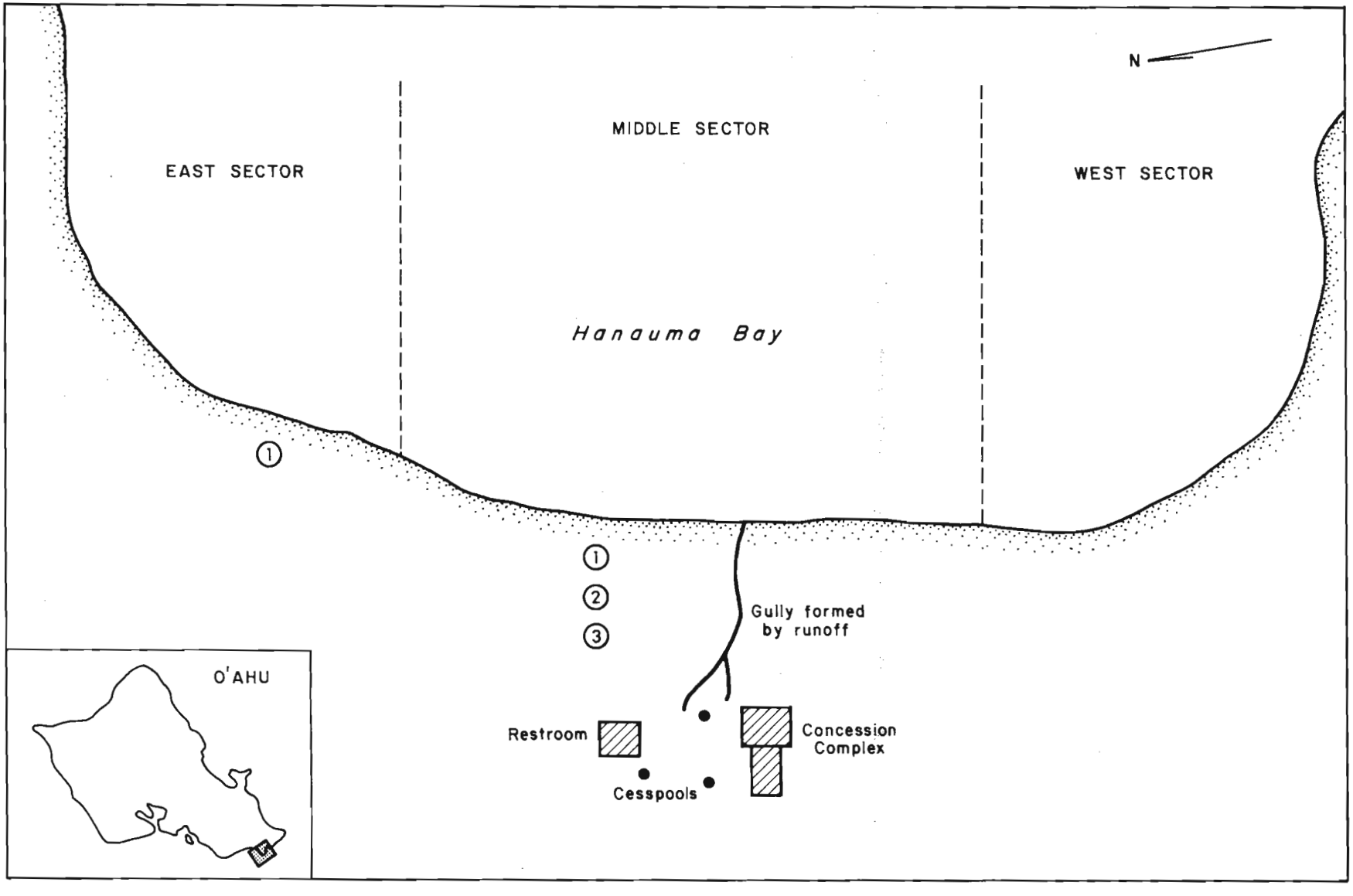


Figure 2. Schematic diagram of three sectors of Hanauma Bay Beach Park and sand sampling sites (1, 2, 3)

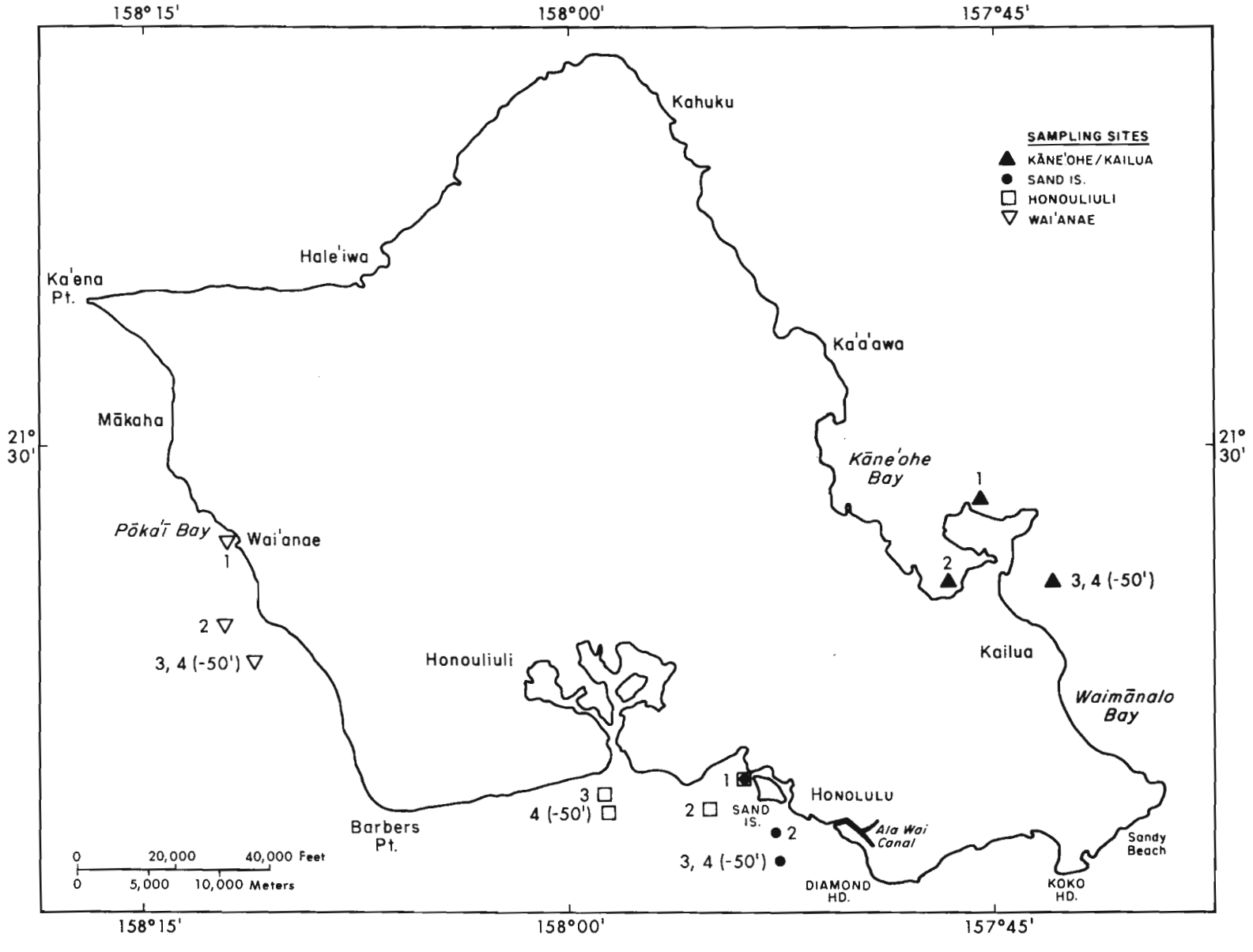
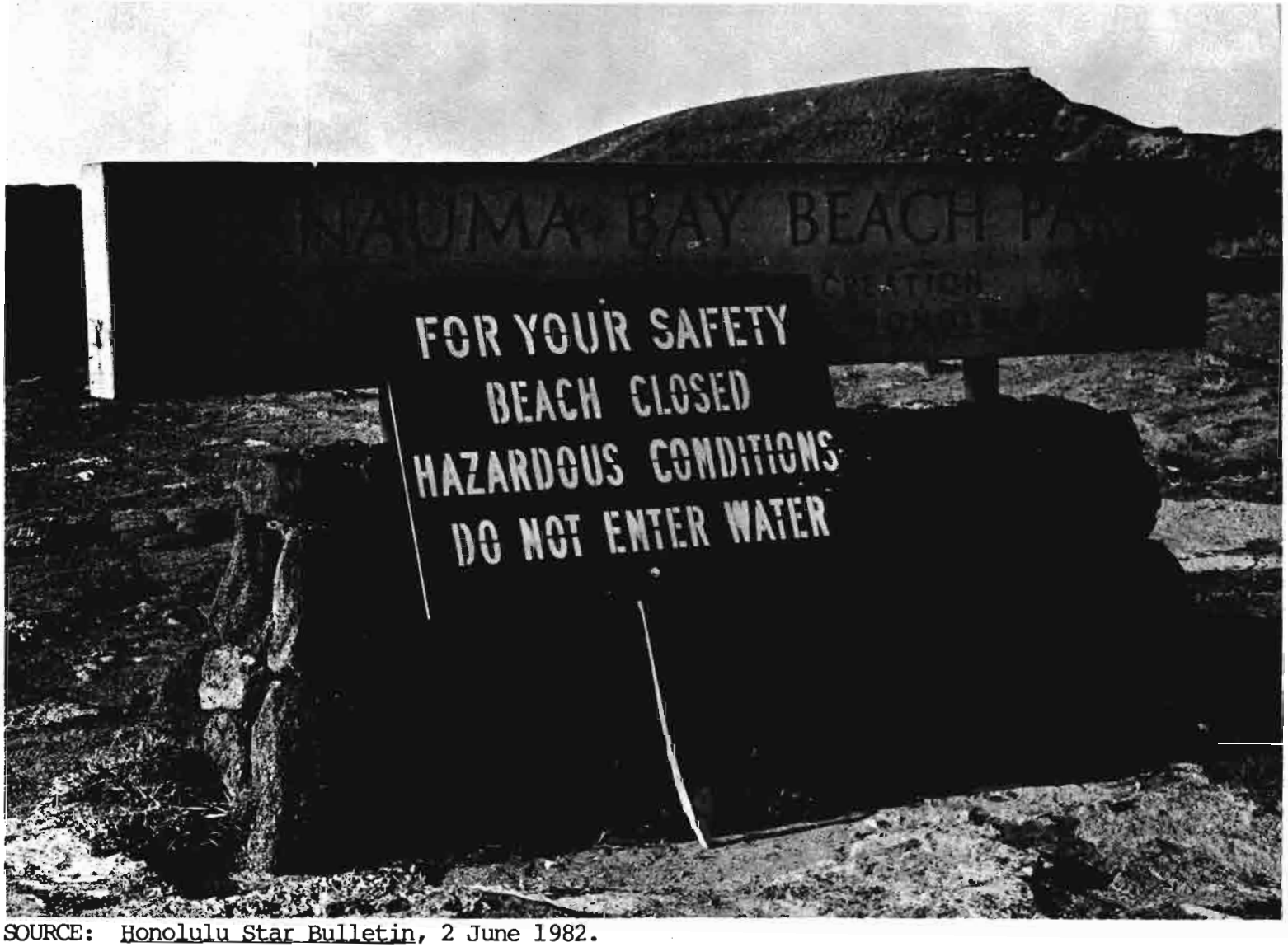


Figure 3. Location of Kāne'ohe, Sand Island, Honouliuli, Wai'anae ocean sites off O'ahu Hawai'i



SOURCE: Honolulu Star Bulletin, 2 June 1982.

Figure 4. Sign posted during closure of Hanauma Bay Beach Park, 1-10 June 1982

significant since it was in this area where most of the public swims, where overflow from the park's cesspool had entered the beach, and where water circulation is minimal. The two adjacent sectors (east and west) are characterized by better water circulation patterns and are less likely to be impacted by picnickers and runoff water from showers or from the cesspool.

Water samples from all sectors, but especially the middle sector, were analyzed for FC, FS, EC, and CP. The results of each individual assay and the mean for each sector are summarized in Table 4. Two significant factors should be considered before drawing conclusions based on the analysis of water samples taken from the three sectors in Hanauma Bay. First, many more samples were taken from the middle sector. Second, one sampling day (13 December 1983) was highly unusual because it had rained heavily just prior to sampling and water samples from only the middle sector were collected on this day. In addition, a new parking lot above the beach park was being constructed during this period and the recently excavated, loose soil was carried in surface runoff onto the beach during the rain. This was the only day in which the beach water was visibly turbid (brown) and the only sample in which the concentrations of FC (2600/100 ml) and CP (170/100 ml) were unusually high, indicating that land runoff is a major source of FC and CP. As a result the mean for all waters analyzed and the mean excluding the 13 December sample were calculated for the middle sector. The mean, excluding the 13 December value, was used to compare against the means for the other sectors and for making general conclusions about the water quality for the middle sector. The results (Table 4) show that the mean FC concentrations in the east (5 FC/100 ml), middle (71 FC/100 ml), and west (14 FC/100 ml) sectors were well below the current recreational water quality standard of 200 FC/100 ml. It should be noted that the Hawaii State Department of Health analyzed 25 water samples taken from the middle sector of Hanauma Bay during the 1982 calendar year and obtained a mean of 109 FC/100 ml. Thus, based on the analysis of beach water samples for FC the hygienic quality of Hanauma Bay can be considered excellent and the beach safe for swimming. This conclusion was substantiated by the low mean recoveries of 0 to 1 CP/100 ml from water samples obtained from the three sectors in the bay.

The same water samples analyzed for FS yielded consistently higher concentrations of presumptive FS in all samples but was especially higher

TABLE 4. CONCENTRATIONS OF FECAL COLIFORM (FC), FECAL STREPTOCOCCUS (FS), ENTEROCOCCUS (EC), AND *CLOSTRIDIUM PERFRINGENS* (CP) RECOVERED FROM HANAUMA BAY SAMPLES, O'AHU, HAWAI'I

Hanauma Bay	Date	FC	FS	EC	CP
		(CFU/100 ml)			
East Sector	06/03/82	0	141	-	0
	06/04/82	0	1,000	-	0
	06/08/82	0	2,770	-	0
	06/09/82	0	700	-	0
	06/22/82	1	200	4	-
	06/29/82	1	124	0	-
	06/30/82	-	45	-	-
	12/16/82	2	460	37	-
	01/27/83	14	610	25	-
	02/10/83	2	100	5	-
	08/08/83	34	1,493	287	-
	09/14/83	0	7,000	179	3
	09/21/83	0	2,890	1,020	0
	Mean	5	1,349	195	0.5
Middle Sector	06/03/82	2	2,630	—	2
	06/04/82	0	740	—	2
	06/08/82	0	3,622	—	0
	06/09/82	0	1,400	—	0
	06/22/82	1	470	34	-
	06/29/82	4	85	0	-
	06/30/82	-	660	0	-
	12/16/82	53	800	62	-
	01/05/83	90	3,100	69	-
	01/27/83	6	580	34	-
	02/10/83	10	250	11	-
	04/04/83	—	1,300	570	-
06/08/83	—	1,300	43	-	-
	07/13/83	770	1,700	1,900	0
	07/20/83	134	7,200	7,000	3
	07/27/83	145	13,000	3,100	0
	08/08/83	77	10,000	9,100	0
	08/29/83	59	5,000	4,000	-
	09/14/83	19	18,200	24,250	0
	09/21/83	1	1,160	590	1
	09/27/83	-	4,600	1,200	-
	10/24/83	80	680	220	0
	12/13/83*	2,600	5,800	1,300	170
	01/04/84	12	3,100	1,100	1
	01/17/84	11	540	88	3
	01/31/84	10	1,950	1,093	0
	Mean†	71	3,363	2,723	1
	Mean (Total)	186	3,456	2,655	16
West Sector	12/16/82	13	300	32	-
	01/27/83	29	910	60	-
	02/10/83	44	15,000	18	-
	08/08/83	0	305	100	-
	09/14/83	0	495	38	0
	09/21 83	0	1084	64	0
	Mean	14	3,016	52	0

*Abnormal sampling day (heavy rain, land runoff). †Excluding 12/13 sample.

in the middle and west sectors as compared to the east sector of the bay. The mean concentration was 1349 FS/100 ml in the east sector, 3016 FS/100 ml in the west sector, and 3363 FS/100 ml in the middle sector. Two conditional interpretations can be drawn from the data obtained. First, assume that the method of recovering FS from these waters is reliable. If this assumption is correct, the recovery of high concentrations of FS from all water samples indicate that the entire bay is significantly contaminated with fecal matter and is therefore unsuitable for swimming. Second, assume that the method of recovering FS from these waters is unreliable and the presumptive FS colonies are not due to true fecal streptococcus bacteria. If this assumption is correct, the presumptive concentrations of FS may be meaningless in assessing the hygienic quality of the water at Hanauma Bay. The question is then raised as to the source and significance of the bacteria producing false-positive colonies on KF agar, and to the reliability of using KF agar for assessing the quality of any marine water in Hawai'i.

Anticipating high presumptive concentrations of FS on KF agar, the same water samples were also assayed for EC, a subgroup of fecal streptococcus on a more restrictive growth medium, M-Ent agar. The results (Table 4) show that the concentrations of EC recovered on M-Ent agar were consistently lower than FS in nearly all samples. The mean concentrations of EC was significantly low in the east (195 EC/100 ml) and the west (52 EC/100 ml) sectors, but comparatively high (2655 EC/100 ml) in the middle sector of the bay. These results indicate that the concentrations of EC bacteria are much higher in the middle sector of the bay as compared with adjacent sectors. Although there are no established concentrations of FS or EC related to the quality of recreational waters, it is safe to assume that high quality recreational waters should not contain more FS or EC than the standard set for FC. Thus, it was assumed that recreational waters should not exceed 200 FS or EC per 100 ml of water. Based on this guideline and assuming that the M-Ent agar is reliable, the results indicate that the quality of water in the east and west sectors of Hanauma Bay is suitable for swimming but that the middle sector of the bay may be unsuitable for swimming.

Analysis of Hanauma Bay Sand

The results obtained showed that the waters of Hanauma Bay contain low levels of FC and CP. Yet after a heavy rain, the concentrations of these bacteria were dramatically elevated, indicating that runoff from soil is a major source of FC and CP entering marine waters. But the concentrations of FS and EC in the same beach water samples taken after the rain storm were not elevated, indicating that soil was not the source of these bacteria. The concentrations of FS throughout the bay and EC only in the middle sector were relatively high, suggesting that shoreline activities, such as picnicking and showering, may be the origin for these bacteria. If this is true, FS and EC may be multiplying in the sand and then being washed into the water during high tide.

To address this possibility, 10-g samples of surface sand obtained from the water's edge were assayed for FC, FS, and CP. The results (Table 5) show that from a single sand sample obtained from the east sector, 0 FC, 8 FS, and 5 CP per 100 g were recovered. From three separate sand samples obtained from the middle sector, 0 to 2 FC, 11 to 79 FS, and 8 to 33 CP per 100 g were recovered. The concentrations of FS recovered from the sand were too low to indicate that sand was the origin of this bacterium. However, since these samples were surface sand samples taken from the water's edge, extensive dilution could have greatly reduced the bacterial concentrations in these sand samples.

To determine whether the source of bacteria could be deeper in the sand, 6 in. (1.8 m) subsurface samples from the middle sector (see Fig. 2) were obtained from the water's edge (site 1), from 30 ft (9.14 m) inshore where picnickers sit (site 2), and farther inshore near the public restroom

TABLE 5. CONCENTRATIONS OF FECAL COLIFORM, FECAL STREPTOCOCCUS, AND *CLOSTRIDIUM PERFRINGENS* RECOVERED FROM SURFACE SAND SAMPLES TAKEN FROM WATER'S EDGE AT HANAUMA BAY BEACH PARK, 8 JUNE 1982

Hanauma Bay	Sample Description	Sampling Sites	FC (MPN/100 g of Sand)	FS	CP
East Sector	Water's edge; clean, wet, surface sand	1	0	8	5
Middle Sector	Water's edge; clean, wet, surface sand	1a	2	79	11
		2b	0	11	33
		3c	0	17	8

TABLE 6. CONCENTRATIONS OF FECAL COLIFORM, FECAL STREPTOCOCCUS, ENTEROCOCCUS, AND CLOSTRIDIUM PERFRINGENS RECOVERED FROM SUBSURFACE SAND SAMPLES TAKEN FROM MIDDLE SECTOR OF HANAUMA BAY BEACH PARK, OCTOBER 1983

Sampling Date	Sampling Site	Site Description	FC	FS	EC	CP
			(CFU/100 ml)			
10/14/83	1	Water's edge; 6-in. depth; wet, clean sand	0	837	11	0
10/21/83			0	150	1	0
10/14/83	2	Inshore; 10 yd from water's edge; 6-in. depth; moist, clean sand	0	9050	1	250
10/21/83			0	1000	0	32
10/14/83	3	Inshore; end of asphalt where shower water drains onto sand; 6-in. depth; moist sand mixed with dirt	800	8550	600	1250
10/21/83			160	3500	73	1900

where waters from the showers and restrooms drain into the sand (site 3). From each of these sites 10-g samples were mixed with 990 ml of sterile artificial seawater, mixed well, and 100 ml of the eluate assayed for FC, FS, EC, and CP by using the standard membrane filtration method. The results of two experiments (Table 6) show that FS was the only bacterium which was elevated in all three sites, although much higher in sites 2 and 3 than at site 1. On the other hand, the concentrations of FC, EC, and CP were recovered at significant levels from site 3, but not from sites 1 and 2.

A reasonable interpretation of these results are as follows:

1. Land activities, such as picnicking but especially showering and washing the restroom floors, are sources of fecal bacteria and nutrients discharged into the sand; thus high concentrations of fecal bacteria are concentrated in certain areas such as site 3
2. These fecal bacteria are transported to the ocean by surface or subsurface water movement, but are also undergoing inactivation
3. FC and EC are rapidly inactivated and therefore cannot be readily recovered from sites 1 and 2; CP is much more stable and was therefore recovered at significant levels from site 2 but not from site 1

4. The recovery of presumptive FS bacteria is probably not fecal bacteria but marine bacteria that can multiply in the sand, especially at site 3 where the nutrients are concentrated.

Analysis of Other Recreational Water Sites on O'ahu

The recovery of low concentrations of FC and CP, but high concentrations of FS, from all water samples was most unusual and raised the question as to the possible uniqueness of the water at Hanauma Bay. To address this question, near-shore water samples from all the major recreational sites on the island of O'ahu were similarly assessed for the various microbial indicators. The results of these analyses (Table 7) show that FC, EC, and CP were recovered at low concentrations from most recreational beaches. The high concentration of FC recovered from Sandy Beach is probably from the nearby sewage outfall. Streams draining into Ala Wai Canal are known sources of FC, FS, and EC bacteria entering this canal. The results show that presumptive FS bacteria can be recovered from all the coastal waters of O'ahu and the concentrations of FS does not correlate with that of other fecal indicator bacteria. Concentrations of FS also seem to increase in waters with higher nutrient load, suggesting that nutrient content controls the concentrations of the bacteria responsible for forming FS colonies on KF agar. To test this hypothesis it was reasoned that enclosed coastal waters, such as bays and harbors, contain relatively high nutrient levels associated with land runoff and that the nutrient load in seawater will decrease proportionately to increases in distance from the shore.

Water samples were obtained from the vicinity of a harbor dock, from 0.5 to 1.0 mile (804.5-1 609 m) offshore, and 2 miles (3 218 m) offshore from four coastal sites in the vicinity of ocean outfalls (Fig. 3, Table 8). These samples were assayed for FC, FS, and EC. The concentrations of FC and EC in these samples were generally low when compared to FS and the concentration of FS was highest in the harbor waters. The results support our preliminary conclusion that the presumptive FS colonies are due to natural marine bacteria and that the concentration of these bacteria in seawater is probably controlled by the nutrients present at a particular time in the water.

TABLE 7. CONCENTRATIONS OF FECAL COLIFORM, FECAL STREPTOCOCCUS, ENTEROCOCCUS, AND *CLOSTRIDIUM PERFRINGENS* RECOVERED FROM VARIOUS COASTAL WATERS OF O'AHU, HAWAI'I

Location	Date	FC	FS	EC	CP
		(CFU/100 ml)			
Sandy Beach	06/29/82	655	370	38	-
	01/05/83	960	570	38	-
	01/05/83	240	740	11	-
Koko Head	01/05/83	0	140	5	-
	09/27/83	-	120	2	-
Kailua Beach	07/13/83	1	480	7	3
	07/20/83	1	1205	16	3
	07/27/83	7	550	6	9
Waikīkī Beach	05/18/83	0	130	2	-
	07/13/83	8	60	0	2
	07/20/83	2	80	10	4
	07/27/83	5	94	2	1
Ala Wai Canal	10/19/82	1100	8300	——	-
	10/26/82	190	1000	——	-
	05/18/83	3500	2400	1400	-
Magic Island	05/18/83	3	290	4	-
Ala Moana Beach	05/18/83	390	290	110	-
	07/13/83	0	130	0	0
	07/20/83	26	290	12	6
	07/27/83	0	200	41	1
Kahe Point	07/22/83	1	210	1	2
Nānākuli Beach	07/22/83	0	250	0	0
Mā'ili Beach	07/22/83	0	113	1	0
Pōka'ī Bay	07/22/83	4	900	20	4
Wai'anae Boat Harbor	07/22/83	4	3000	8	4
Mākaha Beach	07/22/83	1	200	1	1
Yokohama Beach	07/22/83	1	150	2	0
Mokulē'ia Beach	07/29/83	0	690	14	0
Hale'iwa Beach	07/29/83	0	950	3	0
Waimea Beach	07/29/83	7	61	0	1
'Ehukai Beach	07/29/83	2	57	1	0
Sunset Beach	07/29/83	0	93	0	2

NOTE: See Figure 3 for sampling locations.

TABLE 8. CONCENTRATIONS OF FECAL COLIFORM, FECAL STREPTOCOCCUS, AND ENTEROCOCCUS RECOVERED FROM OPEN OCEAN WATER SAMPLES

Site Description	Coastal Location	FC	FS	EC
		(CFU/100 ml)		
Harbor area near boat ramp: 10-50 ft from shore	Honouliuli	31	34,000	45
	Kāne'ohe	10	65	4
	Sand Island	2	3,500	6
	Wai'anae	3	230	1
Open waters: 0.5-1.0 mile offshore	Honouliuli	100	42	20
	Kāne'ohe	1	37	2
	Sand Island	56	32	3
	Wai'anae	0	17	0
Open waters: 2 miles offshore	Honouliuli	1	610	540
	Kāne'ohe	0	20	0
	Sand Island	1	39	0
	Wai'anae	1	0	0
Open waters: 2 miles offshore, 50-ft depth	Honouliuli	1	220	7
	Kāne'ohe	0	91	0
	Sand Island	2	9,900	0
	Wai'anae	0	1	0

Confirmation of Presumptive Colonies

As stated earlier, analysis of marine water samples for FC, FS, EC, and CP by using the membrane filtration method results in presumptive identification and enumeration of these various bacteria. Although all of these methods have been previously determined to be reliable, the high concentrations of FS and occasionally of EC from all marine water samples, including open ocean water, suggest that all marine waters surrounding O'ahu are contaminated with fecal matter. This conclusion is highly unlikely and suggest that the methods used to recover FS and EC may be detecting false positives. To determine the reliability of the methods, presumptive FS colonies on KF agar and presumptive EC colonies on M-Ent agar were randomly picked and subjected to select confirmation tests as described in Standard Methods. The confirmation tests include purification of the isolates on BHI agar, testing these isolates for catalase reaction, growth in BHI broth at 45°C, as well as in BHI broth containing 4% bile at 37°C. Some of these isolates were also tested for the presence of the specific group D antigen which is present in FS and in EC bacteria. The results (Table 9) show that

most of the presumptive FS colonies recovered on KF agar were catalase positive, did not grow in BHI broth at 45°C or in bile broth at 37°C, and most did not contain the group D antigen. These results indicate that the high presumptive concentrations of FS recovered on KF agar were not due to true fecal streptococcus bacteria but instead to the growth of some other bacteria present in the water. These unidentified bacteria were given the name of false fecal streptococci (FFS). On the other hand, most of the presumptive EC colonies on M-Ent agar were catalase negative, grew in BHI broth at 45°C as well as in bile broth at 37°C, and contained the group D antigen. The excellent correlation of the group D antigenicity test with standard tests to confirm or disprove the presence of true FS or EC bacteria is significant because this test is rapid, specific, and has a distinct end point.

As a test on the specificity of the KF agar and the M-Ent agar to support the growth of true fecal streptococcus (FS) as opposed to false fecal streptococcus (FFS), randomly selected red and pink colonies recovered on KF agar were streaked onto M-Ent agar, incubated at 37°C, and observed for regrowth capability. Similarly, red and pink colonies originally recovered on M-Ent agar were streaked onto KF agar to observe their regrowth capabil-

TABLE 9. CONFIRMATION OF PRESUMPTIVELY POSITIVE COLONIES RECOVERED ON KF VERSUS M-ENT AGAR

Sampling Site	Recovery Medium	Catalase Reaction	Growth at 45°C and in Bile Broth	Group D Reaction
		(No. Positive/No. Tested)		
Hanauma Bay (East Sector)	KF agar	55/55	0/10	0/10
	M-Ent agar	0/3	2/3	1/2
Hanauma Bay (Middle Sector)	KF agar	44/55	2/20	0/10
	M-Ent agar	0/31	1/1	4/4
Sandy Beach	KF agar	48/55	5/7	1/10
	M-Ent agar	26/26	24/25	1/1
Sewage (Ala Moana)	KF agar	0/27	14/25	10/10
	M-Ent agar	30/30	----	3/3
S. faecalis (Control)	KF agar	0/1	1/1	1/1
	M-Ent agar	0/1	1/1	1/1

ity. The results (Table 10) show that most red and pink colonies recovered from beach water samples on KF agar were incapable of growing on M-Ent agar. In contrast, most of the pink and red colonies recovered on M-Ent agar from beach water or sewage samples were capable of growing on KF agar. For control, sewage samples known to contain high concentrations of true FS were assayed using KF agar and the red and pink colonies recovered were shown to be capable of growing on M-Ent agar. Based on these results it was concluded that false-positive levels of presumptive FS will be enumerated when KF agar, as prescribed in Standard Methods, is used to recover FS from the marine waters of Hawai'i. On the other hand, M-Ent agar appears to be selective enough to prevent the growth of most of these false-positive bacteria.

Characterization of Typical vs. Atypical Presumptively Positive Colonies on KF Agar

According to Standard Methods (APHA, AWWA, and WPCF 1980), all red to pink colonies observed on KF agar after a 48-hr incubation at 35°C should be counted as presumptive fecal streptococci. The same guideline is applicable when M-Ent agar is used and the size of the presumptively positive colonies ranges from 0.5 to 2 mm in diameter. At least two distinct morphological types of presumptively positive colonies were observed when marine waters were analyzed using KF agar. The first colony morphology which we called "typical" was a red to pink, slightly raised colony with well-defined borders. The second type of colonial morphology which we

TABLE 10. GROWTH OF PRESUMPTIVE FS ON M-ENT AGAR AND PRESUMPTIVE EC ON KF AGAR

Sampling Site	Isolation Medium	Regrowth Medium	No. Positive Growth / No. Isolate Tested
Hanauma Bay (East Sector)	KF agar	M-Ent agar	0/64
	M-Ent agar	KF agar	3/3
Hanauma Bay (Middle Sector)	KF agar	M-Ent agar	10/54
	M-Ent agar	KF agar	31/31
Sandy Beach	KF agar	M-Ent agar	6/61
	M-Ent agar	KF agar	26/26
Sewage (Ala Moana)	KF agar	M-Ent agar	21/24
	M-Ent agar	KF agar	30/30

called "atypical" was similar in color but more flattened and characterized by a distinct red halo surrounding the colony. Typical and atypical presumptively positive colonies were observed on KF agar, whereas only typical colonies were observed on M-Ent agar. In using typical and atypical colonies for confirmation tests during the initial study phase (Tables 9, 10), it was obvious—although not quantitated—that most of the atypical colonies could not be confirmed as true fecal streptococci, whereas only some of the typical colonies could not be confirmed. To quantitate this difference, typical colonies recovered from KF and M-Ent agar were randomly picked and purified. Atypical colonies on KF agar were similarly processed. These isolates were then gram stained and tested for group D antigen. The results (Table 11) show that 100% (14/14) of the typical colonies recovered on KF agar were gram-positive cocci but only 50% (7/14) possessed the group D antigen. In contrast none (0/11) of the atypical colonies recovered on KF agar were gram positive or possessed the group D antigen. These isolates were gram negative, very short rods. By contrast,

TABLE 11. CONFIRMATION OF TYPICAL AND ATYPICAL COLONIES RECOVERED ON KF VS. M-ENT AGAR

KF AGAR						M-ENT AGAR		
Typical Colonies			Atypical Colonies			Typical Colonies		
Isol. No.	Gram + Stain	Group D Antigen	Isol. No.	Gram + Stain	Group D Antigen	Isol. No.	Gram + Stain	Group D Antigen
1	+	+	1	-	-	1	+	+
2	+	-	2	-	-	2	+	+
3	+	-	3	-	-	3	+	+
4	+	+	4	-	-	4	+	+
5	+	+	5	-	-	5	+	+
6	+	-	6	-	-	6	+	+
7	+	-	7	-	-	7	+	+
8	+	+	8	-	-	8	+	+
9	+	-	9	-	-	9	+	+
10	+	-	10	-	-	10	+	+
11	+	-	11	-	-	11	+	+
12	+	+				12	+	+
13	+	+						
14	+	+						
	14/14	7/14		0/11	0/11		12/12	12/12

100% (12/12) of the typical colonies recovered on M-Ent agar were gram-positive cocci and possessed the group D antigen.

Isolates must be purified before they can be properly characterized. Typical colonies recovered on KF agar were readily subcultured on nutrient agar (NA) and on trypticase soy agar (TSA), whereas atypical colonies could be subcultured on TSA but not on NA. Examination of the ingredients of NA and TSA revealed that NA contains no added NaCl while TSA contains 0.5% NaCl. To determine the effect of NaCl on the growth of bacteria recovered on KF agar, NA and TSA were prepared with and without NaCl. Typical and atypical colonies recovered on KF agar were then subcultured on these media. The results (Table 12) show that typical colonies readily grow on NA with or without 2% NaCl and on TSA with or without 0.5% NaCl. On the other hand, the atypical colonies could not be cultured in NA and in TSA in the absence of NaCl but did grow when NaCl was added to these media. It should be noted that sodium ion (Na) is a specific growth requirement for marine bacteria. These results indicate that atypical bacteria are marine bacteria.

The presence of NaCl in bacteriological media is significant because most bacteria which grow in warm-blooded humans and animals require low levels of NaCl (0.8%), but cannot multiply in high concentrations of NaCl approaching that of seawater (3.5%). In this regard M-Ent agar is not supplemented with NaCl whereas KF agar contains 0.5% NaCl. To determine the significance of NaCl in recovering true- and false-positive FS on KF agar, KF agar was prepared with and without the normal concentration of 0.5% NaCl. Samples of seawater were then obtained from Hanauma Bay and from Sand Island and analyzed for concentrations of FC on mFC agar, FS on KF agar with and without NaCl, as well as M-Ent agar. The results (Table 13) show that the seven seawater samples contain low levels of FC

TABLE 12. EFFECT OF NaCl ON PASSAGE OF TYPICAL AND ATYPICAL COLONIES OBTAINED FROM KF AGAR

PRESUMPTIVELY POSITIVE COLONIES ON KF AGAR	GROWTH UPON PASSAGE ONTO			
	Nutrient Agar		Trypticase Soy Agar	
	0% NaCl	2% NaCl	0% NaCl	0.5% NaCl
Typical colonies	+	+	+	+
Atypical colonies	-	+	-	+

TABLE 13. RECOVERY OF PRESUMPTIVELY POSITIVE COLONIES ON STANDARD KF AGAR, KF AGAR WITHOUT SODIUM CHLORIDE, M-ENT AGAR, AND M-FC AGAR FROM OCEAN SAMPLES, O'AHU, HAWAI'I

SAMPLING AREA	SAMPLING SECTOR; SITE	FECAL COLIFORM m-FC Agar (CFU/100 ml)	FECAL STREPTOCOCCUS/ENTEROCOCCUS KF Agar	KF Agar NaCl (CFU/100 ml)	M-Ent Agar
Hanauma Bay (27 Jan. 83)	Middle	6	580	30	34
	East	14	610	30	25
	West	29	910	120	60
Sand Island (31 Jan. 83)	1	2	3500	4	6
	2	56	32	3	3
	3	1	39	0	0
	4	2	9900	0	0

and EC. On the other hand, the concentration of presumptive FS recovered on standard KF agar (with 0.5% NaCl) was very high as compared to the FS recovered on KF agar without NaCl. The concentration of FS recovered on KF agar without NaCl was similar to that recovered on M-Ent agar.

In conclusion, we have determined that in O'ahu's marine water at least two kinds of nonfecal streptococcus bacteria produce presumptively positive colonies on KF agar. One bacterium that produces atypical colonies on KF agar is a gram negative, NaCl requiring bacterium that is probably a marine bacterium. Its inability to grow on M-Ent agar is probably because NaCl is absent in this medium. Another catalase positive, gram positive coccus bacterium forms colonies indistinguishable from true fecal streptococcus bacteria.

Effect of Media Composition on Recovery of FS and EC from Seawater

The chemical composition of KF and M-Ent agar is different (Table 3) and therefore selects for the growth of different groups of fecal streptococci. One of the differences in these two media is NaCl which is present in KF but not in M-Ent agar. This difference was shown to determine whether a gram negative marine bacteria did or did not grow on these media. However, the major selective ingredient in KF and in M-Ent agar is 0.04%

sodium azide which, among other things, is known to inhibit catalase reaction in bacteria. The recovery of a catalase positive bacteria which forms presumptively positive colonies on KF agar suggests that the concentrations of sodium azide in KF agar may be too low.

To determine the role of sodium azide concentration in KF agar as well as the selectivity of other media used to recover EC, a beach-water sample obtained from the middle sector of Hanauma Bay was assayed using KF agar, KF agar containing twice the normal concentration of sodium azide, M-Ent, GTC (gentamycine-thallous-carbonate), and PSE (Pfizer selective enterococcus) media. The results (Table 14) show that 1300 presumptive FS/100 ml were recovered on standard KF agar and that this number was reduced to 720 FS/100 ml when the azide concentration in KF agar was doubled. Lower but still relatively high concentrations of 570 EC/100 ml were recovered on M-Ent agar, whereas 190 EC/100 ml were recovered on GTC and only 7 EC/100 ml on PSE agar. These results show that nonfecal streptococcus bacteria present in seawater have growth properties similar to fecal streptococcus and are therefore forming colonies similar to FS on media developed to selectively recover FS or EC. The large variation in the number of colonies resembling FS or EC when different selective media were used suggests that seawater contains many different kinds of bacteria and that the composition of the isolating media, especially inhibitors, determines which bacteria will be recovered. In this regard, the concentration of sodium azide may be too low in KF agar and even in M-Ent agar. PSE agar contains only 0.025% sodium azide but contains other inhibitors such as bile and esculin. GTC contains no sodium azide but uses selective antibiotics and esculin as inhibitors.

TABLE 14. SELECTIVE MEDIA FOR COMPARATIVE RECOVERY OF PRESUMPTIVE FS AND EC FROM HANAUMA BAY SAMPLES

Sample Origin	Selective Media Used	Presumptive FS/EC (CFU/100 ml)
Hanauma Bay (Middle Sector)	KF agar	1300
	KF agar + 2X azide	720
	M-Ent	570
	GTC	190
	PSE	7

Stability and Growth of False-Positive Bacteria in Seawater

The evidence indicated that marine bacteria are forming false-positive colonies on KF and M-Ent agar. Marine bacteria are normal components of seawater and most are not pathogenic to man; thus, it is of public health significance to show that marine bacteria are growing on media developed to recover human enteric bacteria. One established difference between marine and human fecal bacteria is the ability of marine but not fecal bacteria to multiply in seawater. This difference can be theoretically used to determine whether the bacteria which forms false-positive colonies on KF agar are marine bacteria.

To test this hypothesis seawater from Hanauma Bay and Koko Head were analyzed for presumptive concentrations of FS/EC on KF agar, KF agar containing twice the concentrations of azide, and on M-Ent, immediately after collection and after holding the water for 3 days at 25°C to allow marine bacteria to multiply. The results (Table 15) show that the concentrations of presumptive FS (4600 FS/100 ml) recovered on KF agar from Hanauma Bay water had not significantly changed (3200 FS/100 ml) after the 3-day incubation, which indicated that seawater was neither detrimental nor conducive to the growth of this population of bacteria. On the other hand, much lower concentrations of bacteria were recovered from this same pre-incubated water sample when KF agar containing twice the azide (66 FS/100 ml) or when M-Ent agar (1200 EC/100 ml) was used. After the seawater was incubated for 3 days at 25°C, these bacterial populations were sig-

TABLE 15. SELECTIVE MEDIA FOR COMPARATIVE RECOVERY OF PRESUMPTIVE FECAL STREPTOCOCCUS (FS) AND ENTEROCOCCUS (EC) FROM SEAWATER SAMPLES BEFORE AND AFTER 3-DAY INCUBATION AT 25°C

SAMPLE ORIGIN	SELECTIVE MEDIA USED	PRESUMPTIVE FS/EC	
		Pre-Incubation	Post-Incubation
		(CFU/100 ml)	
Hanauma Bay (Middle Sector)	KF agar	4600	3200
	KF agar + 2X azide	66	17
	M-Ent agar	1200	270
Koko Head	KF agar	120	4
	KF agar + 2X azide	0	0
	M-Ent agar	2	0

nificantly reduced indicating that seawater is detrimental to these bacteria. The results of the Koko Head water sample show a similar response (Table 15) although the concentrations of bacteria recovered from this sample were much lower. The drop in the population of bacteria in seawater after 3 days suggests that the bacterial populations recovered by KF agar containing twice the concentrations of azide and by M-Ent agar are not marine bacteria. On the other hand, no definite conclusion could be drawn based on the insignificant change in the population of bacteria in seawater incubated for three days and recovered by KF agar. This observation indicates that this population of bacteria is simply very stable in seawater or can multiply in seawater but is inhibited by a limiting factor such as nutrients.

As a further test of the hypothesis that seawater will support the growth of marine bacteria but not FS or EC, the same seawater samples from Hanauma Bay and Koko Head as well as from Kailua Beach were obtained and immediately analyzed using the KF and M-Ent agar. Portions of these waters were then supplemented with nutrients (0.01% and 0.1% peptone), inhibitors (azide), peptone plus azide, and incubated for 3 days at 25°C before the waters were again assayed using KF and M-Ent agar. The results (Table 16) show that after 3 days the population of presumptive FS colonies on KF agar which initially numbered 4600/100 ml was reduced to 3200/100 ml in unaltered seawater and to 190/100 ml in the presence of 0.04% azide. Yet when 0.01% peptone was added to seawater, 8.7×10^7/100 ml of presumptive FS were recovered on KF agar after 3 days of incubation at 25°C. The results clearly demonstrated that the bacteria in seawater which form colonies resembling FS on KF agar are capable of multiplying in seawater and were limited by the available nutrients in natural seawater. When nutrients and the inhibitor were added to the seawater, the concentration of recovered bacteria after 3 days was 1×10^7/100 ml, an indication that the inhibitor was overwhelmed by the growing population of bacteria. Similar results were obtained when M-Ent agar was used to analyze the same Hanauma Bay waters. Analysis of Kailua Beach and Koko Head waters showed similar results (Table 16).

In conclusion, bacteria which form false-positive colonies on KF agar and occasionally on M-Ent agar were shown to be capable of multiplying in

TABLE 16. GROWTH OF PRESUMPTIVE FS AND EC IN SEAWATER IN ABSENCE AND PRESENCE OF PEPTONE AND AZIDE

SEAWATER SOURCE	SUPPLEMENT TO WATER SAMPLE	KF AGAR		M-ENT AGAR	
		Pre-Incubation	Post-Incubation*	Pre-Incubation	Post-Incubation*
		(CFU/100 ml)			
Kailua Beach	None	2600	298	38	0
	0.01% peptone	....	7×10^7	—	0
	0.1% peptone	....	2×10^8	—	0
Hanauma Bay	None	4600	3200	1200	270
	0.01% peptone	....	8.7×10^7		6600
	0.01% peptone plus 1X azide	....	1.0×10^7		780
	1X azide	....	190		24
Koko Head	None	120	4	2	0
	0.01% peptone	...	1.9×10^8		1800
	0.01% peptone plus 1X azide	...	6.0×10^7		300
	1X azide	...	0		0

*3 days at 25°C.

seawater, especially when supplemented with nutrients. These results strongly indicate that marine bacteria are responsible for producing false-positive colonies on KF agar.

Identification of False-Positive Bacteria

Although evidence was obtained that marine bacteria are responsible for producing false-positive colonies on KF agar, these bacteria have not been identified. It should be emphasized that the identification of bacteria in marine waters has not been well characterized and as a result the normal flora of marine waters has not been established. However, studies already completed have shown that most marine bacteria are gram negative rods belonging to the following genera: Pseudomonas, Aeromonas, Vibrio, Flavobacter, Cytophaga, Achromobacter, and Alcaligenes. Reports of gram-positive coccus bacteria in marine waters have been reported, but these bacteria have not been well characterized. In contrast to marine bacteria, human bacteria have been well characterized and commercial iden-

tification kits are available to reliably identify these bacteria. The API 20E kit designed to identify gram-negative human enteric bacteria was used to identify the gram-negative, NaCl requiring bacteria which produced atypical colonies on KF agar. Most of these isolates were determined to belong to the genus Pseudomonas or Aeromonas. The API 20S kit designed to identify gram-positive cocci of the genus Streptococcus was used to identify the presumptively positive typical colonies on KF agar or M-Ent agar. The typical colonies, which were subsequently confirmed as true fecal streptococci, were readily identified as belonging to the genus Streptococcus and were further identified as S. faecalis, S. faecium, or S. durans. The typical colonies which could not be confirmed as true fecal streptococci yielded uncharacteristic biochemical profiles when the API 20S kit was used and thus could not be identified.

In conclusion, evidence was obtained to show that the bacteria responsible for the presumptively positive, atypical colonies on KF agar belong to the genus Pseudomonas and Aeromonas. Two types of bacteria produced presumptively positive, typical colonies on KF agar. Those which were subsequently confirmed as true fecal streptococci were readily identified as belonging to the genus Streptococcus (S. faecalis, S. faecium, S. durans). However, the gram positive coccus bacterium which could not be confirmed as a true fecal streptococcus could not be identified using the API 20S kit. As stated earlier, the API 20 kits were designed to identify human bacteria, thus, their inability to identify environmental bacteria is not unexpected.

ESTABLISHMENT OF METHODS TO RECOVER TRUE FS FROM MARINE WATER SAMPLES

The cumulative data from this study clearly show that bacteria naturally present in the marine waters of O'ahu will form colonies similar to FS on KF agar and thus invalidate the reliability of this technique. It is clear that a method to reliably measure the concentrations of true FS in the marine waters of Hawai'i is required. To address this problem, it was reasoned that marine bacteria naturally present in the coastal waters of Hawai'i are probably aerobic as compared to FS bacteria which are known to be facultative anaerobes. Thus, incubation under anaerobic conditions may

be a technique to selectively culture true FS bacteria and to suppress the growth of marine bacteria.

To test this hypothesis, samples of seawater were obtained from the three sectors of Hanauma Bay and assayed on KF agar and M-Ent agar under aerobic and anaerobic incubation at 37°C. In this experiment, two different lot numbers of KF agar manufactured by Difco, one from BBL, and one prepared in our laboratory from individual components called scratch KF were used. The results (Table 17) show that the recovery of presumptively positive colonies was consistently lower in all samples incubated under anaerobic conditions as compared to aerobic conditions. Presumptively positive, typical colonies were then picked from plates incubated under aerobic and anaerobic conditions and these isolates examined for gram reaction and catalase reaction. The results (Table 18) show that all 55 isolates recovered after aerobic incubation were gram-positive cocci but only 35% (19/55) were catalase negative, indicating again the growth of false-positive bacteria primarily on KF agar but to some degree on M-Ent agar. The commercially prepared KF agar recovered more false-positive bacteria than the scratch KF agar prepared in our laboratory, probably reflecting the active concentration of sodium azide in these media. When these same samples were incubated under anaerobic conditions, 100% (16/16) of the selected colonies recovered on any of the medium were gram-positive cocci and catalase negative, indicating that anaerobic conditions selectively inhibited the growth of the false-positive bacteria while allowing the growth of true FS. To confirm that false-positive bacteria will grow under aerobic but not under anaerobic conditions, while true FS will grow under aerobic and anaerobic conditions, five isolates of false-positive bacteria (gram-positive cocci, catalase positive) and true FS (gram-positive cocci, catalase negative) were grown in tryptic soy broth under aerobic conditions for three days at 25°C. These cultures were then assayed on KF agar under aerobic and anaerobic conditions. The results (Table 19) show that S. faecalis used as a control, as well as the true FS bacteria, were detected at concentrations of 10^8 to 10^9/ml under aerobic and anaerobic conditions, whereas the false-positive bacteria were detected at concentrations of 10^9/ml under aerobic conditions but could not be detected on KF agar when assayed under anaerobic conditions.

In conclusion, incubation of media developed to selectively recover

TABLE 17. RECOVERY OF TYPICAL COLONIES ON KF AND M-ENT AGAR MEDIA UNDER AEROBIC VS. ANAEROBIC INCUBATION AT 35°C

HANAUMA BAY SAMPLE SECTOR	ISOLATION MEDIA	INCUBATION	
		Aerobic	Anaerobic
		(CFU/100 ml)	
East	KF Difco 701241	100	10
	KF scratch	37	5
	KF Difco 672017	59	6
	KF BBL K5DMWO	30	3
	M-Ent Difco 640936	5	1
Middle	KF Difco 701241	250	10
	KF scratch	12	11
	KF Difco 672017	120	29
	DF BBL K5DMWO	36	17
	M-Ent Difco 640936	11	4
West	KF Difco 701241	1500	10
	KF scratch	61	19
	KF Difco 672017	1200	31
	KF BBL K5DMWO	480	20
	M-Ent Difco 640936	18	12

FS and EC bacteria under anaerobic rather than aerobic conditions will inhibit the growth of bacteria in marine waters from forming false-positive colonies on KF or M-Ent agar. This technique is therefore recommended when marine waters from Hawai'i are to be analyzed for fecal streptococcus or enterococcus bacteria by using KF agar or M-Ent agar.

SUMMARY

Based on a physician's report that the beach water may have been contaminated with fecal-borne pathogens, Hanauma Bay was closed to the public in June 1982. The Hawaii State Department of Health and the City and County of Honolulu Division of Wastewater Management analyzed numerous samples of beach water for fecal coliforms (FC) to determine the hygienic quality of the water. However, all beach water samples contained low levels of FC, indicating that the beach water was not contaminated with

TABLE 18. CONFIRMATION RATES OF TYPICAL COLONIES RECOVERED ON KF AND M-ENT AGAR MEDIA UNDER AEROBIC VS. ANAEROBIC CONDITIONS

ISOLATION MEDIA AND LOT NUMBER	AEROBIC INCUBATION		ANAEROBIC INCUBATION	
	No. Gram Pos. Cocci / No. Isolates Tested	No. Catalase Neg. / No. Isolates Tested	No. Gram Pos. Cocci / No. Isolates Tested	No. Catalase Neg. / No. Isolates Tested
KF agar (Difco: 701241)	10/10	0/10	2/2	2/2
KF agar (Difco: 672017)	6/6	0/6	7/7	7/7
KF agar (BBL: K5DMWO)	10/10	3/10	3/3	3/3
KF agar (BBL: D3DK1W)	7/7	1/7	1/1	1/1
KF agar (Scratch)	11/11	7/11	2/2	2/2
M-Ent agar (Difco: 640936)	11/11	8/11	1/1	1/1
	55/55	19/55	16/16	16/16

TABLE 19. GROWTH OF CONFIRMED AND FALSE-POSITIVE FECAL STREPTOCOCCUS ISOLATES ON KF AGAR UNDER AEROBIC VS. ANAEROBIC INCUBATION

ISOLATES TESTED	ISOLATE NUMBER	INCUBATION	
		Aerobic	Anaerobic
		(CFU/50 ml)	
Confirmed Fecal Streptococcus	1	3.7×10^9	3.6×10^9
	2	6.0×10^8	5.2×10^9
	3	1.6×10^9	2.2×10^9
	4	2.0×10^9	3.5×10^9
	5	2.3×10^9	4.2×10^9
False Positive Fecal Streptococcus	1	6.0×10^9	<1.0
	2	3.7×10^9	<1.0
	3	3.3×10^9	<1.0
	4	4.4×10^9	<1.0
	5	4.7×10^9	<1.0
Streptoccus faecalis (ATCC 19433)	1	3.6×10^9	2.6×10^9

NOTE: Incubation 35°C, 3 days.

fecal matter. FC is currently the only indicator bacteria stipulated by laws to assess the quality of recreational waters. However, due primarily to the instability of FC, the reliability of FC as an indicator for the presence of fecal pathogens has been questioned and the use of other indicator microorganisms has been proposed. Of these alternative indicators, fecal streptococcus (FS) bacteria have been most often used. Standard Methods (APHA, AWWA, and WPCF 1980) prescribes the use of KF agar to selectively recover FS from all natural waters and states that the reliability of KF agar is such that nearly 100% of the presumptive colonies on KF agar can be expected to be confirmed as true fecal streptococcus bacteria. The Water Resources Research Center (WRRC) showed that FS was much more stable than FC in marine waters and has recommended its use to assess the quality of marine recreational waters. Based on this recommendation, the Division of Wastewater Management also analyzed Hanauma Bay beach water samples for FS. The high concentrations of FS recovered from many of these samples were the only evidence suggesting that the beach water was contaminated with fecal matter. These findings were confirmed but showed that many of

the positive colonies observed on KF agar were not due to the growth of FS bacteria and therefore appeared to be false positives. This study was initiated to evaluate the reliability of KF agar to recover FS bacteria from the marine waters of O'ahu as a means of assessing its recreational quality. The design of this study was to obtain water samples from many coastal sites, primarily from Hanauma Bay but also from open ocean waters and to analyze these samples for four fecal bacterial indicators (FC, FS, EC, and C. perfringens).

Most marine water samples were shown to contain concentrations of FC well below the 200 FC/100 ml standard for recreational water quality. These same water samples were shown to contain low concentrations of C. perfringens, a very stable and conservative indicator of water pollution. Increased levels of FC and C. perfringens were occasionally recovered from marine water samples but these samples were associated with sewage outfalls, stream discharge, or land runoff. Based on the results of these two indicators, we conclude that O'ahu's coastal water is hygienically safe for recreational uses.

The use of KF agar to recover FS bacteria from O'ahu's marine water was determined to be unreliable because of the formation of false-positive colonies. Two components of KF agar were shown to readily allow the growth of false-positive bacteria. First, KF agar contains 0.5% NaCl and provides the Na required for the growth of marine bacteria. Second, this medium is highly enriched and therefore the concentration of sodium azide is insufficient to inhibit some of the false-positive bacteria. Thus, by doubling the concentrations of sodium azide or removing the NaCl, much lower concentrations of false-positive bacteria were recovered from the modified KF agar. At least two major groups of bacteria were shown capable of forming red to pink presumptively positive FS colonies on KF agar. One group of false-positive bacteria was a gram-negative rod, required NaCl for growth, and formed atypical colonies characterized by a red halo surrounding each colony. This group of bacteria was shown to be capable of growing in seawater, was identified as Psuedomonas sp. and Aeromonas sp., and was concluded to be marine bacteria. Because of the formation of the atypical colonial morphology, this group of false-positive bacteria could be distinguished from the colonies of the true fecal streptococcus bacteria on KF agar. The second group of false-positive bacteria was similar to true

fecal streptococcus bacteria in that they were gram-positive cocci and formed typical colonies on KF agar. However, this group of bacteria was catalase positive, did not contain the group D antigen, did not grow in the presence of bile or at 45°C, and therefore could be distinguished from true fecal streptococci by using simple biochemical tests. Isolates of these bacteria were not identified, were shown to multiply in seawater, and were thus considered marine bacteria.

M-Ent agar selectively recovers a subgroup of fecal streptococcus bacteria called enterococcus (EC). In most marine water samples, the recovery of EC using M-Ent agar was much less than the concentration of FS recovered on KF agar. Also, atypical colonies were not observed on M-Ent agar and most of the presumptively positive colonies could be confirmed as true fecal streptococci. However, false-positive bacteria were recovered on M-Ent agar when marine waters containing high concentrations of false-positive bacteria were processed. The presence of high concentrations of false-positive bacteria in marine water samples appeared to be related to the availability of nutrients in the water to support the multiplication of these bacteria. Most of the coastal waters surrounding O'ahu are nutrient poor and therefore the concentrations of false-positive bacteria in these waters are low. However, some coastal waters (e.g., harbors, middle sector of Hanauma Bay) have poor circulation, receive land runoff which increases their nutrient content and allows false-positive bacteria to multiply to higher concentrations.

It was clear that a reliable method which eliminated the growth of false-positive bacteria was required to recover FS and EC from O'ahu's marine waters. Such a method was devised by modifying the technique using KF agar to recover FS and M-Ent agar to recover EC. This modification was based on the knowledge that FS and EC are enteric bacteria able to grow under aerobic and anaerobic conditions (facultative anaerobe). On the other hand, marine bacteria growing in the shallow coastal waters of Hawai'i were presumed to be aerobic. When water samples placed onto KF agar and M-Ent agar were incubated under anaerobic conditions instead of the standard aerobic conditions, the false-positive bacteria did not grow while allowing FC or EC bacteria to grow and to be reliably enumerated. It is therefore recommended that Hawai'i's marine waters should be analyzed for FS on KF agar and EC on M-Ent agar and incubated under anaerobic rather

than aerobic conditions. This procedure should eliminate the formation of false-positive colonies on these media which otherwise can be expected.

The recovery of marine bacteria which readily forms false-positive colonies on KF agar and occasionally on M-Ent agar has not been reported by scientists using these media to analyze the coastal waters of the continental U.S.A. Thus, the population of marine bacteria in O'ahu's subtropical coastal waters must differ from the marine bacterial population in the temperate coastal waters of the continental U.S. Significant environmental differences between the continental U.S. and Hawai'i should be expected and have been verified from time to time. These findings emphasize that standards and methods developed under continental U.S. conditions may not be applicable to Hawai'i's conditions. Understanding these differences and modifying standards and methods to apply to Hawai'i are the responsibilities of scientists, engineers, and planners in Hawai'i.

COMMENTS AND RECOMMENDATIONS ON RECREATIONAL WATER QUALITY STANDARDS FOR HAWAI'I

Existing Water Quality Standards: Impact on Hawai'i

The existing recreational water quality standard of 200 fecal coliforms (FC) per 100 ml applies to marine and fresh waters. The use of FC in establishing water quality standards for recreational waters was based on the best available technology at the time it was promulgated in 1968 and on the principle that FC was the best indicator to measure the degree of fecal contamination in any water. Yet the concentrations of FC in the water had not been correlated to the rates of gastroenteritis associated with swimming.

In Hawai'i, marine recreational waters usually contain low levels of FC. Based on these results, the marine recreational waters in Hawai'i are considered to be unpolluted and safe for use. In contrast the freshwater streams of Hawai'i generally contain concentrations of FC exceeding 200 FC/ 100 ml. These streams, most of which do not receive known discharges of fecal wastes, are classified for recreational use. Based on these results, the freshwater streams in Hawai'i do not generally meet current recreational water quality standards. The source and significance of these high concentrations of FC and even higher concentrations of fecal streptococcus

(FS) are not known and should be addressed.

Although FC continues to be the indicator used to assess recreational water quality standards, overwhelming evidence from independent laboratories throughout the world has shown that FC is too unstable in recreational waters to be a reliable indicator for the absence of enteric pathogens which may be present in the same water. Cabelli (1983) and Dufour (1982) recently reported that concentrations of FC in marine and fresh recreational waters could not be correlated with rates of swimming associated gastroenteritis among users of these waters.

Fecal streptococcus and Clostridium perfringens are two other fecal bacteria which are known to be more stable than FC in environmental waters and thus have been considered as alternative indicators of fecal contamination. Yet the use of these indicators suffer from the same inherent weakness as the use of FC; that is, the concentrations of these bacteria have not been shown to correlate with rates of swimming associated gastroenteritis.

EPA Proposed New Indicators and Standards: Impact on Hawai'i

Recognizing the problem of using FC to assess the quality of recreational waters, the EPA initiated a series of studies from 1972 through 1982 to establish a correlation between rates of swimming associated gastroenteritis and concentrations of several indicator bacteria in these waters. Based on the results of this well-designed, epidemiological and microbiological monitoring study, clear evidence was obtained to show that swimming in sewage-contaminated waters increases the risk to gastroenteritis. Also, the concentrations of some of the currently used indicators (FC, FS, Clostridium perfringens) did not correlate with risks to gastroenteritis. However, the concentrations of two new indicators (E. coli, enterococci) in recreational waters did correlate with rates of gastroenteritis among users of these waters. The results of these studies clearly showed that E. coli and enterococci could be used to assess the quality of fresh recreational waters, but that only enterococci could be used for marine waters. The basis for this difference was best explained on the differential stabilities of E. coli and enterococci in natural waters. E. coli is stable in fresh waters but not in marine waters. On the other hand, enterococci are highly salt-tolerant bacteria and are stable in fresh and marine waters.

Having successfully completed this study, EPA now insists that indicators of recreational water quality should be based on risks to swimming associated gastroenteritis and no longer be based on indicators which simply suggest the presence or absence of fecal contamination.

In the May 1984 issue of the Federal Register, EPA published their proposed new water quality criteria which are summarized as follows:

> Fresh recreational waters should not exceed a log mean of 77 E. coli/100 ml or 20 enterococci/100 ml
>
> Marine recreational waters should not exceed a log mean of 3 enterococci/100 ml.

It should be noted that the data base for the EPA studies was obtained from study sites in New York, Massachusetts, New Orleans, Pennsylvania, and Oklahoma. Also, new bacterial media not commercially available are required to assay for E. coli and enterococci. Thus, the data base using these bacteria is only now beginning to be obtained throughout the rest of the U.S. The new proposed criteria, especially for marine waters, is very restrictive. If implemented as water quality standards applicable to Hawai'i, there is a strong possibility that many of the popular recreational beaches in Hawai'i will have to be closed to the public. If this were to occur, the result would have a severe psychological and economic impact on the state of Hawai'i. As a result of this possibility and evidence accumulated by the WRRC laboratory that the waters in Hawai'i are very different from waters in the continental U.S., we have already initiated a limited study to analyze the recreational waters in Hawai'i for E. coli and enterococci. The results of our preliminary study indicate that all freshwater streams and some of the popular beaches on O'ahu would not meet the new EPA-proposed criteria.

At the present time, the EPA is still considering the implementation of the new proposed criteria and is considering the response from throughout the nation. Depending on the comments received by the EPA, the proposed criteria may remain unchanged or may be made less restrictive. It should be noted, however, that once the EPA establishes the criteria, each state will be asked to establish a standard based on these criteria. Each state will have the opportunity to modify the proposed criteria if it is believed to be too restrictive. To obtain this variance, however, the applying state must provide justifiable reasons and documented evidence that the population at risk is not being exposed to higher risks as a

result of changing the water quality standards. Thus, it is imperative that the state of Hawai'i obtain as much data as it can on the concentrations of E. coli and enterococci in recreational waters of the state. These results, if transmitted to EPA during this interim period can influence the final water quality criteria to be recommended by the EPA.

In conclusion a study to survey the recreational waters of Hawai'i for enterococci and E. coli by using the new EPA-developed media and technique should be conducted as soon as possible. This study should determine whether false-positive enterococci will be a problem when using the new EPA mE medium, especially since the results of this present study have shown that many false-positive fecal streptococci will grow on KF agar. A second part of this study should address the source and public health significance of the high concentrations of fecal coliform, E. coli, fecal streptococci, and enterococci which appear to be present in freshwater streams in Hawai'i.

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