PATHOGENIC ENTERIC VIRUSES IN THE HAWAIIAN OCEAN ENVIRONMENT: VIABILITY AND DIE-OFF

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by

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ABSTRACT

Human enteric viruses were recovered from 0.19 to 0.76 m^3 samples obtained from various natural marine water sites using the portable virus concentrator (Aquella). The concentration and frequency of viruses and coliform bacteria were highest within the sewage plume over the ocean discharge pipe and proportionately decreased as the sample distance from the plume was increased. Viruses were recovered at a maximum distance of 3 218 m from the plume but never from a station 6 436 m from the plume. However, since the same type of sewage-borne viruses were also recovered from boat marinas and from a stream entering the ocean, the sewage ocean outfall may not be the only source of viruses entering the ocean. Significantly, viruses were occasionally recovered from samples which were negative for coliform bacteria.

The expected stability of human enteric viruses in the marine waters was determined to be approximately 48 hours using type 1 poliovirus as the model virus. All marine waters obtained from various sites off the coast of Hawaii were determined to be virucidal and evidence was obtained that marine microorganisms are the natural virucidal agents in these waters.

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OBJECTIVES

Disposal of sewage into the ocean is a common practice and currently 22.71×10^4 m^3/day (60 mgd) of Honolulu's sewage, representing nearly half of O'ahu's output is discharged untreated into Māmala Bay via the Sand Island ocean outfall. It was previously determined that pathogenic human enteric viruses are present in all raw sewage of O'ahu examined and that significant concentrations of these viruses are daily entering the Hawaiian ocean environment. However, no data are available regarding the fate, dispersion, and public health significance of these sewage-borne viruses after they enter the ocean. The objectives of this study are:

1. To determine whether infectious sewage-borne viruses can be recovered from the ocean waters near and around the sewage outfall site in Māmala Bay
2. To determine the types and concentrations of these viruses that are present
3. To determine the distribution and manner of dispersion of these viruses in the ocean
4. To determine the survival time of these viruses in the ocean environment and to determine the natural component(s) of sea water responsible for the inactivation of viruses; and to also monitor additional parameters, which can affect survival rates, such as temperature, ionic composition, and pH
5. To evaluate whether the current practice of ocean disposal of raw sewage poses any health threat to the use of ocean waters in Māmala Bay by the general public, especially at Ala Moana and Waikīkī beaches
6. To monitor other ocean sites on O'ahu which may be likely sources for sewage-borne viruses.

RESEARCH ACCOMPLISHMENTS

Aquella Virus Concentrator

The isolation of viruses from ocean water involves two inherently unique problems: (1) sampling size and method and (2) high salt concentration and its effect on virus concentration methods.

The extensive dilution that occurs to sewage when it is discharged into the ocean makes it imperative that unusually large volumes of water (0.19-0.38 m^3 or 50-100 gal) be sampled for virus analysis. The bulk and weight of such a sample make it most difficult to transport this large volume of water back to the laboratory for virus analysis. Furthermore, the efficiency as well as the facility of most existing virus concentration methods decreases as the volume of the sample increases.

The other factor is the high salt concentration of sea water which may affect methods for concentrating viruses. For example, the protamine sulfate method (England 1972) cannot be used in sea water and the PE-60 (Wallis, Melnick, and Fields 1971) and the two-phase methods (Shuval et al. 1969) are highly sensitive to salt (ionic) concentration. In contrast, high salt concentration has been determined to be an asset to the membrane adsorption method (Wallis, Henderson, and Melnick 1972).

On the basis of the reasons enumerated above the experimental portable virus concentrator, Aquella (Metcalf, Wallis, and Metnick 1973), capable of processing 52.6 x 10^{-6} m^3/s to 63.1 x 10^{-6} m^3/s (50-60 gph) of water at the field sample site, was selected for use to recover viruses from the ocean. The Aquella (Fig. 1) was leased from the Carborundum Company, Niagara Falls, New York. Operationally, the Aquella pumps the water sample through a series of selective filters (Fig. 2). The first three filters are the clarifying filters which retain particles larger than 1 μm while allowing the water and viruses to pass freely through. The resulting clarified sample is then "conditioned" by the addition of appropriate amounts of $AlCl_3$, adjusted to the proper pH with HCl, and then pumped through the last two filters which adsorb preferentially the viruses from the water sample. All filters are then recovered and the adsorbed viruses eluted into a smaller volume of buffer (1-2 ℓ/filter) for further concentration and assay. While simple in principle, the effective operation of the Aquella virus concentrator involves considerable skill. Furthermore, depending upon the kind of sample taken, we have found that the Aquella required extensive modifications in procedure before a reasonable efficiency of recovery of virus was achieved. Thus, while the Aquella's efficiency of virus recovery from 0.19 m^3 (50 gal) of sea water was initially less than 1% when operated under the recommended conditions, it was increased to 15 to 20% after undergoing a series of extensive modifications. It is important that the efficiency of the Aquella be further improved and efforts

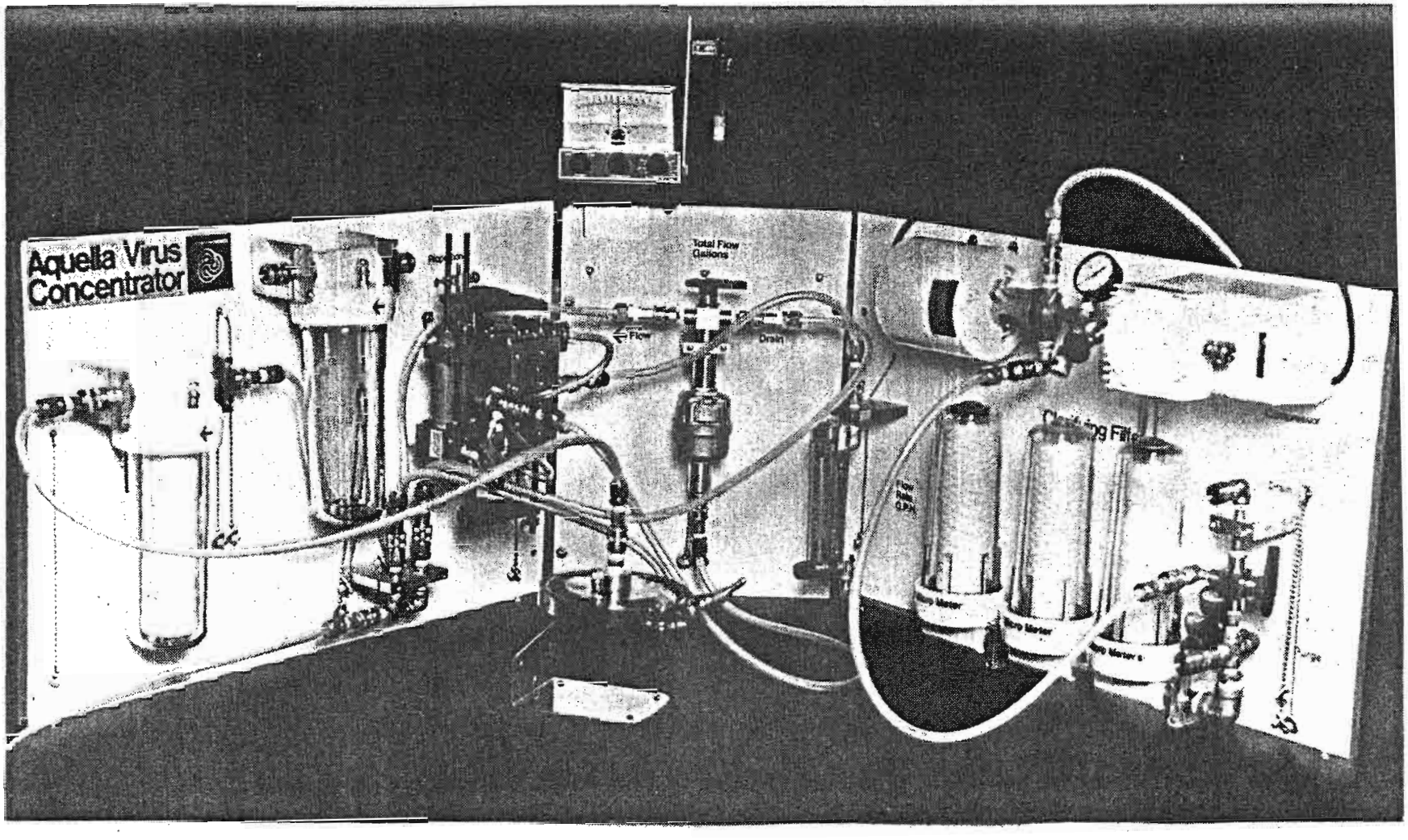


FIGURE 1. THE AQUELLA, PORTABLE VIRUS CONCENTRATOR.

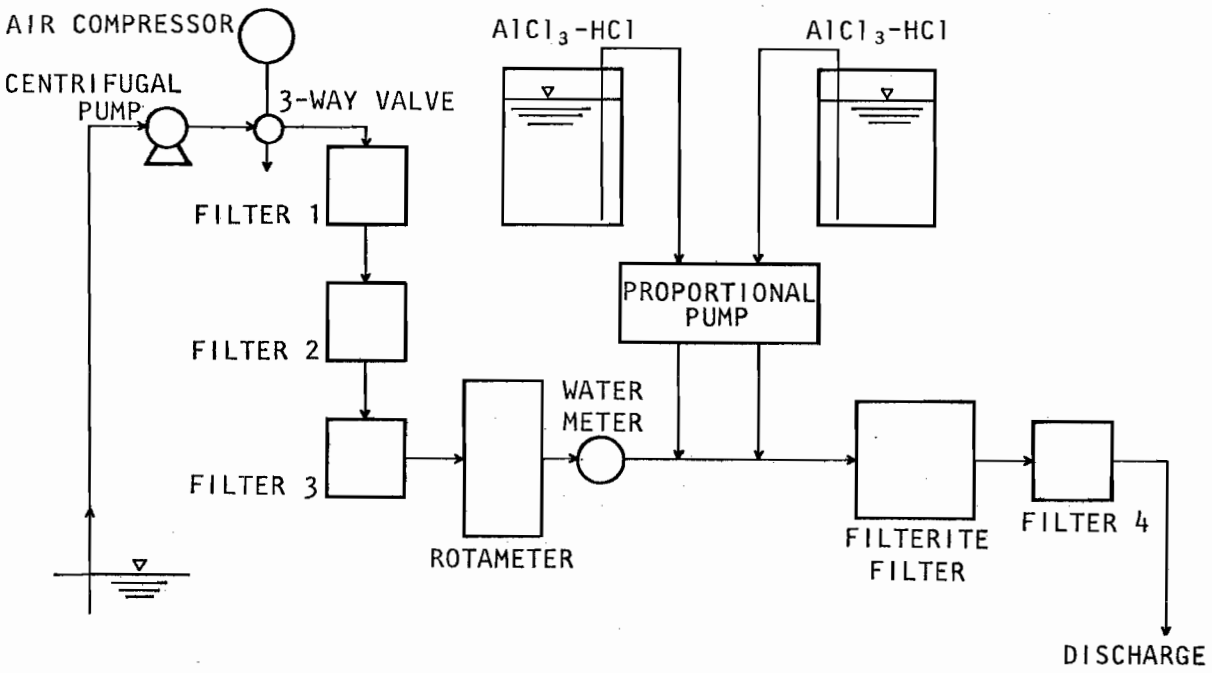


FIGURE 2. FLOW DIAGRAM OF AQUELLA CONCENTRATOR.

toward this direction are currently being made in this laboratory.

Sample Sites and Sample Analyses

The approximately 22.71 x 10^4 m^3/day (60 mgd) of raw sewage from the city of Honolulu, which is discharged 1 128 m (3700 ft) offshore from Sand Island into Māmala Bay at a depth of 12.2 m (40 ft), rises to the surface of the water as a visible area, designated the "boil", of approximately 3 m (10 ft) in diameter. Most of the sewage spreads outward from the boil and remains primarily at the water surface, resulting in a large area of visible discoloration of the water surrounding the boil. This discolored area, which is easily visible from airplanes or from high points surrounding Māmala Bay, is designated the "plume". Although the location of the boil is fairly fixed, the size and shape of the plume surrounding the boil varies greatly in response to changing environmental factors, such as the direction and velocity of winds and water currents.

In the eastern (Diamond Head) and northern (Sand Island) direction of the boil, the boundaries of the plume were generally well defined and remained relatively close (within 91 m or 100 yd) to the boil. On occasions,

the edge of the plume has been observed as far away as 1,609 m (1 mile) Diamond Head of the boil and 804.5 m (0.5 miles) landward of the boil. The plume generally extended farthest in the western (Ewa) and southern (seaward) direction of the boil. However, the boundaries of the plume in these directions varied considerably and were often so ill defined that the exact edge could not be located. On numerous occasions, the visible edge of the plumes were observed approximately 3 218 m (2 miles) in the southwesterly direction of the boil.

Since microbial pathogens (bacteria, viruses) are often present in sewage, these agents do enter Māmala Bay via the boil and presumably move outward from the boil as part of the plume and, perhaps, farther away. However, because of their differences in size, concentration and stability, the movement and distribution of these viruses, bacteria, and other sewage components vary considerably in the ocean environment. Since the primary objective of this study is to determine the presence, distribution, and survivability of sewage-borne viruses in the ocean environment surrounding the boil, samples of 0.19 to 0.76 m^3 (50-200 gal) of water taken from depths of 0.3 to 1.2 m (1-4 ft) below the ocean water surface were processed with the Aquella virus concentrator. In addition, these samples were frequently analyzed for sewage-borne bacteria (fecal coliform, fecal streptococcus), as well as turbidity and salinity in an attempt to establish a relationship between the presence of the virus and the more easily measured sewage components.

Virus Isolation and Identification

PHASE 1: RECOVERY OF SEWAGE-BORNE VIRUSES IN AND NEAR THE PLUME IN MĀMALA BAY. The Phase 1 objectives are: (1) to demonstrate that viruses can be recovered from Māmala Bay using the Aquella virus concentrator, (2) to demonstrate that viruses can be consistently recovered from the plume area, (3) to determine whether viruses can be recovered from waters outside of the plume area. Sampling sites were selected with reference to the plume observed on the sampling day. More samples were taken east (Diamond Head) of the plume in order to obtain data in relation to the pressing question of whether sewage-borne viruses can be transported in the direction of the popular swimming beaches of Ala Moana and Waīkīki.

In this study, sampling times were often arranged at the convenience of the personnel from the City and County of Honolulu Division of Wastewater Management and upon the availability of the boat. This practice resulted in many samples being taken over changing tide patterns and, consequently, the effect of tidal action on virus movement could not be properly evaluated.

The Phase 1 results are summarized in Table 1 and the sites identified in Figure 3. Of seven samples assayed for virus within the sewage plume, six were positive for virus. The only negative sample was taken at a depth of 1.8 m (6 ft) rather than the standard 0.3-m (1-ft) depth. These results indicate that viruses are present at the surface of the water and can be consistently recovered from the plume area by the Aquella. Outside of the plume area, virus was recovered in 6 of 12 samples taken east (Diamond Head) of the plume at distances of up to 685.5 m (750 yd) from the edge of the plume. A single sample taken outside the reef off Waikīkī Beach (6 436 m or 4 miles from the boil) was negative for virus. One sample taken 91.4 m (100 yd) north of the plume was positive for virus while 2 of 3 samples obtained west of the plume and 1 of 2 samples obtained south of the plume were positive for virus at a maximum distance of 228.5 m (250 yd) from the edge of the plume. These results show that viruses can be recovered in all directions outside of the plume area.

On the basis of the following observations: (1) the frequency of virus isolation within the plume was higher than outside the plume, (2) the concentration of virus was greatest within the boil (0.4 to 3.3 PFU/gal), and (3) the viruses recovered were serologically identified to be those belonging to the variety of human enteric viruses which are commonly present in O'ahu's domestic sewage. Thus, it was concluded that the viruses recovered from Māmala Bay originated from the sewage discharge into the ocean.

PHASE 2: MOVEMENT OF SEWAGE-BORNE VIRUSES EAST OF THE PLUME IN MĀMALA BAY. The results of Phase 1 indicated that viruses from the sewage boil were being disseminated by the ocean water. However, the extent of virus movement as well as the role of physical factors, such as tides and winds, which may affect virus movement in the ocean water were not established.

Towards this end, Phase 2 of the project was directed specifically

TABLE 1. RECOVERY OF VIRUSES FROM MAMALA BAY, O'AHU, HAWAI'I, PHASE 1

EXPERIMENT NO.		DATE	DISTANCE (m)	VOL. (m^3)	VIRUS*	TURB. (NTU)	SS (mg/ℓ)	Cl (mg/ℓ)	COLIFORM Total (MPN/100 mℓ)	COLIFORM Fecal (MPN/100 mℓ)	FECAL STREP. (MPN/100 mℓ)	FC:FS RATIO
Within Plume					+CB5							
1	A-3	07/30/75		0.17	+CB2							
					+P-2							
2	A-15	10/07/75		0.19	+P-3							
3	A-16	10/07/75		0.08	+CB4							
4	A-20	11/18/75		0.19	+E-7							
5	A-21	11/18/75		0.19	+P-1							
6	A-44	02/09/76		0.16	Neg.	1.0	96	18.7	....	.....	.....	....
7	A-51	03/15/76		0.13	+CB4	5.3	..	19.0	$1x10^5$	$3.4x10^4$	$6.5x10^4$	0.52
					+P-3							
East of Plume												
1	A-28	12/12/75	91.4	0.19	+CB4							
2	A-33	12/30/75	91.4	0.23	+E-7	0.59	7.6	19.9	9	0	94	.....
					+CB4							
3	A-34	01/06/76	228.6	0.38	Neg.	0.48	12	19.9	1	1	10	.....
4	A-35	01/06/76	457.2	0.38	Neg.	0.37	12.4	19.9	1	1	5	
5	A-36	01/15/76	228.6	0.38	+CB4	0.92	16.0	19.8	$1.32x10^3$	$6.1x10^2$	$1.94x10^4$	0.031
6	A-38	01/27/76	6437.4	0.38	Neg.	....	....	....	.....	.....	.....	.....
7	A-40	02/02/76	457.2	0.38	+E-7	0.29	65	19.8	$3.4x10^3$	$1.3x10^2$	$1.6x10^4$	0.008
8	A-41	02/04/76	685.8	0.38	+P-1	0.30	74	19.9	10	<1	<1	.....
9	A-42	02/04/76	228.6	0.38	Neg.	0.30	57	19.8	<1	<1	10	.....
10	A-47	03/01/76	228.6	0.20	+E-7	....	..	....	..	..	..	.....
11	A-48	03/08/76	228.6	0.38	Neg.	0.2	11	19.5	<1	<1	<1	.....
12	A-49	03/08/76	251.5	0.35	Neg.	1.2	12	19.0	$3x10^4$	60	$4.2x10^4$	0.001
West of Plume												
1	A-39	02/02/76	228.6	0.28	+P-1	0.28	58	19.8	1	3	40	.....
2	A-43	02/09/76	137.2	0.38	Neg.	2.2	85	19.2	30	2	20	.....
3	A-50	03/15/76	228.6	0.36	+E-7	1.1	..	19.4	$1x10^4$	$1x10^3$	$1x10^4$	0.1
South of Plume												
1	A-29	12/16/75	91.4	0.23	Neg.	0.4	4.2	19.9	2	2	9	.....
2	A-32	12/30/75	91.4	0.23	+CB4	0.46	9.3	20.0	215	120	905	0.132
North of Plume												
1	A-30	12/16/75	91.4	0.23	+CB4	0.55	10.5	20.1	44	39	5	7.8

NOTE: All samples taken at depths 0.3-0.6 m below water's surface except A-44 where sampling depth was 1.8 m.
*CB = Coxsackie B; E = ECHO; Neg. = Negative; P = Polio.

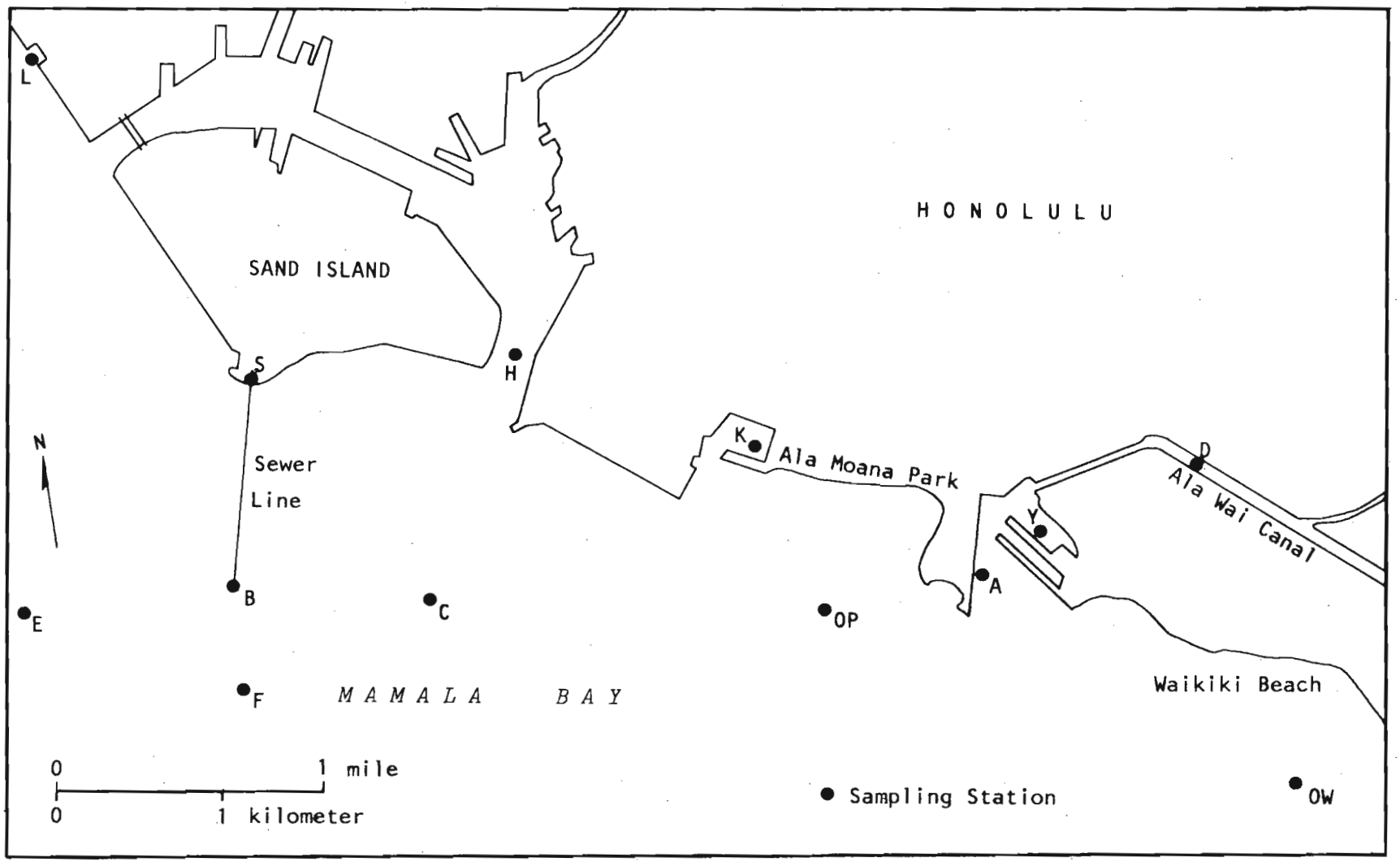


FIGURE 3. SAMPLING STATIONS IN AND AROUND MĀMALA BAY.

to answer the following questions: (1) Can viruses from the boil reach Ala Moana and Waikīkī beaches? (2) What are the environmental conditions that are conducive for the movement of viruses toward Waikīkī? (3) Are surface waters entering Māmala Bay a significant source of viruses? To achieve answers to the preceding questions, and on the basis of what is known concerning the tides and currents in Māmala Bay which may ultimately affect the movements of viruses, the following plan was adopted:

a. Stations were selected between the boil and Waikīkī Beach.

   Station B: The boil

   Station C: Honolulu Channel and 1 206.75 m (0.75 miles east of boil and 1 609 m (1 miles) south of the mouth of Honolulu Harbor

   Station OP: Outside the reef of Ala Moana Beach and 3 218 m (2 miles) east of the boil

   Station OW: Outside the reef of Waikīkī Beach and 6 436 m (4 miles) east of the boil

b. Stations were selected at entrances of surface runoff waters entering Māmala Bay.

   Station A: Ala Wai Channel (entrance of Ala Wai Canal water into Mamala Bay)

   Station D: Dock-site in Ala Wai Canal (near entrance of Ala Wai Canal water into Ala Wai Channel)

   Station H: Honolulu Channel (entrance of Honolulu Harbor water into Māmala Bay.

c. Stations were selected in boat marinas and coastal waters other than in Māmala Bay.

   Station Y: Ala Wai Yacht Harbor

   Station K: Kewalo Basin Harbor

   Station L: Ke'ehi Lagoon Harbor

   Station PH: Pearl Harbor

   Station KB: Kāne'ohe Bay

d. Sampling Regimen: Since it had been previously shown that sewage material from the plume are moved about rapidly at the water's surface, all samples were taken at depths of 0.3 to 0.6 m (1-2 ft) from the water's surface. Furthermore, sampling time was conducted under either rising or ebb tide conditions to evaluate the effect of tides on virus movement. It was previously reported by Bathen* that rising tides tend to drive sewage material toward the land while ebb tide conditions tend

to drive sewage material away from the land. In addition, the velocities and direction of the wind and water currents at the sampling sites were also measured.

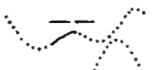
The results of Phase 2 are summarized in Table 2 and the sites identified in Figure 3. Two samples taken from the boil (Sta. B) under rising tidal conditions were positive for virus while three samples taken from Station C under rising and ebbing tidal conditions were negative for virus. However, 2 of 3 samples at Station OP were positive for virus under rising tidal conditions. At station OW under both rising and ebbing tidal conditions all three samples were negative for virus. The isolated viruses were serologically identified as the common sewage-borne viruses (Table 2). Thus, evidence was obtained that under normal (moderate) weather conditions viruses from the boil can be recovered in detectable concentrations 3 218 m (2 miles) east of the boil but not as far away as 6 436 m (4 miles) away.

It must be cautioned that the sewage discharge pipe in Māmala Bay cannot be assumed to be the only source of virus entering Māmala Bay. Surface runoff waters from the land which enter the bay may also be a source of virus. To assess this possibility, the major points where such land originated waters enter the area of study (Stas. A, H) were also assayed for virus and bacteria. At Station H (Honolulu Channel) 1 of 2 samples taken under rising tidal conditions was positive for virus. Since rising tidal conditions result in a net transport of water into Honolulu Harbor, the positive virus isolations may represent virus which originated from the boil. Two samples taken from Station A (Ala Wai Channel) during ebb tide conditions were negative for virus, suggesting that the amounts of viruses entering Māmala Bay via this source were negligible. However, a single sample taken from Ala Wai Canal proper under rising tidal conditions was positive for virus. It was not possible to determine whether this virus originated from the land or was brought into Ala Wai Canal from the ocean by the rising tidal conditions.

It must be concluded that due to the small number of samples taken, no definite conclusions can be drawn from these results and the question of whether land based waters are a significant source of virus remains to be resolved.

*K. H. Bathen 1977: personal communication

TABLE 2. RECOVERY OF VIRUSES FROM MĀMALA BAY, O'AHU, HAWAI'I, PHASE 2

EXPERIMENT NO.	DATE	VOL. (m^3)	TIDE	VIRUS*	TURB. (NTU)	SS --(mg/ℓ)--	Cl	COLIFORM Total	COLIFORM Fecal	FECAL STREP.	FC:FS RATIO
								---------(MPN/100mℓ)---------			
				STATION B (Boil)							
A60	05-11-76	0.09		+CB4 +P-2	2.8	44	18.2	$8x10^4$	$3.6x10^4$	$5x10^4$	0.72
A72	07-28-76	0.19		+P-1 +P-2	3.5	31	18.5	$4x10^5$	$2.4x10^4$	$4x10^4$	0.60
				STATION C							
A52	03-22-76	0.58		Neg.	0.12	17	19.5	< 2	< 2	< 2	....
A59	05-11-76	0.42		Neg.	0.2	29	19.3	18	2	14	0.14
A67	06-14-76	0.35		Neg.	0.23	27	19.2	..	2	6	0.33
				STATION OP							
A56	04-26-76	0.48		Neg.							
A55	04-21-76	0.56		Neg.	0.38	15	19.3	<10	<10	<10	....
A61	05-14-76	0.41		+P-1	0.2	21	19.3	< 2	< 2	< 2	....
A73	08-02-76	0.38		+P-2	0.2	13	19.4	<10	<10	<10	....
				STATION OW							
A62	05-14-76	0.42		Neg.	0.2	31	19.3	< 2	< 2	< 2	
A69	06-28-76	0.53		Neg.	0.1	31	19.1	< 1	< 1	< 1	
A71	07-28-76	0.56		Neg.	0.3	24	19.5	<10	<10	<10	
				STATION A							
A58	05-03-76	0.11		Neg.	1.0	19	16.3	10	30	10	3.00
A70	07-21-76	0.51		Neg.	4.0	12	15.9	600	100	60	1.67
				STATION H							
A63	05-24-76	0.37		Neg.	0.8	44	19.0	70	<10	20	....
A82	09-22-76	0.47		+P-1							
				STATION D							
A65	05-26-76	0.34		+P-2	2.0	18	9.8	$2.3x10^4$	$2.7x10^3$	$1.7x10^1$	158

NOTE: All samples taken at depths 0.3-0.6 m below water's surface.
*Neg. = Negative; CB = Coxsackie B; P = Polio; E = ECHO.

RECOVERY OF VIRUSES FROM SITES OTHER THAN MĀMALA BAY. Although Māmala Bay receives the largest volume of sewage discharge in O'ahu, other bodies of waters, such as Pearl Harbor and Kāne'ohe Bay, are also known to receive sewage and in the present study a few of these receiving waters were occasionally examined for virus using the Aquella virus concentrator. Of six samples assayed 1 078 m (0.66 miles) south of the Pearl City STP effluent discharge pipe in Pearl Harbor (Fig. 4), one sample was positive for virus (Table 2). Of two samples taken approximately 402.25 m (0.25 mile) south of the Kāne'ohe STP discharge pipe into Kāne'ohe Bay (Fig. 5), one sample was positive for virus.

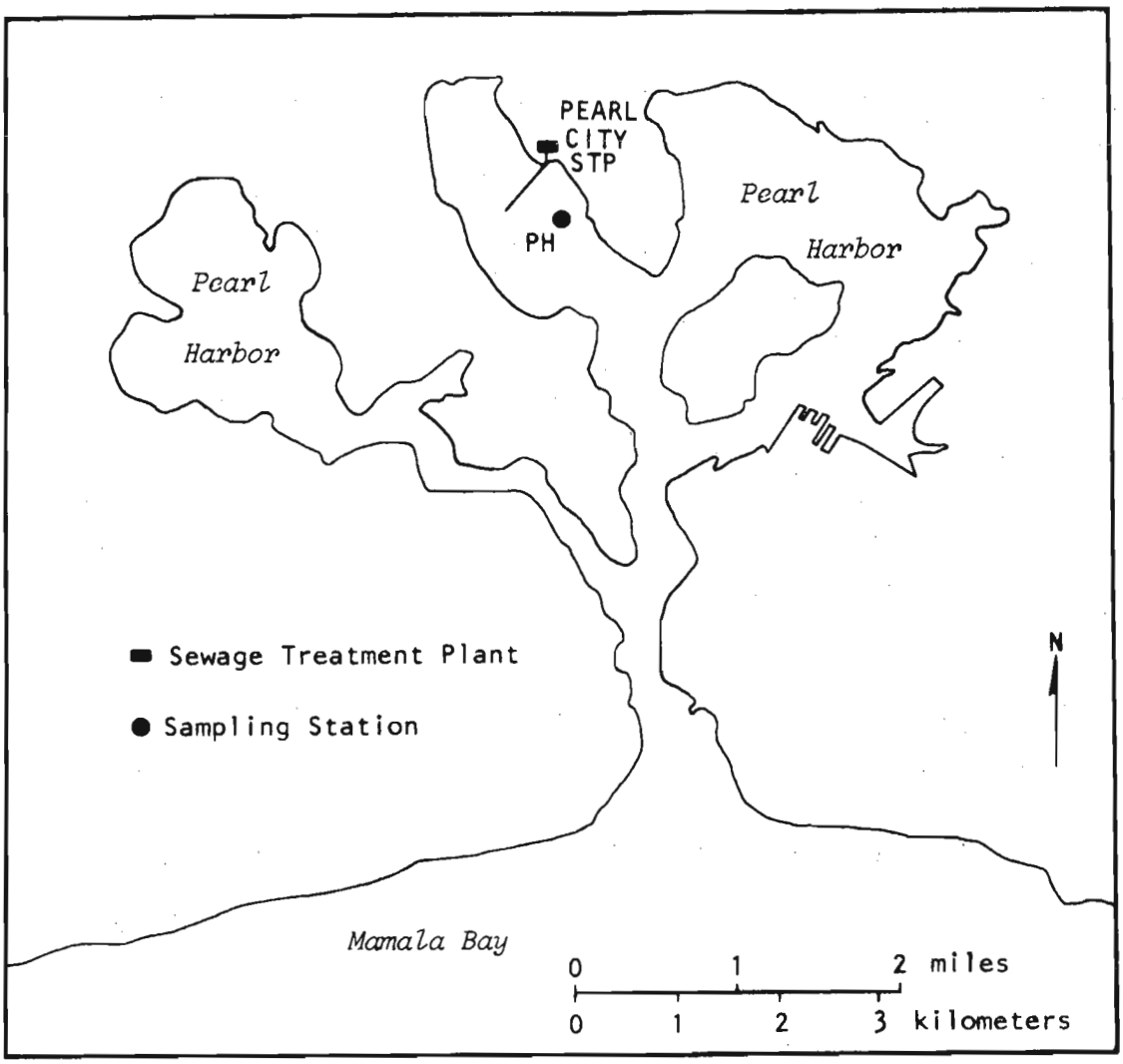


FIGURE 4. SAMPLING STATION IN PEARL HARBOR (PH)

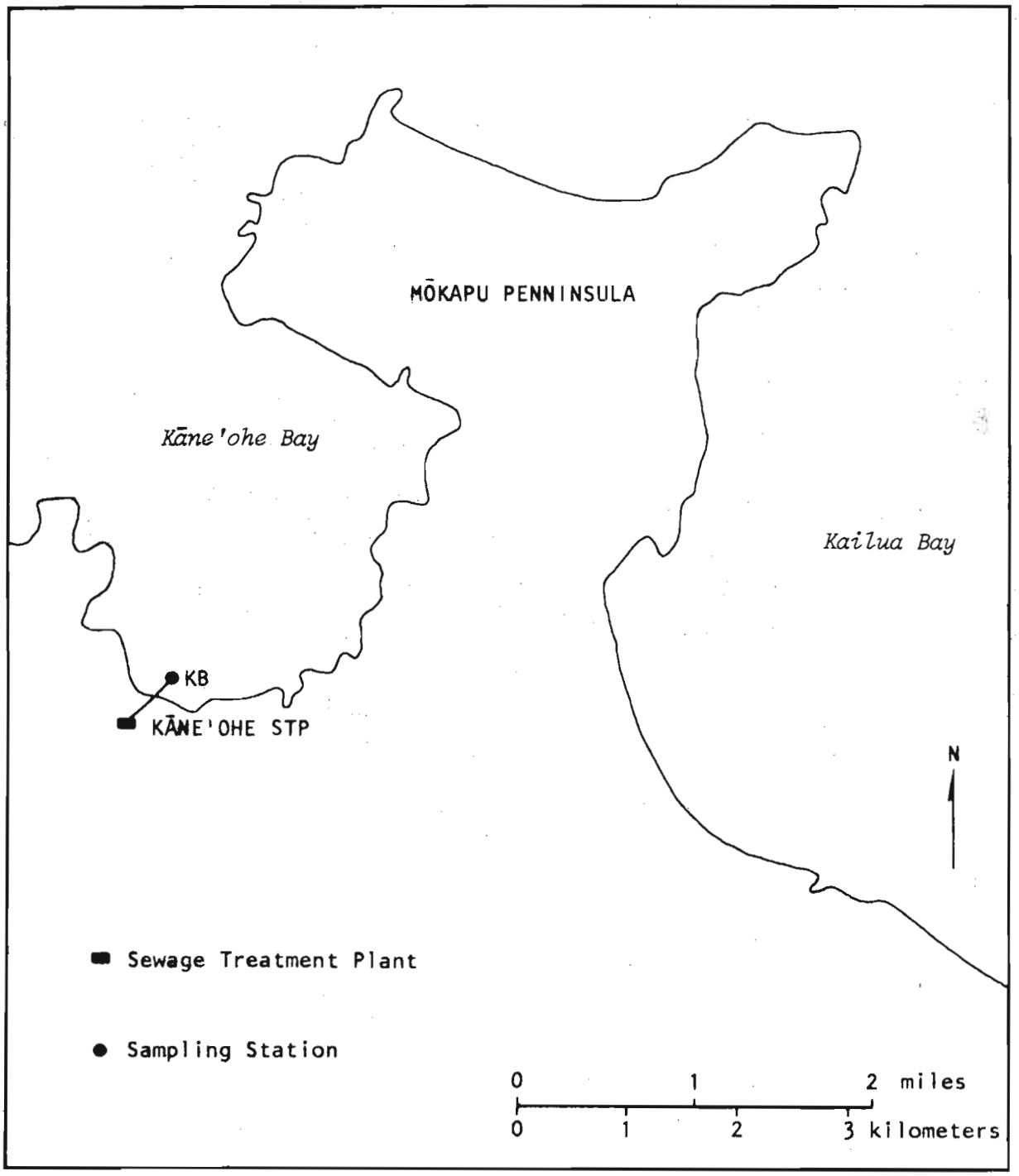


FIGURE 5. SAMPLING STATION IN KĀNE'OHE BAY (KB)

Besides the discharge of sewage by sewage treatment plants, marinas with live-on boats have been suspected of directly discharging waste material into the harbor. Three such marinas were assayed for virus. Positive virus isolations were obtained from 1 of 2 samples taken from Ala Wai Yacht Harbor and 1 of 1 sample taken from Ke'ehi Lagoon Harbor while 2 of 2 samples taken from Kewalo Basin were negative for virus. Since Ala Wai Yacht Harbor and Ke'ehi Lagoon Harbor cater primarily to live-on boats while Kewalo Basin Harbor primarily handles commercial boats, there appears to be a positive correlation between live-on boats and the presence of virus in the harbors. Although only a limited number of samples were taken outside of Māmala Bay, the results suggest that any body of water known to receive sewage should be considered as a potential source for human enteric viruses.

Bacterial Analysis of Water Samples

The health hazard potential of any natural waters contaminated by domestic sewage is currently determined by assaying the receiving waters for common sewage-borne bacteria (fecal coliforms and fecal streptococci). Furthermore, the ratio of fecal coliform to fecal streptococci (FC:FS) is used to interpret whether a freshwater source is contaminated with human or nonhuman waste. Existing Public Health Regulations for water quality standards classify Māmala Bay in the Class A category which is suitable for water-recreational activities with stipulated limits on coliform bacteria density (coliform index). However, published studies elsewhere have indicated that the coliform index is not a reliable indicator for the absence of viruses. Moreover, the FC to FS ratio indices have not been established to be valid for ocean water conditions. In the present study, in order to determine whether for the warm tropical ocean water like the Hawaiian coastal waters there is a definitive correlation between the coliform index and the presence of viruses and, also, to determine the validity of the established interpretation of the FC to FS ratio under ocean water conditions, water samples that were assayed for virus were also frequently assayed for total coliform, fecal coliform, fecal streptococcus, as well as turbidity and chloride content.

The results summarized in Tables 1 and 2 show that coliform were always present at high concentrations within the plume and exceeded the

concentration limits for Class A waters. Since virus isolations were also most frequent within the plume and close to the plume, it suggested a positive correlation between high coliform index and the presence of virus. However, outside the plume, the bacteria counts declined rapidly and were consistently well within Class A standards, especially at the distant stations (C, OP, OW). The positive virus isolations at sites with corresponding low to negligible coliform counts (Tables 1 and 2) confirm the previous published observations that human enteric viruses are relatively more stable in ocean water than coliform bacteria. The present results suggest that coastal waters near known source of sewage discharge and determined to meet Class A standards on the basis of low coliform counts would not necessarily be free of human enteric viruses.

The FC to FS ratio is generally greater than four for both raw domestic sewage and fresh waters contaminated with human waste. In the present study the value of the ratio was determined to be considerably less than one for the sewage contaminated ocean water samples analyzed (Tables 1 and 2). These results support earlier findings that fecal coliform bacteria is considerably less stable in sea waters than in fresh waters and, furthermore, that fecal streptococci bacteria is more stable than fecal coliform bacteria in sea waters. Thus, the present data suggest that the interpretation of FC to FS ratios established for fresh water would not necessarily apply under ocean water conditions. No statistical correlation using present data is found to exist between total coliform concentration and chloride ($r = 0.09$) and only poor correlation exists between total coliform and turbidity ($r = 0.54$). Further statistical analysis is in progress. Further studies, however, are needed to confirm these results.

Virus Stability and Antiviral Factors in Sea Waters

A major factor determining the recovery and distribution of infectious viruses in the ocean is their relative stability in sea water. Previous reports have indicated that enteric viruses are inactivated in sea water although at a much slower rate than bacteria. Thus, the T90 or time for 90% inactivation of poliovirus in Baltic sea water at 15°C was reported to be 48 hr (Magnusson, Hedstrom, and Lycke 1966). The virus-inactivating activity in the sea water was associated with a

biological agent(s) which remained still to be identified. During the course of our study on the survival of animal viruses, such as poliovirus in sea water, data was obtained in the laboratory to indicate that water samples taken from either Māmala Bay or Pearl Harbor contained components which inactivated the virus. Sea water samples were taken from these sites and brought back to the laboratory where they were either used immediately or allowed to incubate at room temperature (25°C) or at 4°C for various intervals of time. Poliovirus was then added to these water samples and their infectivity determined daily for up to one week. Most of these experiments were conducted at 25°C, a temperature similar to that found in the Hawaiian ocean environment. The results showed that in Māmala Bay and Pearl Harbor water at 25°C, the T90 for poliovirus was respectively 48 hr and 24 hr. It was further determined that for both Māmala Bay and Pearl Harbor waters, the component(s) responsible for virus inactivation can be: (1) maintained in an active state for weeks at 4°C; (2) destroyed by heat sterilizing the waters (boiling for 15 min; autoclaving, 121°C, 15 min); (3) inhibited by treating the waters with either penicillin and/or streptomycin; and (4) removed by filtering the water through membrane filters of 0.22- to 0.45-μm porosity. These results strongly suggest that the virus inactivating agent(s) found in Hawaiian sea water is not a chemical but a biological component, most likely bacteria. It also indicated that this virus inactivating agent(s) flourishes better in nutrient-rich Pearl Harbor water than in nutrient-poor Māmala Bay waters and consequently the virus inactivation capacity of Pearl Harbor water is more rapid than Māmala Bay water. From the above data, it can be concluded that viruses discharged into Māmala Bay would remain viable for longer periods of time and, consequently, would more likely be transported to greater distances than those discharged into the waters of Pearl Harbor.

Studies are currently in progress to isolate, characterize, and identify the virus inactivating agent(s) found in natural sea waters off O'ahu with the hopes of eventually using the agent(s) as a means of biological control of human viruses contaminating any natural waters in the state of Hawai'i.

APPLICATION OF RESEARCH RESULTS

The data from this project will be used by the City and County of Honolulu Division of Wastewater Management in evaluating the effectiveness of their sewage treatment and disposal system and in planning new and more effective treatment and disposal systems. In addition, the results would aid agencies, such as the Hawaii State Health Department and the Environmental Protection Agency in properly assessing the health hazards of sewage-borne viruses in the ocean and to formulate regulations necessary for the maintenance of the cleanliness and safety of the marine environment. Lastly, the information from this project would assist the U.S. Department of Fish and Game Wildlife Service in evaluating the effect of these human viral pathogens on marine life and the suitability of employing such "contaminated" marine waters for mariculture.

PROJECT AND FUTURE STATUS

The accumulated results provide evidence that human pathogenic enteric viruses can be isolated and identified from the sewage discharge area and also strongly indicate that the present methodologies employed are sound. For the remainder of the project period the following investigations are planned:

1. Continued sampling of sea water taken at various sites in Māmala Bay and other surrounding areas for viruses; monitoring of the following parameters, fecal coliforms and streptococci, salinity, pH, suspended solids, temperature, tide, wind speed and current; and the processing of all virus positive samples for identification and typing of agents.

2. Continued characterization of the properties of the virucidal component(s) present in the sea waters off the Hawaiian islands, particularly around Māmala Bay. Additional studies will be made to isolate, characterize and identify the microbial agent(s) involved.

3. Continued investigations into the survivability of known viruses in the ocean water but under field conditions.

4. Continued modification and improvement of the efficiency of the Aquella virus concentrator.

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APPENDIX A.
Field Investigations

Field studies were primarily conducted with the City and County of Honolulu Division of Wastewater Management's 5.5 m (18 ft) Glaspar boat. As an alternative for harbor surveys and calm near-shore sites, the WRRC 4.3-m (14 ft) McKee boat was used when the larger boat and crew were unavailable. The launching site for most of the survey sites was at the Ke'ehi Lagoon boat ramp. As an alternate, the Ala Wai Boat Harbor ramp was used but because of the lack of a suitable pier for loading, only the WRRC boat was launched there for the Waikīkī, Ala Moana and Ala Wai harbor study sites when the sewer's division boat was not used.

The two largest and heaviest pieces of equipment consisted of the self-contained Aquella virus concentrator and the portable electric generator (Onan, 2850 W) that supplied AC power for the motor driving the centrifugal pump and the compressor-vacuum pump motor. In a closed position, the Aquella is 0.46 by 0.9 m (1.5 ft x 3 ft) occupying 0.42 m^2 (4.5 ft^2) of area. However, in its operating position a trapezoidal area of 0.62 m^2 (6.7 ft^2) is required along with the generator which occupied 0.32 m^2 (3.4 ft^2) of space. When used in the small WRRC boat loaded with bottles of $AlCl_3$, test equipment, gasoline, and spare filters, space is extremely limited and movement of personnel on board, restricted.

Virus Filtration and Sample Preparation

At the study site water was pumped through the filtration by means of a centrifugal 0.12 m (3/4-in.) rubber impeller Teel pump driven by a 1/3 hp AC motor. The intake line was suspended over the side of the boat usually at 0.30 m (12-in.) depth with the intake end covered with a small mesh screen to prevent large particles from entering the system. In areas of clear water where high suspended solids concentration did not exist, normal filtration runs were at 2- or 4-hr duration depending upon the total number of runs desired for the day. At the sites two prefilters were used in conjunction with the two absorbing filters. At the sewage boil sites one of the prefilters has a larger pore size, thus lessening the clogging of the other filters. However, these runs were limited to one hour or less with a through put rate of 0.08 to 0.15 m^3 (20 to 40 gal). At the clear water sites 0.19 m^3/hr (50 gph)

could be pumped through the filters with little reduction in rate attributed to filter clogging. The pH of the water passing through the absorbing filters is lowered to 3.5 to 3.8 by injecting 0.03 M $AlCl_3$ and HCl through a Johanson proportional pump (Fig.1). This value of pH is maintained and checked throughout the run.

After measuring and recording the total volume passed through the filters with a Kent water meter, the various filters were eluted with glycine. The adsorbing filters (Filterite and No. 4 filter) are first removed of excess water by blowing out with air from the compressor and then rinsed with 1 ℓ of saline solution with a pH of 3.5. The first elution is done with 1 ℓ of 0.1 M glycine buffer, pH 11.5. This eluate is neutralized with 0.5 M glycine, pH 1.5. The second elution is done with 1 ℓ of 0.05 M glycine, pH 11.5 and then neutralized with 0.5 M glycine, pH 1.5. Similarly, the clarifying filters are eluted but the saline rinse is omitted. The eluted samples are transported in polycarbonate bottles to the laboratory.

Location Fixing of Sampling Sites and Environmental Conditions

Position finding of the sampling site was determined by sighting with a Davis handbearing compass on visible landmarks on shore. In the area of the old existing outfall and the surrounding areas bearings were taken of Aloha Tower, Tripler Hospital, the Dole pineapple water tower, the KGU radio tower and the end of land of Diamond Head. At the Ala Moana and Waikīkī beach areas, the channels 2 and 4 TV towers, KGU radio tower, Sheraton Hotel, and Diamond Head point were used for position fixing. At all study sites salinity and temperature profiles were measured using a YSI Model 33 salinity, temperature, and conductivity meter. Measurements were also done at sampling depths during various intervals of the run to obtain an average of the water quality entering the filter system. Ocean current velocity was measured with a pygmy-type current meter. Because of the noise of the generator, revolutions were counted by sighting on a marked cup instead of utilizing the standard clicks heard with earphones. Current direction during the run were recorded at various intervals. Wind velocity and direction which may affect surface currents were recorded using a small portable anemometer (Sims model BT) and a small wind vane. Other environmental conditions, such as rain, visible

suspended particles, and air temperature, were noted and recorded.

Bacteriological and Water Quality Sample

A feature of the Aquella unit is that the compressor used for blowing out excess liquid through the system also contains an intake which serves as a vacuum source for the filtration of bacterial samples. All bacteria work was done using the Millipore method. Media for total coliform, fecal coliform, and fecal streptococcus were preprepared in the WRRC laboratory and refrigerated in an ice chest before sampling. Filtration of appropriately diluted sample with buffered water was done using a Millipore filter funnel and filter flask on type HA 0.45-μ, 47-mm filters. The filters were placed in petri dishes containing agar media and stored in a light-proof container for transport to the laboratory for incubation. The following media was used for each bacterial type: total coliform; M-Endo agar LES, fecal coliform, M-FC with rosolic acid and fecal streptococci; M-Enterococcus agar. During the bacterial analysis work, a 1 ℓ water sample was taken in a plastic bottle, refrigerated in the ice chest, and transported back to the laboratory for suspended solids, turbidity, and chloride analyses.