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

The discharge of domestic sewage into the ocean waters off Oahu is a common practice. Since infectious enteric human viruses have been determined to be present in all sewages, viruses are also being discharged into the ocean environment. This study was undertaken to determine the fate, dissemination, and public health significance of sewage-borne viruses after they enter the ocean environment. Using an experimental, portable virus concentrator, indicator bacteria as well as human enteric viruses were consistently recovered from water samples taken from the surface sewage plume, the result of sewage discharge from the original ocean outfall pipe extending 129.8 m into Mamala Bay at a depth of 12.2 m. Recovery of sewage-borne bacteria and viruses decreased as the distance from the plume increased. However, viruses were recovered from a station as far away as 3 218 m east of the plume and within the vicinity of Ala Moana Beach. Significantly, viruses were occasionally recovered in the absence or negligible concentrations of coliform bacteria, indicating that the standard coliform test for water quality may be inadequate as an assessment for the presence of viruses. When the old sewage outfall pipe into Mamala Bay was diverted to the new outfall pipe which extends approximately 2 743 m from shore (exclusive of a 914 m long, multi-porthole diffuser) at a depth of 73.2 m, no surface plume was observed and viruses were only sporadically recovered from ocean waters above the discharge pipes (zone of mixing). Viruses were recovered from ocean waters near other ocean sewage outfalls, such as in Pearl Harbor, Kaneohe Bay, and Kailua Bay, as well as from boat marinas and a stream emptying into the ocean. Other studies showed that human enteric viruses can be expected to survive for 24 hr in the marine environment before they are actively destroyed. Moreover, evidence was obtained that microorganisms naturally present in all the marine waters off Oahu are responsible for the inactivation of human enteric viruses.

SURVIVAL, DISSEMINATION, AND PUBLIC HEALTH SIGNIFICANCE OF HUMAN ENTERIC VIRUSES IN OCEAN WATERS OFF O'AHU: VIABILITY AND DIE-OFF; ROLE AND EFFECTS OF ANTIVIRAL AGENT(S)

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May 1980

Final Technical Completion Report
for
Pathogenic Human Enteric Viruses in Hawaiian Ocean Waters:
Role and Effects of Antiviral Agent(s)

Principal Investigators: L. Stephen Lau and Philip C. Loh
Associate Investigator: Roger S. Fujioka
Project Period: 1 September 1975 to 30 November 1979
Grant Agreement Nos.: 14-34-0001-6012, 7025, 7026, 8013, 9013
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ABSTRACT

The discharge of domestic sewage into the ocean waters off O'ahu is a common practice. Since infectious enteric human viruses have been determined to be present in all sewages, viruses are also being discharged into the ocean environment. This study was undertaken to determine the fate, dissemination, and public health significance of sewage-borne viruses after they enter the ocean environment. Using an experimental, portable virus concentrator, indicator bacteria as well as human enteric viruses were consistently recovered from water samples taken from the surface sewage plume, the result of sewage discharge from the original ocean outfall pipe extending 129.8 m into Māmala Bay at a depth of 12.2 m. Recovery of sewage-borne bacteria and viruses decreased as the distance from the plume increased. However, viruses were recovered from a station as far away as 3 218 m east of the plume and within the vicinity of Ala Moana Beach. Significantly, viruses were occasionally recovered in the absence or negligible concentrations of coliform bacteria, indicating that the standard coliform test for water quality may be inadequate as an assessment for the presence of viruses. When the old sewage outfall pipe into Māmala Bay was diverted to the new outfall pipe which extends approximately 2 743 m from shore (exclusive of a 914 m long, multi-porthole diffuser) at a depth of 73.2 m, no surface plume was observed and viruses were only sporadically recovered from ocean waters above the discharge pipes (zone of mixing). Viruses were recovered from ocean waters near other ocean sewage outfalls, such as in Pearl Harbor, Kāne'ohe Bay, and Kailua Bay, as well as from boat marinas and a stream emptying into the ocean. Other studies showed that human enteric viruses can be expected to survive for 24 hr in the marine environment before they are actively destroyed. Moreover, evidence was obtained that microorganisms naturally present in all the marine waters off O'ahu are responsible for the inactivation of human enteric viruses.

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INTRODUCTION

This is the final completion report for the project, "Pathogenic Enteric Viruses in Hawaiian Ocean Environment: Viability and Die-Off," for the 1 September 1975 to 31 August 1979 period. The project originated from a request by the City and County of Honolulu Department of Public Works for information on the presence, survival, and distribution of pathogenic human enteric viruses related to the discharge of untreated sewage effluent into the Hawaiian ocean environment. Funded jointly by the Department of Public Works, City and County of Honolulu, and the University of Hawaii Sea Grant College Program of the National Oceanic and Atmospheric Administration, the project was conducted by the Water Resources Research Center (WRRC) and the Department of Microbiology of the University of Hawaii at Manoa.

The report presents field and laboratory studies of the isolation, survival, and distribution of human enteric viruses in Māmala Bay, O'ahu, Hawai'i, and other ocean sites receiving sewage discharge. Also presented are (1) an evaluation of the effectiveness of the design of the new Sand Island sewage outfall in alleviating the problem of sewage-borne human enteric viruses being discharged into the bay and (2) evidence of the presence of marine antiviral agents of a biological nature in all of the ocean sites sampled off the island of O'ahu.

BACKGROUND AND MOTIVATION

The intestinal tract of infected individuals may harbor more than 100 different types of human enteric viruses (adenovirus, poliovirus, coxsackievirus, echovirus, reovirus, rotavirus, parvovirus, infectious hepatitis) which are responsible for a wide variety of human diseases (poliomyelitis, hepatitis, conjunctivitis, myocarditis, meningitis, gastroenteritis) (Table 1). These viruses infect humans when ingested and are excreted with feces, and thus are found in sewage. That these sewage-borne viruses survive even secondary sewage treatment and chlorination and are commonly present in all sewages has been recently established by the Environmental Virus Laboratory of the Water Resources Research Center, University of Hawaii (Ruiter and Fujioka 1978; Fujioka and Loh 1978) and other laboratories (Berg et al. 1976; England 1972; Goyal, Gerba, and Melnick 1978). The Environmen-

TABLE 1. HUMAN ENTERIC VIRUSES THAT MAY BE PRESENT IN WATER

Virus Group	No. of Types	Disease or Symptoms
Enteroviruses		
Poliovirus	3	Meningitis; paralysis, fever
Echovirus	34	Meningitis, respiratory disease; rash, diarrhea, fever
Coxsackievirus A	24	Herpangina, respiratory disease, meningitis; fever
Coxsackievirus B	6	Myocarditis, meningitis, respiratory disease, pleurodynia; congenital heart anomalies, rash, fever
New enteroviruses	4	Meningitis, encephalitis, respiratory disease, acute hemorrhagic conjunctivitis; fever
Hepatitis Type A (probably an enterovirus)	1	Infectious hepatitis
Gastroenteritis Type A (probably an enterovirus)	2	Epidemic vomiting and diarrhea, fever
Rotavirus (reovirus family; gastroenteritis type B)	?	Epidemic vomiting and diarrhea (chiefly of children)
Reovirus	3	Not clearly established
Adenovirus	>30	Respiratory disease, eye infection
Parvovirus (Adeno-associated virus)	3	Associated with respiratory disease of children, but etiology not clearly established

SOURCE: Melnick, Gerba, and Wallis (1978).

tal Virus Laboratory has recovered viruses from *all* of the raw sewage samples taken from three different waste water treatment plants (WWTP) on O'ahu at concentrations ranging from 27 to 19,000 plaque-forming units per liter (PFU/ℓ). Since ocean disposal of sewage is practiced in Hawai'i, sewage-borne human enteric viruses must also be entering the ocean environment.

Although sewage-borne pathogens may enter the ocean, they become a potential health problem only if they are transmitted via waters used by the community for recreation or for seafood production. While it has been reported (City and County of Honolulu 1971) that most of the coliform bacteria entering the ocean is destroyed within the hour (e.g., the time required to destroy 90% [T_{90}] of the coliforms in Māmala Bay waters off Sand Island was

40 min), little is known as to the survivability of human enteric viruses in ocean water. These viruses have been reported to vary in their survival periods in different water environments (Hetrick 1978), Thus, survival periods of 2 to 168 days in tap water, 2 to 130 days in sea water, and up to 90 days in oysters have been reported (Hetrick 1978). Since viruses are much more persistent than coliform bacteria in ocean waters, coliform counts obtained from ocean waters would not be an accurate indicator for the absence of viruses. However, generalizations on virus survival can be dangerous. Factors such as temperature and purity of the water, play a role in the survival time of viruses. The influence of other factors is, at present, inadequately understood and the amount and combinations of these factors in nature are numerous. It should be noted that there is increasing evidence that enteric viruses are often associated with sediments in the various aqueous environments (Gerba and Schaiberger 1975). This association of viruses with solids does not result in inactivation; in fact, virus survival appears to be prolonged. Even less is known concerning the movement of viruses in the oceans. However, since viruses are so minute, they can be expected to either remain suspended in the water and to be transported by currents or, if they are adsorbed onto particulate materials (a common phenomenon), to be sedimented.

A major ocean sewage disposal site in the state of Hawai'i is Māmala Bay off the southern coast of O'ahu Island. More than 2.6 m^3/s (60 mgd) of the city of Honolulu's sewage composed of urban domestic waste water, including hospital wastes and some industrial wastes, are discharged into the bay. It should be noted that two of the most popular swimming and recreational beaches (Ala Moana and Waikīkī) are only 4.8 to 8.0 km (3-5 miles) away from this discharge site.

Prior to the present project, information was completely lacking on the survival and distribution in sea waters of pathogenic human enteric viruses from sewage disposal practices in Hawai'i. It is undoubtedly true that dilution and die-off of viruses will take place as the sewage enters the ocean environment and is subjected to solar irradiation, antiviral microflora inactivation, and other factors. However, the lack of data regarding the fate of viruses in the ocean environment of Hawai'i prevented rational and scientific assessments of ocean sewage disposal which could have important implications for the welfare of human and marine life.

The Department of Public Works (DPW), City and County of Honolulu is the action agency responsible for waste water management facilities for the island of O'ahu with its greater than 700,000 population. In 1975 the agency expressed a crucial need for data and information for pathogenic human enteric viruses in the ocean environment that could aid in the planning, design, treatment, and operation of sewage facilities and sewage disposal into the ocean. With the participation of the WRRC Environmental Virus Laboratory and the Department of Microbiology, University of Hawaii at Manoa, a project was designed and organized to examine this problem. The project can be divided into two periods: (1) the period prior to December 1976 when the major portion of O'ahu's sewage was discharged untreated into shallow 12.2 m (40 ft) coastal waters, 1 097.3 m (3600 ft) off Sand Island in Māmala Bay, and (2) after December 1976 when the sewage was diverted to a new sewage outfall extending approximately 2 743 m (9000 ft) from shore—exclusive of a multiporthole diffuser some 914 m (3000 ft) long and 73 m (240 ft) deep.

OBJECTIVES

The project objectives were as follows:

1. To determine whether infectious sewage-borne human enteric 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 dissemination of these viruses in the ocean
4. To determine the die-off rates or survival times of these viruses in the ocean environment and to determine whether there are any natural component(s) of sea water responsible for the inactivation of viruses; and to evaluate additional parameters, such as temperature, ionic composition, and pH, which can affect survival rates
5. To evaluate whether the current practice of ocean disposal of untreated sewage may pose 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 evaluate the effectiveness of the new Sand Island sewage outfall in alleviating the problem of the dissemination of human enteric viruses in Māmala Bay
7. To monitor other ocean sites on O'ahu which are receiving sewage discharge for sewage-borne viruses
8. To examine the relationship and significance between the presence of fecal bacteria, such as coliforms and streptococci, to the presence of human enteric viruses in the sewage-contaminated ocean water in Māmala Bay.

Anticipated benefits from this project are (1) guidelines for the proper methods of disinfection and disposal of sewage in Hawai'i where a major portion of the untreated sewage is presently discharged into the ocean; (2) prevention of the contamination and/or destruction of marine resources in the ocean water of Hawai'i; (3) provision for alternative methods for the disinfection of sewage wastes and sewage-contaminated waters; (4) methods for the routine surveillance of human enteric viruses in ocean water contaminated with sewage waste; (5) assistance to agencies, such as the Hawai'i State Department of Public Health and the Environmental Protection Agency, in properly assessing the health hazards of sewage-borne viruses in the ocean and in formulating safe management policies for the marine environment; and (6) assistance to the Division of Fish and Game in evaluating the effect of viral pathogens on marine resources.

ENVIRONMENTAL VIRUS LABORATORY

In 1972 the University of Hawaii at Manoa Water Resources Research Center and the Department of Microbiology collaborated to form this laboratory whose principal function is to examine environmental problems involving pathogenic human enteric viruses contaminating water and land resources. The laboratory is capable of isolating and identifying human enteric viruses from raw and treated sewage, from streams receiving sewage water, as well as from soil and soil percolates which have been irrigated with sewage effluent, and from estuarine and coastal waters. Nearly all of the reported major methods to concentrate viruses from water have been evaluated and used by the laboratory. The laboratory has developed the only environmental viral information source in Hawai'i; methodologies for concentration, isola-

tion, and identification of human enteric viruses from relatively large volumes of water and waste water samples; and provides experienced personnel to collect, identify, and evaluate data. Methodologies for the growth, enumeration, and identification of animal viruses and the growth, maintenance, and preservation of all kinds of animal cell cultures have been also established in the laboratory.

MATERIALS AND METHODS

Cells, Media, Virus, and Virus Assay

The BGMK, a continuous line of African green monkey kidney cells obtained from G. Berg (EPA, Cincinnati, Ohio), was used for all virus isolation, identification, and assay. The cell line was grown under 5% CO_2 atmosphere at 36°C in roller tubes, in 2 or 32 oz prescription bottles using Eagle's basal medium (EBM) supplemented with 5 to 10% fetal calf serum (FCS). The Sabin type-1 attenuated poliovirus strain (LSc2ab) was used in all seeding experiments and was grown in BGMK cells and purified by differential centrifugation and isopycnic banding in a CsCl gradient. To assay for virus, from 0.25 to 1.0 mℓ of the sample was added to monolayers of cells grown in 2-oz bottles and adsorbed for 1 to 1½ hr at 36°C. The bottles were then overlaid with 4 mℓ of complete medium containing 5% calf serum with or without 1.5% agar. After incubation for 1 to 5 days at 36°C, the set of bottles with medium containing agar was overlaid with another 4 mℓ of complete medium containing 1.5% agar and 0.1% neutral red and the cells observed for plaques over the next 5 days. All plaques were counted and representative plaques were picked and regrown in BGMK cells. The other set of bottles which had initially received 4 mℓ of liquid medium was observed for cytopathology (CP). If no CP was observed within 4 to 5 days, part of the original medium was removed, 3 mℓ of fresh, complete medium were added, and the cells observed for another 3 to 5 days. Bottles showing CP, as well as representative bottles not showing obvious CP after 7 days of incubation, were frozen and samples were inoculated into roller tubes of fresh cells to determine for the presence of virus. Tubes showing CP were subsequently purified by the plaque method and the recovered virus identified using the Lim-Benyesh Melnick (1960) antisera pool.

Sampling Areas

SAND ISLAND OUTFALL IN MĀMALA BAY. Studies conducted in this area can be divided into two phases: (1) the old sewage outfall (pre-December 1976) and (2) the new sewage outfall (post-December 1976). The old sewage outfall extended 1 097.3 m (3600 ft), at a depth of 12.2 m (40 ft), offshore from Sand Island into Māmala Bay (Fig. 1). The new sewage outfall extends approximately 2 743 m (9000 ft) from shore exclusive of a multi-porthole diffuser some 914.4 m (3000 ft) long and 73.2 m (240 ft) deep (Fig. 2). The new outfall was designed to provide a high and rapid dilution of the sewage (200:1); to cause a rapid and efficient dispersion of the sewage; to be minimally affected by winds, tides, and currents; and to take advantage of the normal thermocline of the ocean water at the depths involved (73.2 m) which causes the sewage to remain submerged.

PEARL CITY OUTFALL. The sewage outfall pipe extends 701 m (2300 ft), at a depth of 10.7 m (35 ft), into the Pearl Harbor Middle Loch. The site of the virus sampling station (PH) was 550 m southeast of the discharge pipe (Fig. 3).

KĀNE'OHE OUTFALL. Samples were taken from the bay water 460 m northeast of the old sewage outfall pipe (KB) which extended 701 m (2300 ft), at a depth of 9.1 m (30 ft), into Kāne'ohe Bay (Fig. 4).

MŌKAPU OUTFALL. Sewage from the waste water treatment plants (WWTPs) of Kāne'ohe, Kailua, and Kaneohe Marine Corps Air Station (KMCAS) was diverted to the new Mōkapu Outfall pipe which extends 1 541.07 m (5056 ft) into the ocean just slightly south of Mōkapu Point. The last 293.5 m (963 ft) of the outfall is a diffuser with 80 sideports that discharges at depths varying from 27 to 33 m (89-109 ft). The Kāne'ohe and Kailua WWTPs were connected to the Mōkapu Outfall on 21 December 1977, and the KMCAS WWTP in May 1978.

The sampling stations in the various study areas are described in Table 2.

Concentration of Viruses from Sewage

Samples of Honolulu's raw sewage just before it enters the ocean outfall pipe (Sta. S) and some of the ocean virus samples were collected on the same day and returned to the laboratory. Two-liter aliquots were concen-

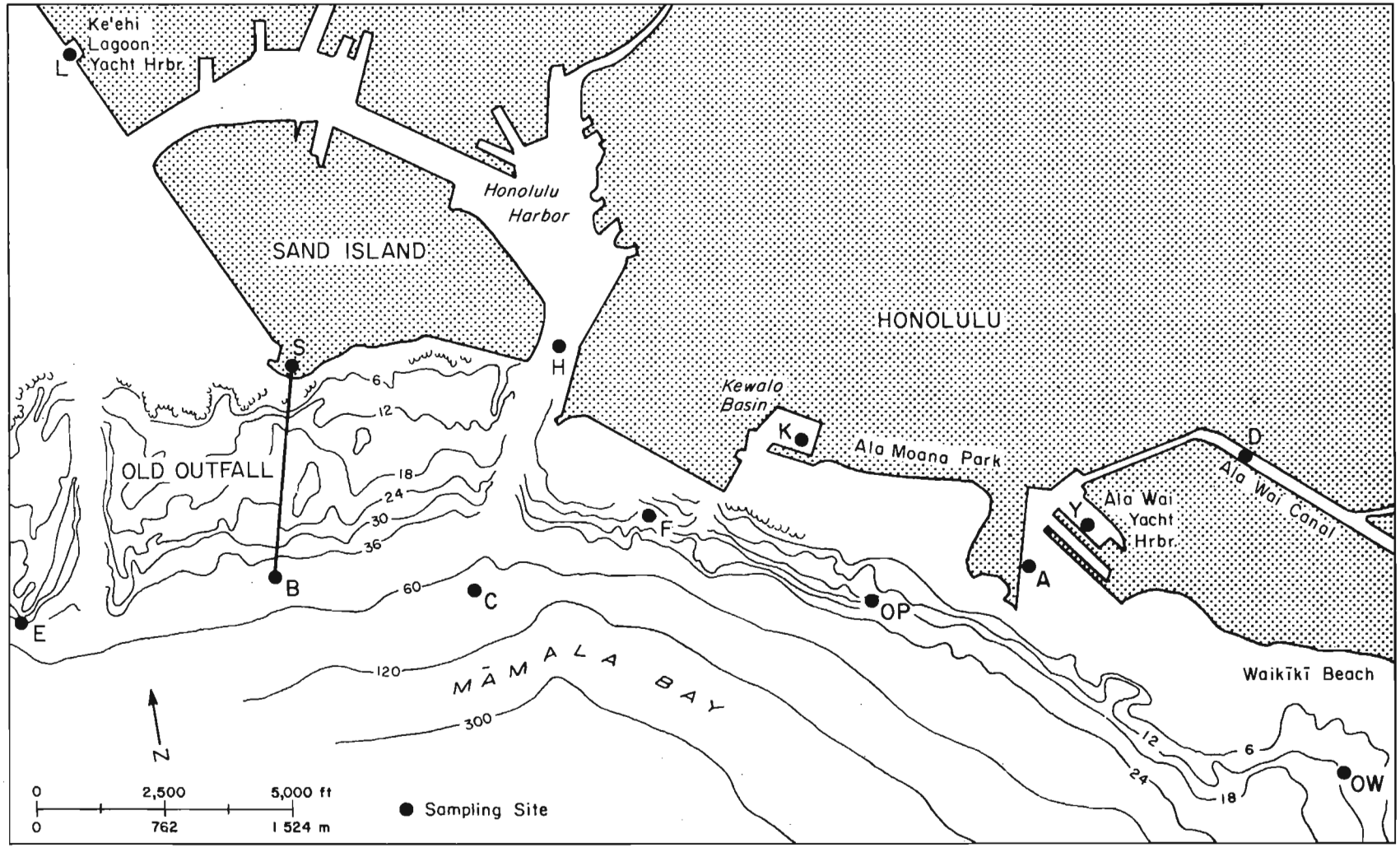


Figure 1. Virus sampling stations in and off Māmala Bay

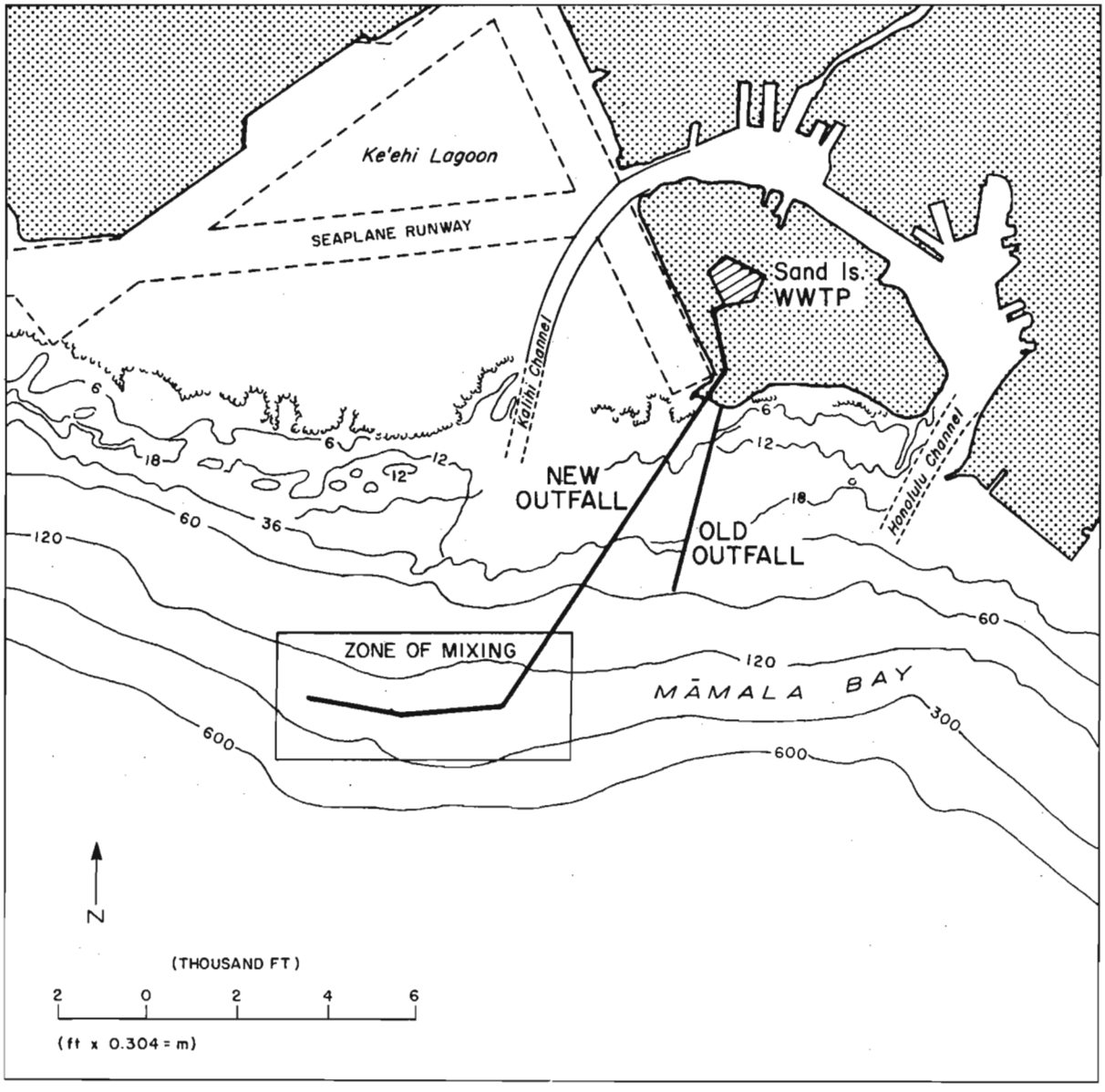


Figure 2. Location of new Sand Island ocean sewage outfall in relation to old outfall pipe in Māmala Bay

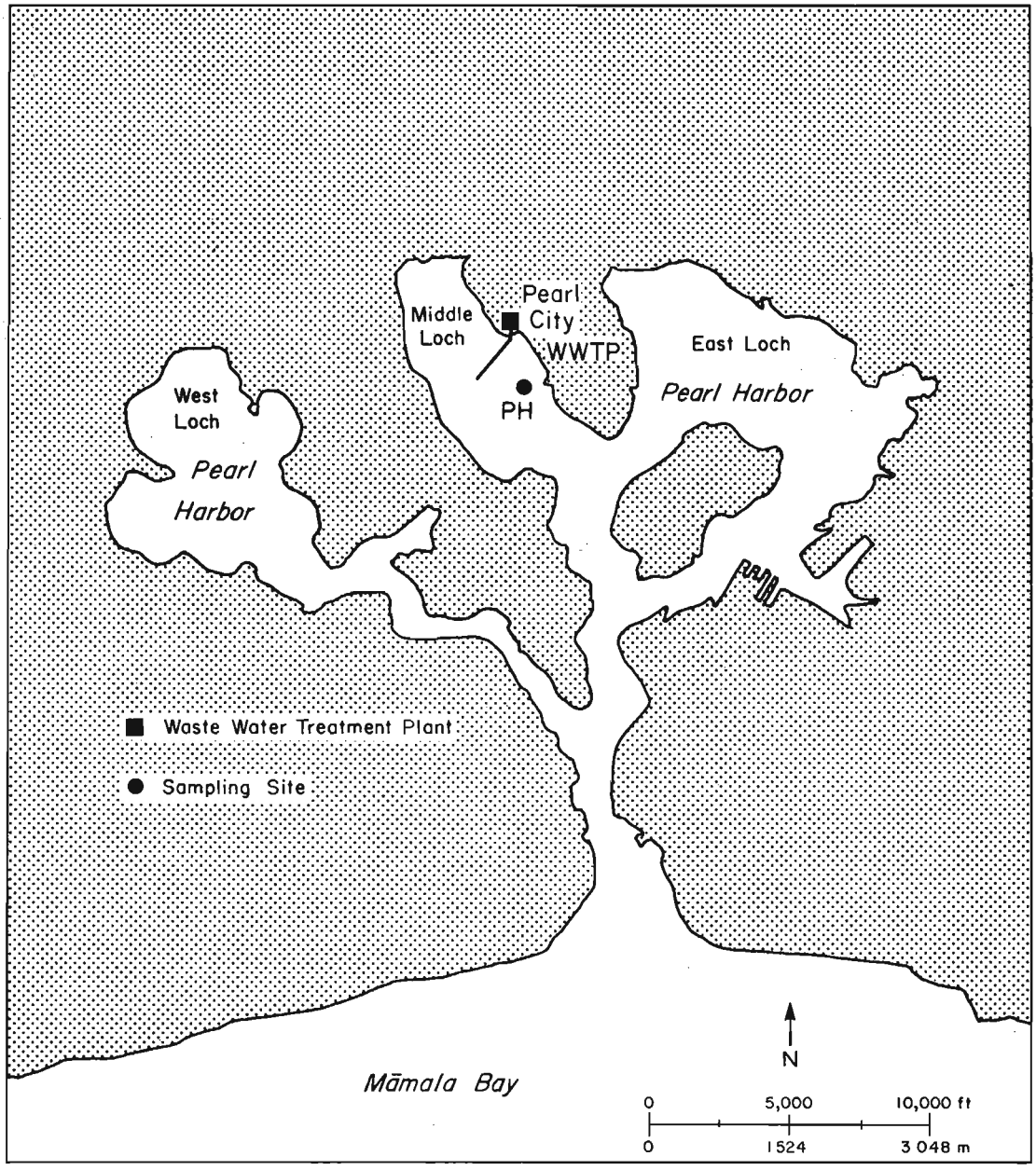


Figure 3. Sampling station in Pearl Harbor

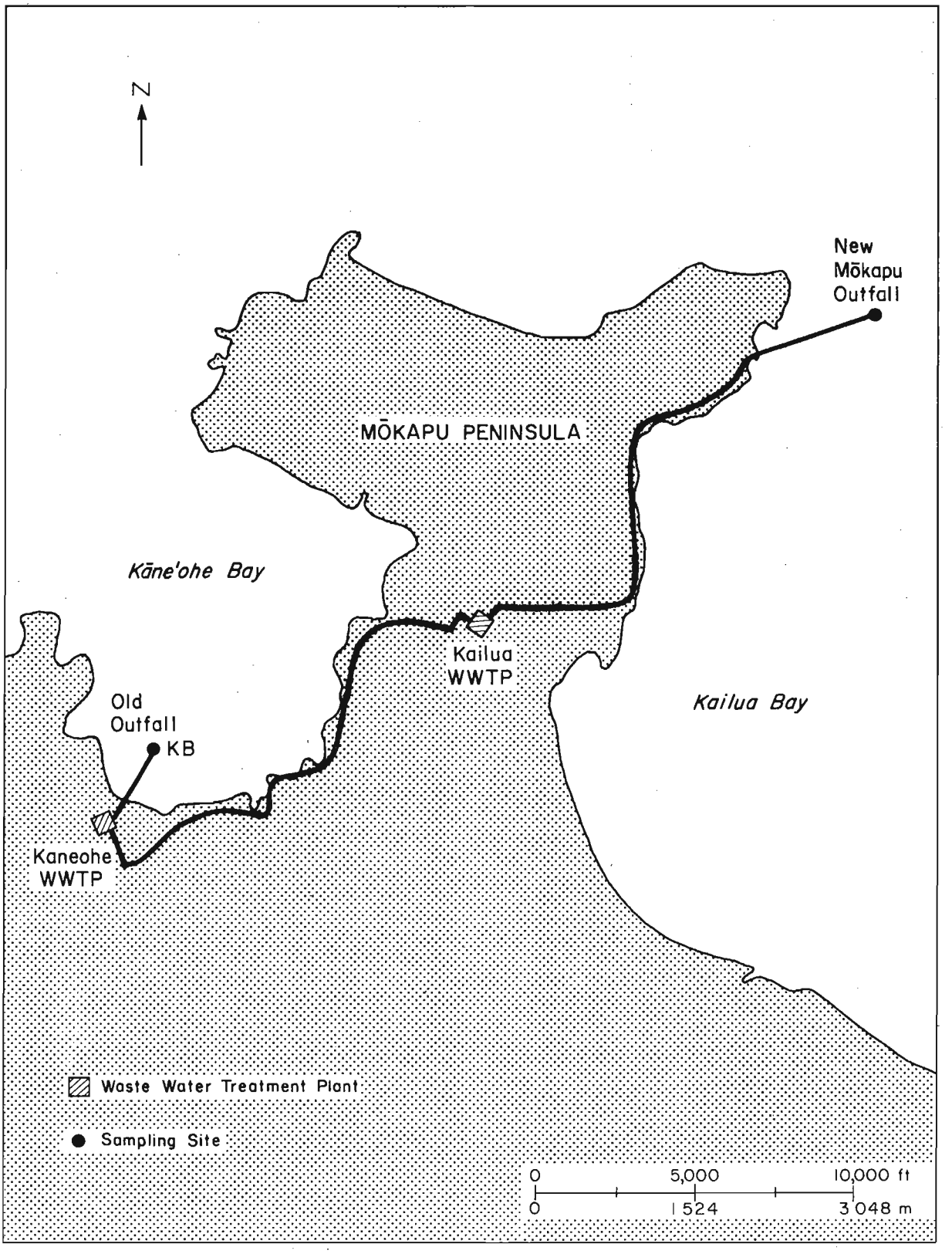


Figure 4. Sampling station in Kāne'ohe Bay and the new Mōkapu outfall pipe

TABLE 2. VIRUS SAMPLING STATIONS, O'AHU, HAWAI'I

Station	Description and Relative Distance from Outfall Site
Māmala Bay	
S	Sand Island WWTP outfall pipe just before discharge enters ocean pipe.
B	The boil or upwelling of the sewage at the sea water surface. This site is fairly constant and is located at the end of the old 1 097.3 m sewage outfall. The boil is the source of the plume whose size, shape, and general direction relative to the boil varies with environmental factors, such as winds, tides, and currents.
E	Approximately 1 600 m west of the boil in 31 m of water.
F	Approximately 0.8 m northeast of the boil in 37 m of water.
C	Approximately 1 200 m east of the boil in 12 m of water.
H	Mouth of Honolulu Harbor into Māmala Bay, approximately 2 400 m northeast of boil. A major stream running through Honolulu empties into Honolulu Harbor and flows into Māmala Bay.
OP	Approximately 3 200 m east of the boil and 800 m offshore from Ala Moana Beach in 9 m of water.
OW	Approximately 600 m east of the boil and 800 m offshore from Waikīkī Beach in 7.5 m of water.
D	Western end of Ala Wai Canal which collects fresh water from several streams from the Mānoa and Pālolo watersheds and empties into Māmala Bay.
A	Mouth of Ala Wai Canal, the point of discharge into Māmala Bay.
Y	Ala Wai Yacht Harbor, used primarily to dock pleasure and house boats with no direct hookup to the city's sewage line.
K	Kewalo Basin, a harbor used primarily for commercial boats.
L	Ke'ehi Lagoon Yacht Harbor, a marina is used for both commercial and pleasure boats, and house boats with no direct hookup to the city's sewage line.
Middle Loch, Pearl Harbor	
PH	Approximately 550 m south of the sewage discharge point into Pearl Harbor at a depth of only 3 m.
Kāne'ohe Bay	
KB	460 m northeast of the old sewage discharge point from the Kāne'ohe WWTP into Kāne'ohe Bay at a depth of 6 m.
Kailua Bay	
	20 to 40 m south of discharge pipe into Kailua Bay.

trated by the protamine sulfate methods as described previously.

Water Quality Parameters of Water Samples

Aliquots of the water samples assayed for virus were also analyzed for various other water quality parameters including total and fecal coliforms, fecal streptococci, suspended solids, turbidity, and salinity. The methods for these measurements are described in *Standard Methods* (APHA, AWWA, WPCF 1976).

Concentration of Viruses from Sea Waters

A commercial model (Aquella) of the portable virus concentrator developed and evaluated by Wallis et al. (1972) was leased from Carborundum Company (Niagara Falls, N.Y.) (Figs. 5, 6; Table 3; App. A). This virus concentrator pumps the water samples through a series of clarifying filters after which a proportioner pump injects $AlCl_3$ and HCl to condition the water to a final pH of 3.5 and an Al^{+++} concentration of 0.0005 to 0.001 *M*. The conditioned water is then pumped through a second set of filters which preferentially adsorbs the viruses from the water. The adsorbing filters are then rinsed with 0.004 m^3 (1 gal) of physiological saline at pH 3.5 to remove excess Al^{+++} as well as other heavy metals before the filters are eluted. All filters attached to the virus concentrator are 0.26 m, wound tubular filters (Commercial Filters, Inc., Lebanon, Indiana) housed individually in transparent plastic cartridge holders. The first 3 clarifying filters have the following chemical composition and porosity: filter no. 1, orlon, 10 µm; filter no. 2, orlon, 1 µm; and filter no. 3, cellulose nitrate, 1 µm. Filter no. 4 (K-27), which is of fiberglass with a porosity of 3 µm, is used as a virus adsorbent. In addition, a sandwich of two 293 mm diameter, epoxy-fiberglass membrane filters of 5 and 0.45 µm porosities, respectively, housed in a stainless steel membrane filter holder (Cox Instrument Company, Detroit, Michigan) is also used to adsorb viruses. The complete set of three clarifying filters plus the K-27 and the Cox membranes as adsorbing filters were used during the initial phase of this study to primarily sample water from within the plume area of the ocean outfall. When cleaner waters outside the plume were sampled, only filter nos. 2 and 3 were used as clarifiers and two K-27 in series were used to adsorb viruses.

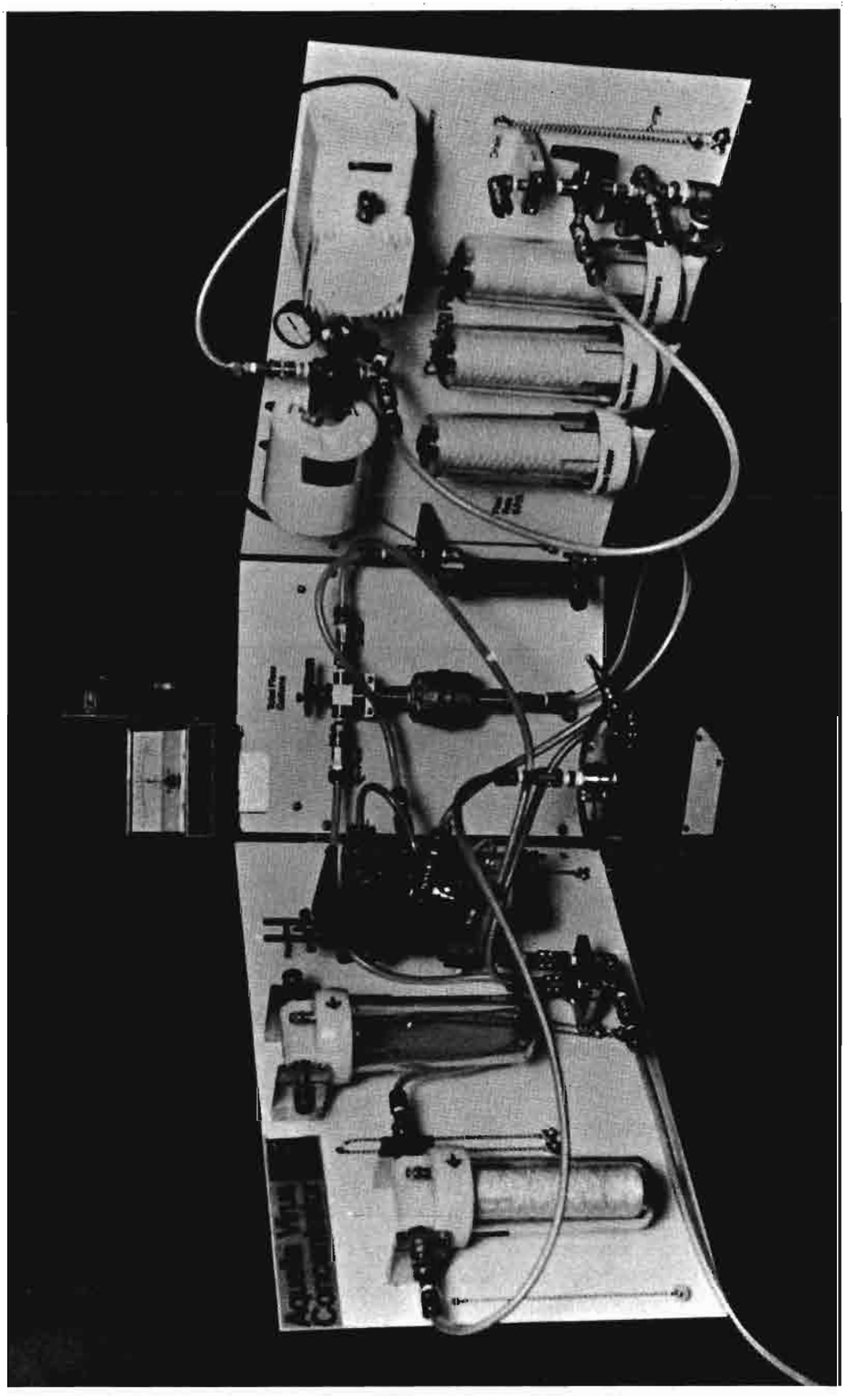


Figure 5. Aquella portable virus concentrator

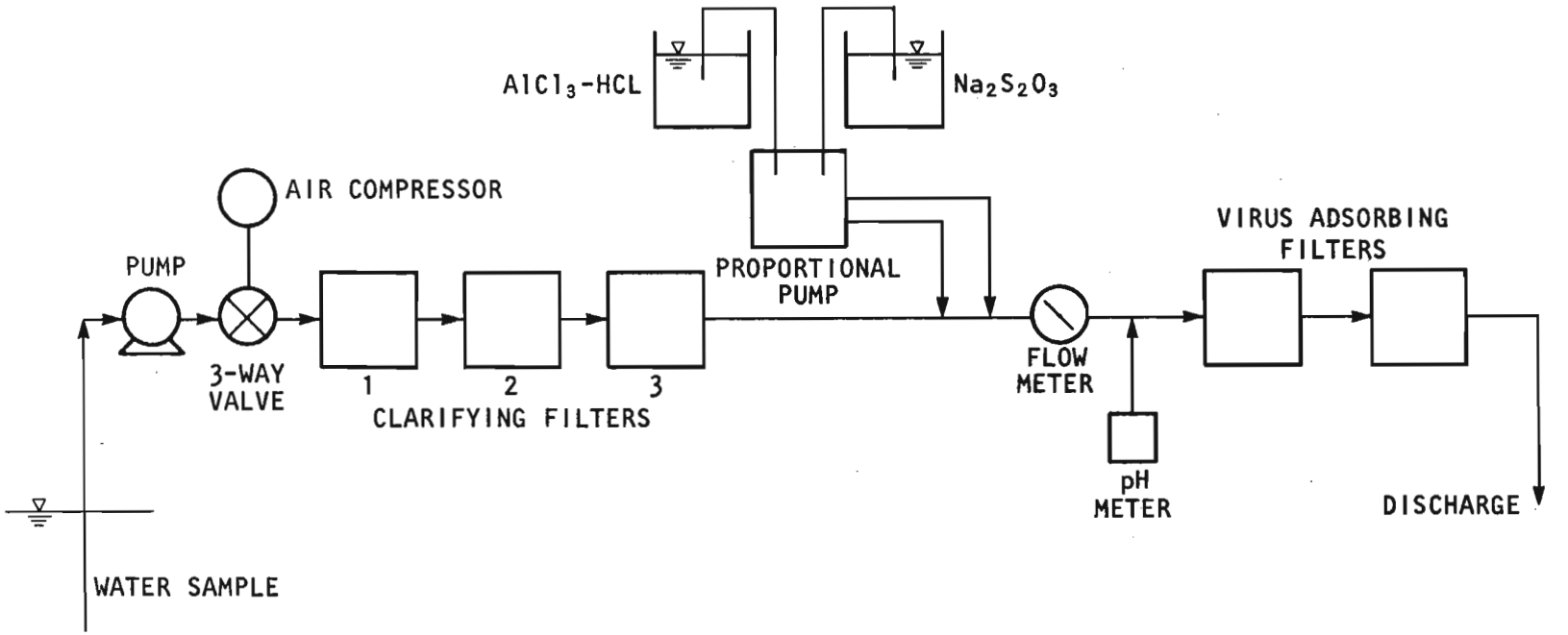
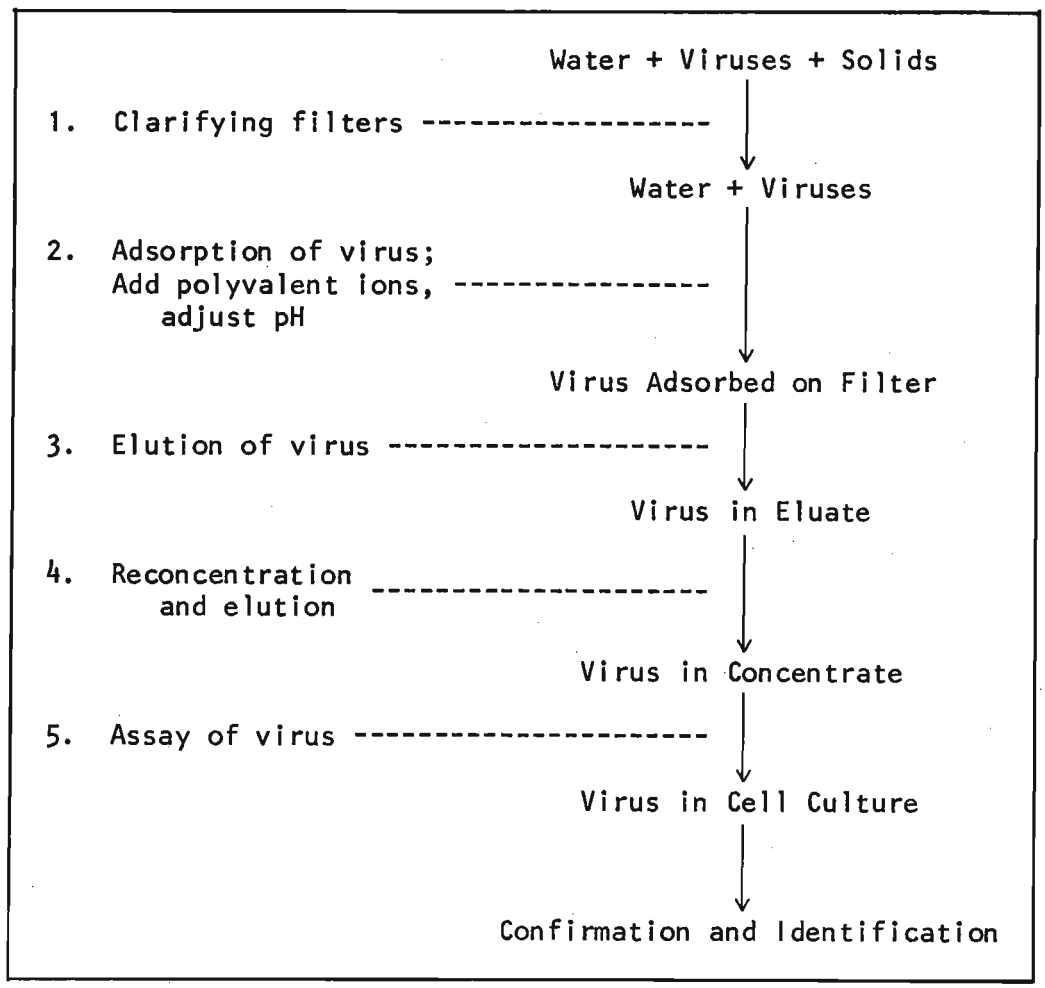


Figure 6. Operational diagram of automatic virus concentrator

TABLE 3. SEQUENCE OF STEPS TO RECOVER VIRUSES



The virus concentrator was transported to the ocean site by a boat anchored for the 1- to 4-hr duration of sampling. Unless otherwise specified, all ocean water samples were taken 0.3 to 0.6 m (1-2 ft) from the surface of the ocean.

Elution and Reconstruction of Viruses from Filters

To elute viruses, 1 to 2 ℓ of pH 11 to 11.5 glycine (0.05-0.1 *M*) were forced through all filters used (maximum contact time 1-2 min). The eluate was immediately neutralized with pH 2 glycine (0.1-0.5 *M*) resulting in a final volume of 2 to 4 ℓ which necessitated another concentration process (reconcentration) to reduce the volume to 5 to 15 mℓ before the samples were inoculated into cell cultures. The following methods were used to reconcentrate the eluate.

Membrane Adsorption

The procedure as described previously by Sobsey et al. (1973) was used. Briefly, the eluate (1-4 ℓ) was acidified to pH 4 with pH 2 glycine and 0.6 *N* HCl followed by the addition of $AlCl_3$ to a final concentration of 0.0005 *M*. The mixture was slowly filtered through a sandwich of 5 μm and 0.45 μm epoxy-fiberglass filter of 125-mm diameter (Cox Instrument Company, Detroit, Michigan). The viruses adsorbed to these filters were then re-eluted by slowly filtering through 100 mℓ of 0.05, glycine, pH 10.5, and the eluate neutralized immediately with pH 2, 0.05 *M* glycine. This virus containing eluate was then further concentrated by filtration through a 0.45 μm epoxy-fiberglass filter of 47-mm diameter (Cox Instrument Company, Detroit, Michigan), which was subsequently eluted with 5 to 10 mℓ of pH 10, 5, 0.05 *M* glycine and immediately neutralized with pH 2, 0.05 *M* glycine.

Precipitation with $AlCl_3$ or $FeCl_3$

Both methods have been previously described by Sobsey et al. (1973) and are very similar except that $AlCl_3$ was added to a final concentration of 0.003 *M* while $FeCl_3$ was added to the neutralized eluate followed by the addition of 1 *M* Na_2CO_3 until the pH was adjusted to pH 7.0. After mixing for 30 min, the mixture was allowed to stand for 30 to 45 min to allow the floc which formed to settle. The supernatant was then aspirated off and discarded while the suspended floc was centrifuged at 10,000 rpm for 10 min to sediment the floc. The packed floc which has an appearance of a gel was then thoroughly mixed for 4 min with twice its volume of either 0.05 *M* glycine or borate buffer at pH 10.5 plus 10% calf serum followed by centrifugation at 10,000 rpm for 5 min. The supernatant was saved and immediately neutralized with pH 2 glycine.

Two Phase Separation

A modification of the method as described by Shuval (1971) was used. Since the eluate normally contains relatively high concentrations of salts, it was initially diluted with distilled water until the conductivity was less than 80 000 Ω (<0.2 *M* NaCl). This was followed by the addition of 64.5 g/ℓ of polyethylene glycol 6000 (PEG) (J.T. Baker), 2 g/ℓ of sodium

dextran sulfate (Pharmacia Fine Chemicals) and NaCl to raise the conductivity to 133 000 Ω or 0.3 *M* NaCl. After thorough mixing and allowing the phases to separate at 4°C for 15 to 20 hr, the lower phase plus the interphase were collected and either 50 mg/ℓ of NaCl or KCl was added. The top aqueous phase which formed was then collected and was either diluted 1 to 7 with distilled water or dialyzed against phosphate buffered saline (PBS) before being used to inoculate cell cultures.

RESULTS

Efficiency of the Virus Concentrator

To determine the expected efficiency of recovering viruses from sea water using the virus concentrator (Aquella), poliovirus type 1 used as a marker virus (final concentration of 1-3 × 10^3 PFU/mℓ) was seeded into 0.15 to 0.19 m^3 (40-50 gal) of Māmala Bay water and processed through the concentrator at the maximum flow rate of 5.25 to 6.3 × 10^{-5} m^3/s (50-60 gph). Initially, three marker type experiments were performed using filter nos. 1, 2, and 3 to clarify the water and filter no. 4 plus the 293-mm Cox membranes to adsorb the virus. The two adsorbing filters were eluted with 1 ℓ of 0.05 *M* glycine (pH 11.5) which was immediately neutralized with 1 ℓ of 0.05 *M* glycine (pH 2), and the eluate reconcentrated by the membrane-adsorption-elution method. The results of these three marker experiments revealed that 10 to 25% of the input virus was recovered in the eluate of the adsorbing filters, but that less than 1% of the virus was recovered in the final concentrate, indicating that virus reconcentration by the membrane adsorption-elution method was very inefficient. To further evaluate the efficiency of the virus concentrator and to examine ways to increase its efficiency, three additional marker type studies were carried out. However, in these experiments, 4-ℓ samples of the 38th, 76th, 114th, and 152d ℓ of the processed water leaving the concentrator (the effluent) were assayed for virus. All filters were individually eluted as described previously and their eluates assayed for virus. The eluates of the K-27 filter (no. 4) and the Cox membranes were then divided in half with one half reconcentrated by the membrane adsorption-elution method and the other by the $AlCl_3$ precipitation method.

The results of these experiments revealed that less than 1% of the

viruses was recovered in the effluent samples, thus indicating that greater than 99% of the input virus was being removed from the water by the virus concentrator. Furthermore, less than 1% of the virus was recovered from the eluates of each of the clarifying filters, while 28, 24, and 33% of the virus were recovered from the eluates of the K-27 filters, and 2, 5, and 8% of the virus were recovered from the eluates of the Cox membranes. Thus, only 29 to 41% of the input virus was recovered from the adsorbing filters and about 60 to 70% of the virus could not be accounted for. Reconcentration of the eluates from the virus-adsorbing filters (filter no. 4, Cox membranes) by the membrane adsorption-elution technique resulted in recovering only 3, 1, and 5%, respectively, of the virus; whereas, when the $AlCl_3$ precipitation method was used, 10, 13, and 23%, respectively, of the virus were recovered.

In the initial field studies, especially when samples were taken from within the plume area, the complete set of 3 clarifying plus 2 virus adsorbing filters was used. Under these conditions, the clarifying filters accumulated visible debris and, as a result of the clogging of the Cox membranes, the flow rate decreased. Although viruses were most frequently recovered from the concentrates of the K-27 filter eluate, viruses were occasionally recovered from the eluate concentrates of the clarifying filters, and, on a single occasion, in the absence of virus recovery from the adsorbing filters. The additional steps of recovering viruses from the clarifying filters consequently greatly increased the time and effort required to process one sample. To simplify and facilitate the processing procedure while still maintaining a high flow rate, only clarifying filter nos. 2 and 3 plus two K-27 filters were used in sampling the "cleaner" waters outside of the plume area. The virus recovery efficiency of this modified arrangement was evaluated as previously described using two 0.15-m^3 (40-gal) samples of sea water seeded with poliovirus. The results revealed that 1 and 3% of the virus were recovered in the effluent indicating that 97 to 99% of the input virus were being removed by this modified virus concentrator. In the first experiment, 2 and 7% of the virus were recovered from clarifying filter nos. 2 and 3, respectively; while in the second experiment, less than 1% of the virus was recovered from the eluates of these clarifying filters. Most of the virus (27 and 31%) was recovered from the first K-27 adsorbing filter while 8 and 10% were recovered from the second

K-27 adsorbing filter. Reconcentration of the eluates by the $AlCl_3$ precipitation method resulted in a recovery of 12 and 17% of the input virus from the first K-27 filter while 2 and 5% of the virus were recovered from the second K-27 filter. Thus, 35 and 41% of the input virus were recovered by eluting the two K-27 filters, but only 14 and 22% of the virus were recovered after concentrating the eluates from these two adsorbing filters. Again, more than 50% of the input virus was not accounted for and was probably due to inefficient elution of the virus from the filters, although inactivation and aggregation resulting from the processing procedure may also be involved. To further improve the efficiency of reconcentrating viruses from filter eluates, poliovirus type 1 was seeded into the eluates of K-27 filters and the following methods for reconcentration evaluated: 200-mℓ aliquots were reconcentrated by precipitation with either $AlCl_3$, $FeCl_3$, or by the polymer 2-phase method (Farrah et al. 1976; Shuval et al. 1969; and Sobsey et al. 1973). With clear eluates, all three methods concentrated 50 to 60% of the added virus. However, when the eluates were turbid, interference with the efficiency of virus recovery was greatest using the polymer two-phase and least with the $AlCl_3$ precipitation method. Consequently, eluates of filters were preferentially reconcentrated using the $AlCl_3$ precipitation method although the $FeCl_3$ precipitation, polymer two-phase, or membrane adsorption methods were also used on occasion.

Recovery of Human Enteric Viruses from the Old Sewage Outfall Area in Māmala Bay

In this phase of the study, the primary objective was to determine whether infectious human enteric viruses could be recovered with the virus concentrator from sites within and surrounding the plume of the ocean sewage outfall in Māmala Bay. The visible sewage boil and the plume surrounding the boil in Māmala Bay were taken as points of reference in the selection of sampling sites. The sampling regimen was to collect samples at and in all directions from the boil but within the plume, and outside of the plume until viruses could no longer be recovered. After it was demonstrated that viruses could be recovered from sites other than the sewage boil, several water quality measurements of water samples, such as turbidity, salinity, and indicator bacteria were also initiated.

The results summarized in Table 4 indicated that within the sewage

TABLE 4. RECOVERY OF VIRUSES FROM MĀMALA BAY, O'AHU, HAWAI'I, PHASE 1

EXPERIMENT NO.		DATE	DISTANCE (m)	VOL. (m^3)	VIRUS*	TURB. (NTU)	SS (mg/ℓ)	Cl (g/ℓ)	COLIFORM Total	COLIFORM Fecal	FECAL STREP.	FC:FS RATIO
									------(Colonies/100 mℓ)------			
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 +P-3	5.3	--	19.0	1×10^5	3.4×10^4	6.5×10^4	0.52
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 +CB4	0.59	7.6	19.9	9	9	94	-----
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	1.32×10^3	6.1×10^2	1.94×10^4	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	3.4×10^3	1.3×10^2	1.6×10^4	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	3×10^4	60	4.2×10^4	0.001
West of Plume												
1	A-39	02/02/76	228.6	0.28	+P-1	0.18	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	1×10^4	1×10^3	1×10^4	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	12/30/76	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 0.3- to 0.6-m depths below water's surface except A-44 where sampling depth was 1.8 m.

NOTE: SS = suspended solids; Cl = chloride; NTU = nephelometric turbidity units.

*CB = Coxsackie B; E = Echo; P = Polio; Neg. = Negative.

plume 6 of 7 samples ranging from 0.08 to 0.19 m^3 (20-50 gal)/sample were positive for virus. The recovered viruses were determined to be typical of human sewage origin (polio 1, 2, 3; coxsackie B-2, -4, -5; and echo-7). Significantly, 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 sewage-borne viruses are present and can be consistently recovered from the surface of the ocean water within the sewage outfall plume by the virus concentrator. To determine whether viruses could also be recovered from sites outside of the plume, 0.19- to 0.38-m^3 (50- to 100-gal) samples were taken at various distances and directions from the plume. More samples were taken east of the plume than in any other direction because recreational beaches are in this direction and the major concern is of the dispersion of sewage-borne viruses to these areas.

Of 12 samples taken east of the plume, 6 were determined to be positive for virus at distances ranging from 91.4 to 685.8 m (100-750 yd) away from the plume (Table 4). A single sample, 6 436 m (4 miles) east of the plume and off Waikīkī Beach, was negative 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 distances ranging from 91 to 228.6 m (100-250 yd) away from the plume. The viruses recovered outside of the plume were identified and determined to be similar to those recovered from within the plume (Table 4). These results indicate that viable sewage-borne viruses were being dispersed from the plume to visibly cleaner waters in all directions and as far away as 685.8 m (750 yd) from the plume.

Source and Dispersion of Sewage and Viruses in Māmala Bay

To determine the source and dispersion patterns of sewage and viruses in Māmala Bay, fixed sampling stations (Table 2, p. 12) were selected and samples were collected from these stations during either rising or ebbing tide conditions. In addition to measuring the direction and velocity of wind and water currents at these stations, water samples were analyzed for human enteroviruses, indicator bacteria as well as for turbidity, and chloride concentrations. Analysis of the raw sewage entering the ocean (Table 5) revealed high turbidity, 26 to 60 NTU; low chloride, 9 to 20 g/ℓ; and high enteric bacteria concentrations of total coliform, 8 to 85 × 10^6/100 mℓ; fecal coliform, 4 to 51 × 10^6/100 mℓ; and fecal streptococcus, 1 to 6 × 10^6/

TABLE 5. ANALYSIS OF UNTREATED SEWAGE AT STATION S, MĀMALA BAY, O'AHU, HAWAI'I

1976	Chloride (g/ℓ)	Turbidity (NTU)*	Total Colif. (× 10^6/100 mℓ)	Fecal Colif. (× 10^6/100 mℓ)	Fecal Strep. (× 10^6/100 mℓ)	Fecal Colif. Fecal Strep. Ratio	Virus Conc. (PFU/ℓ)	Virus Identified†
05/11	NA‡	NA	NA	NA	NA	NA	122	P1
05/14	9.2	50	8	4.8	1.8	2.67	420	CB4
05/24	9.0	60	78	51	6	8.5	399	CB4
06/14	8.7	50	39	16	2	8.0	122	E7
06/28	11.0	60	85	12	4	3.0	42	CB4
08/02	13.0	26	17	4	2	2.0	175	CB2
08/26	NA	NA	NA	NA	NA	NA	35	P2
09/01	NA	NA	18	5	1	5.0	100	P2
09/13	15.0	NA	12	8	3	2.7	18	E22, E23
09/22	19.0	36	19	1.9	1.4	1.36	28	CB2
09/27	20.0	37	38	3.6	1	3.6	10	CB2
10/05	17.0	42	31	21	3	7.0	402	NA
10/20	16.0	40	72	22	2	11.0	57	NA

NOTE: 2-ℓ samples taken (just before sewage entered outfall pipe leading to Māmala Bay) and concentrated by protamine sulfate method to recover virus.

*NTU = Nephelometric turbidity units.

†P1 = Poliovirus type 1
P2 = Poliovirus type 2
CB4 = Coxsackievirus B4
CB2 = Coxsackievirus B2
E7 = Echovirus type 7
E22 = Echovirus type 22
E23 = Echovirus type 23.

‡NA = Not analyzed.

100 mℓ. Of the samples assayed, 100% (12/12) were positive for virus at concentrations ranging from 10 to 470 PFU/ℓ. Unlike virus and bacterial populations in marine waters, the turbidity and chloride measurements are consistent and stable and can be used to determine the approximate dilution of sewage after it enters the ocean. The results (Table 6) show that at the boil (sta. B), the average turbidity and chloride values were respectively 3.1 NTU and 18.3 g/ℓ, indicating that the sewage is diluted by a factor of 13 to 16 times as it reaches the ocean surface. Based on chloride analysis, all other open ocean sites (stas. E, F, C, OP, OW) were found to be completely diluted in sea water. However, turbidity measurements of samples taken from station E suggested a slight contamination of sewage material and supports the visual observation that the sewage plume tails off gradually in the direction of this station. Indicator bacteria were recovered from stations B, S, and E but not from stations C, OP, and OW, confirming the implication of sewage contamination by turbidity measurements. Significantly, enteroviruses were recovered from stations revealing evidence of sewage pollution (stas. S, B, E) as well as from sites where no contamination by sewage material (stas. C, OP) was detectable. This strongly implies that viruses are more stable than indicator bacteria in marine waters and that indicator bacterial analysis of marine waters should not be interpreted to mean the absence of human enteric viruses. It is of further significance that while the FC to FS ratios (fecal coliform:fecal streptococcus) of the raw sewage entering the ocean was greater than 4, indicating that the sewage was predominantly of human origin, the ratios of samples obtained from ocean water sites were generally much less than 4 and suggest that the significance of this ratio may not be valid under marine conditions. Preliminary studies indicated that fecal coliform bacteria were considerably less stable in sea water than in fresh water and that fecal streptococci were relatively stable in sea water.

The presence and effective movement of viruses from the ocean sewage boil throughout Māmala Bay are dependent principally on the stability of the viruses in the marine environment and ocean currents. However, ocean currents are influenced by several factors, such as tides and winds, and can be further complicated by eddies formed and modified by the topography of the land. Since tides have a predictable and sustained effect on the direction and velocity of ocean currents, ocean water samples were collected

TABLE 6. RECOVERY OF VIRUSES FROM MĀMALA BAY, O'AHU, HAWAI'I

STA. NO.	EXPERIMENT NO.	DATE	VOLUME (m^3)	TIDE*	VIRUS†	TURBIDITY (NTU)	SUSPENDED SOLIDS (mg/ℓ)	CHLORIDE (g/ℓ)	COLIFORM Total (Colonies/100 mℓ)	COLIFORM Fecal (Colonies/100 mℓ)	FECAL STREP. (Colonies/100 mℓ)	FC:FS RATIO
B‡	A-60	05/11/76	0.09	R	+CB4 +P-2	2.8	44	18.2	8×10^4	3.6×10^4	5×10^4	0.72
	A-72	07/28/76	0.19	R	+P-1 +P-2	3.5	31	18.5	4×10^5	2.4×10^4	4×10^4	0.60
C	A-52	03/22/76	0.58	E	Neg.	0.12	17	19.5	<2	<2	<2	----
	A-59	05/11/76	0.42	R	Neg.	0.2	29	19.3	18	2	14	0.14
	A-67	06/14/76	0.35	R	Neg.	0.23	27	19.2	--	2	6	0.33
OP	A-56	04/26/76	0.48	R	Neg.	----	--	----	--	--	--	----
	A-55	04/21/76	0.56	R	Neg.	0.38	15	19.3	<10	<10	<10	----
	A-61	05/14/76	0.41	E	+P-1	0.2	21	19.3	<2	<2	<2	----
	A-73	08/02/76	0.38	R	+P-2	0.2	13	19.4	<10	<10	<10	----
OW	A-62	05/14/76	0.42	R	Neg.	0.2	31	19.3	<2	<2	<2	----
	A-69	06/28/76	0.53	R	Neg.	0.1	31	19.1	<1	<1	<1	----
	A-71	07/28/76	0.56	E	Neg.	0.3	24	19.5	<10	<10	<10	----
A	A-58	05/03/76	0.11	E	Neg.	1.0	19	16.3	10	30	10	3.00
	A-70	07/21/76	0.51	E	Neg.	4.0	12	15.9	600	100	60	1.67
H	A-63	05/24/76	0.37	R	Neg.	0.8	44	19.0	70	<10	20	----
	A-82	09/22/76	0.47	R	+P-1	---	--	----	--	--	--	----
D	A-65	05/26/76	0.34	R	+P-2	2.0	18	9.8	2.3×10^4	2.7×10^3	17	158
	A-102	02/28/77	0.57	R	+P-1	4.7	--	17.5	510	0	0	90
	A-103	03/07/77	0.34	E	----	3.0	--	17.6	1.1×10^3	8	8	390
	A-104	03/14/77	0.38	R	----	2.6	--	18.1	7.2×10^3	0	0	140
E	A-66	06/15/76	0.39	R	----	0.48	--	19.1	NA	-	80	50
	A-74	08/02/76	0.38	E	----	0.32	--	19.4	40	-	10	420
	A-76	08/26/76	0.68	E	----	NA	--	NA	NA	-	NA	NA
	A-78	09/01/76	0.41	R	----	NA	--	NA	80	-	10	30
	A-83[a]	09/27/76	0.36	E	----	0.47	--	19.3	370	-	150	790
	A-84[b]	09/27/76	0.34	E	+P-1	0.54	--	19.4	20	-	<10	<10
F	A-79	09/01/76	0.36	R	----	NA	--	NA	<2	-	<2	<2
	A-80	09/13/76	0.55	E	+P-1	NA	--	19.4	<10	-	<10	<10
K	A-87	10/12/76	0.57	E	----	2.6	--	19.3	0	-	0	0
	A-64	05/24/76	0.36	R	----	0.8	--	19.0	30	-	<10	<10
L	A-54	04/13/76	0.20	E	+P-1	1.6	--	19.3	6	-	20	2

NOTE: All samples taken at 0.3- to 0.6-m depths below water's surface.
NOTE: NTU = Nephelometric turbidity units; NA = Not analyzed.
*R = Rising; E = Ebbing.
†Neg. = Negative; CB = Coxsackie B; P = Polio; E = Echo.
‡At boil.
[a]Sample taken from Zone of Mixing.
[b]Sample taken outside of Zone of Mixing.

from selected stations in Māmala Bay during rising or ebbing tides on the rationale that the rising tide results in a net flow of water landward or northeast of the boil while ebbing tide results in a net flow of water seaward or southwest of the boil.

As expected, viruses were most frequently recovered (4 positive isolations from 5 attempts) from relatively small volumes (0.08 to 0.19 m^3 [21-50 gal])/sample) at the boil (sta. B) (Table 6). At station F (804.5 m [½ mile] south of boil), a 0.37-m^3 (97-gal) sample taken during rising tide was negative for virus while a 0.55-m^3 (145-gal) sample taken during ebbing tides was positive for virus. At station E (1 609 m [1 mile] west of the boil), only 1 of 6 samples was positive for virus when 0.33- to 0.68-m^3 (88- to 180-gal) samples were taken during both rising and ebbing tides. The positive virus isolations at stations E and F were made during ebbing tides when the net flow of water was towards those stations.

Since a major concern of this study was the movement of viruses east of the boil towards recreational beaches, stations C, OP, and OW located respectively 1 206.75 m (0.75 mile), 3 218 m (2 miles), and 6 436 m (4 miles) were selected along an imaginary line drawn between the boil and 804.5 m off Waikīkī Beach. Although 3 of 3 samples (0.35-0.58 m^3 [92-153 gal]/sample) taken from station C during rising tidal conditions were negative for virus, 4 of 5 samples taken at sites near this station during Phase 1 were positive for virus. At station OP (804.5 m off Ala Moana Beach), 3 of 5 samples (0.38-0.56 m^3 [100-149 gal]/sample) taken were positive for virus. Two of the positive samples were taken during the end of a rising tidal cycle while one of the positive samples was taken during the end of an ebbing tidal cycle. The two negative samples were taken during early and late rising tidal conditions. At station OW (804.5 m off Waikīkī Beach), 4 samples (0.38-0.76 m^3 [100-200 gal]/sample) taken during early and late rising tidal conditions were negative for virus. A single 0.76-m^3 sample taken at this same site during Phase 1 of this study was also negative for virus. Thus, the maximum distance and direction from the boil from which viruses were recovered were 804.5 m south (sta. F), 1 609 m (1 mile) west (sta. E), and 3 218 m (2 miles) east (sta. OP). It should be noted that the depths of the water at these sampling stations vary: 36.6 m (120 ft) at station F south of the boil; 30.48 m (100 ft) at station E west of the boil; 12.19 m (40 ft) at station C, and 6.1 m (20 ft) at stations OP and OW, respectively, northeast of the boil. Thus, the theoretical dilution of sewage and its

components are not equal in all directions of the boil. Although more positive virus isolations were made when samples were taken from stations in the direction of the tidal currents, the association between movement of viruses and tidal patterns was not always predictable.

The results were not unexpected since the samples were taken near the surface of the ocean and surface water currents are greatly affected by the velocity and direction of the wind and eddies formed by water deflecting off the island's coastline. Although wind and water current measurements were taken at the sampling sites, it was observed that these measurements varied considerably over the 1 to 4 hr sampling period. Thus, the direct effect of tides, winds, and surface water currents on the dispersion pattern of viruses from the sewage ocean outfall throughout Māmala Bay could not be definitely established.

Although ocean currents are responsible for the movement of viruses, the maximum dissemination of viable viruses will be limited by their relative stability in the marine environment. To determine the relative stability of enteric viruses in Māmala Bay, poliovirus type 1 (LSc2ab) was selected as the representative virus for study. To 1-ℓ samples of sterile phosphate-buffered saline (PBS), as well as Māmala Bay waters obtained from the plume and from 6 436 m (4 miles) east of the plume (sta. OW), were added 1 mℓ of purified poliovirus (10^7 PFU $m\ell^{-1}$). The samples were continuously mixed at 24 ±1°C and, at various time intervals, 0.5-mℓ aliquots were removed and assayed for virus. The results in Figure 7 show that under these conditions, poliovirus in PBS was stable for 4 days. However, in both Māmala Bay water samples, poliovirus was stable for about 1 day and was rapidly inactivated during the next 3 days. These results suggest that human enteric viruses can remain viable for at least 24 hr after being discharged into Māmala Bay, after which time they are rapidly inactivated. Under these experimental conditions, the T_{90} of poliovirus type 1 is approximately 48 hr. The results also indicate that the factors responsible for inactivating poliovirus in the waters of Māmala Bay are present in waters contaminated with sewage as well as those which are apparently free of sewage pollution. However, if viruses remain viable up to 24 hr after they enter the ocean environment, they may be extensively dispersed before they are inactivated.

Although all viruses recovered from Māmala Bay were determined to be

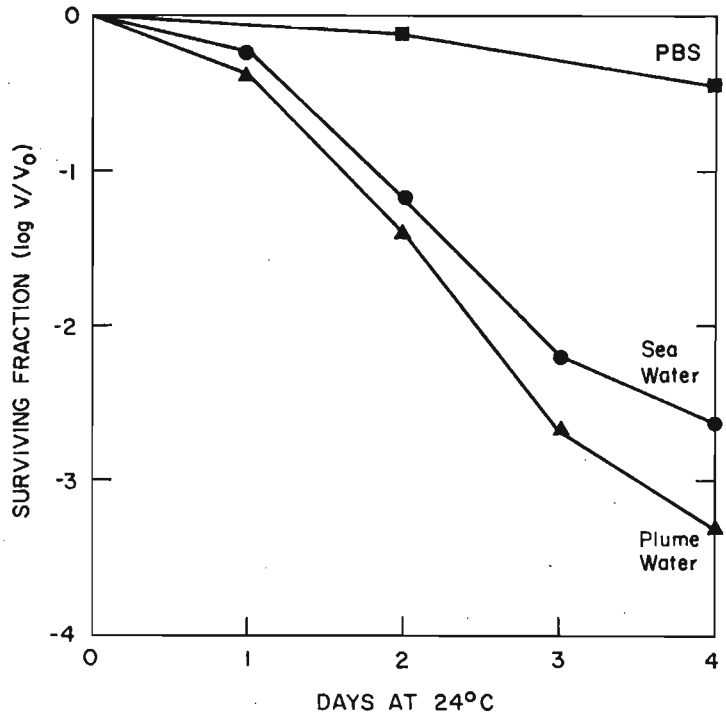


Figure 7. Inactivation of poliovirus in Māmala Bay waters

those found commonly in human sewage (Tables 4-6), it cannot be assumed that the ocean sewage outfall is the sole source for these viruses. To assess the possibility that harbor waters used heavily by man or that streams which empty into Māmala Bay may also serve as sources of viruses, several sampling stations were selected near these sources (no NPDES permits for sewage effluent discharge were issued for the area): the two major entrances of fresh water into the study area in Māmala Bay are Honolulu Channel (sta. H) and Ala Wai Canal (sta. A). The movement of viruses in and out of these channels was assumed to follow the tidal pattern since the flow of water from these channels into Māmala Bay was observed during rising tides. Consequently, when 1 of 2 samples taken from station H during rising tides was positive for virus, it was concluded that the recovered virus probably originated from the ocean outfall rather than from within Honolulu Harbor. However, the possibility that the isolated virus could have originated from a source within Honolulu Harbor could not be ruled out. The two samples taken from station A (Ala Wai Canal) during ebb tides were negative for virus, thus suggesting that viruses were not entering this channel from Māmala Bay. However, one of two samples taken from the Ala Wai Yacht Harbor (sta. Y) and 2

of 4 samples taken from the Ala Wai Canal (sta. D) were positive for viruses. These results suggest that viruses may be recovered from waters close to land and that they do not appear to originate from the ocean sewage outfall. Since the Ala Wai Yacht Harbor (sta. Y) was positive for virus, waters from two other major small-boat harbors off Māmala Bay were also analyzed for virus. Two samples taken from Kewalo Basin (sta. K) were negative for virus, while a single sample from Ke'ehi Lagoon Harbor (sta. L) was positive for virus. Since Ala Wai Yacht Harbor and Ke'ehi Lagoon Harbor moor primarily houseboats while Kewalo Basin handles commercial boats, there appears to be a positive correlation between the presence of human enteric viruses in harbors with houseboats.

Recovery of Human Enteric Viruses from the New Sewage Outfall Area in Māmala Bay

Sewage was diverted from the old to the new outfall in December 1976. Surveillance for the presence of human enteric viruses was conducted at the old and new sewage outfall areas beginning in February 1977 until February 1978.

Using the virus concentrator and sampling conditions which regularly isolated virus in the old sewage outfall area (volumes of 0.19-0.28 m^3 [50-75 gal] at depths of 0.3-0.6 m [1-2 ft]), *no* human enteric virus was detected in either the old or new outfall areas (Table 7). Of 8 samples taken, no virus was recovered from the new outfall area, including the Hawai'i State Department of Health-designated zone of mixing, 457.2 m (1500 ft) wide (on either side of the diffuser) and 2 057.4 m (6750 ft) long (Fig. 2). This is an area where all recreational activities are prohibited. However, when sampling volumes were doubled (0.38-0.76 m^3 [100-200 gal]), human enteric viruses could then be isolated *only* in the zone of mixing (12/18) but not outside the area (0/8). Thus, the new sewage outfall has not only markedly reduced the concentration of human enteric viruses present in the discharge area (by at least two to ten-fold), but it has also confined the isolation of virus to the zone of mixing.

The above study, in conjunction with other related projects, has provided the necessary baseline data for the Department of Public Works of the City and County of Honolulu to apply to the EPA for a waiver of secondary treatment of the municipal waste water for ocean disposal in O'ahu.

TABLE 7. RECOVERY OF VIRUSES FROM THE NEW SEWAGE OUTFALL AREA, MĀMALA BAY, O'AHU, HAWAI'I

Sampling Area	Volume (m^3)	No. Samples Containing Virus/ Total No. Samples
Within Zone of Mixing	0.19-0.28 (50-75 gal)	0/8
Within Zone of Mixing	0.38-0.76 (100-200 gal)	12/18
Outside Zone of Mixing	0.38-0.76 (100-200 gal)	0/8

NOTE: Sampling depth = 0.3 to 0.6 m (1-2 ft).

Recovery of Viruses from Ocean Water Near Other Sewage Outfalls

Since human enteric viruses were recovered from Māmala Bay near the ocean sewage outfall, two other stations near other ocean sewage outfalls were selected and assayed occasionally for virus. One of six samples taken from the Middle Loch of Pearl Harbor approximately 1 100 m south of the discharge of sewage from the Pearl City WWTP (Fig. 3, sta. PH) was positive for virus, while 1 of 2 samples taken from Kāne'ohe Bay approximately 400 m south of the discharge of sewage from the Kaneohe WWTP was positive for virus. The recovered viruses were again those found commonly in human sewage, and it was concluded that they probably originated from the ocean sewage discharge.

The new Mōkapu Outfall, which became operational in December 1977, has a 1.2 m (48 in.) reinforced concrete pipe, of which the land portion is 1 213.6 m (47,780 ft) and that of the ocean, 1 541.1 m (5056 ft). The last 293.5-m (963-ft) section of the ocean outfall is a multiport diffuser whose discharge depth varies from 27.1 to 33.2 m (89-100 ft). Surveillance and sampling was initiated in the discharge area slightly south of Mōkapu Point in Kailua Bay (Fig. 4).

A major problem restricting sampling of the new outfall area was the extremely rough and hazardous water conditions which required a larger boat provided by the Hawaii Institute of Marine Biology at Coconut Island. Human enteric virus was recovered from one of two successful ocean runs which suggests that viruses from the Mōkapu Outfall may reach the water surface after

discharge. Several other attempts were made, but aborted, because of the rough water conditions.

Marine Antiviral Agents Role in the Survival of Human Enteric Viruses

A major factor that determines the recovery and distribution of infectious viruses in the ocean is their relative stability in sea water. Furthermore, sewage-borne viral pathogens entering the ocean become a potential problem only if they are able to survive and be disseminated to populated areas, recreational areas, and seafood-producing coastal waters. During the course of this study on the survival of human enteric viruses, such as poliovirus in sea water, data indicated that sea water samples taken from either Māmala Bay or Pearl Harbor contained components which inactivated the virus. Additional studies were made on the antiviral components which resulted in the following findings.

DISTRIBUTION. Additional studies on the distribution of marine antiviral agents (MAVAs) indicated that they are widely distributed in the ocean waters surrounding the island of O'ahu and in the open ocean 40 225 m (25 miles) off the coast of Maui. Although MAVAs were detected in brackish water samples taken from lower Nu'uanu Stream in central Honolulu and at the mouth of the stream as it flowed into Māmala Bay (estuarine type water), they were *absent* in *freshwater* samples taken from upper Nu'uanu Stream (near forest reserve). Such freshwater samples did not exhibit any antiviral activity.

KINETICS OF INACTIVATION. The virus-inactivation rates of sea water samples from two different sites, Waikīkī and Pearl Harbor, were examined. While both samples exhibited antiviral activity, the kinetics of inactivation were different. The Waikīkī sample showed an initial 24-hr lag period before inactivation of poliovirus type 1 was observed. In contrast, the effect of the Pearl Harbor water was immediate (Fig. 8). In 2 to 3 days, both samples gave a 3 to 4 log decrease in the infectivity titer of poliovirus. This difference in inactivation kinetics was attributed to differences in the initial concentration of bacteria in the water samples: the Waikīkī water containing an initial count of 10^3 bacteria/mℓ and the Pearl Harbor water, 10^5 bacteria/mℓ. However, the Waikīkī water attained a density of 10^6 bacteria/mℓ after 24-hr incubation, making it comparable with

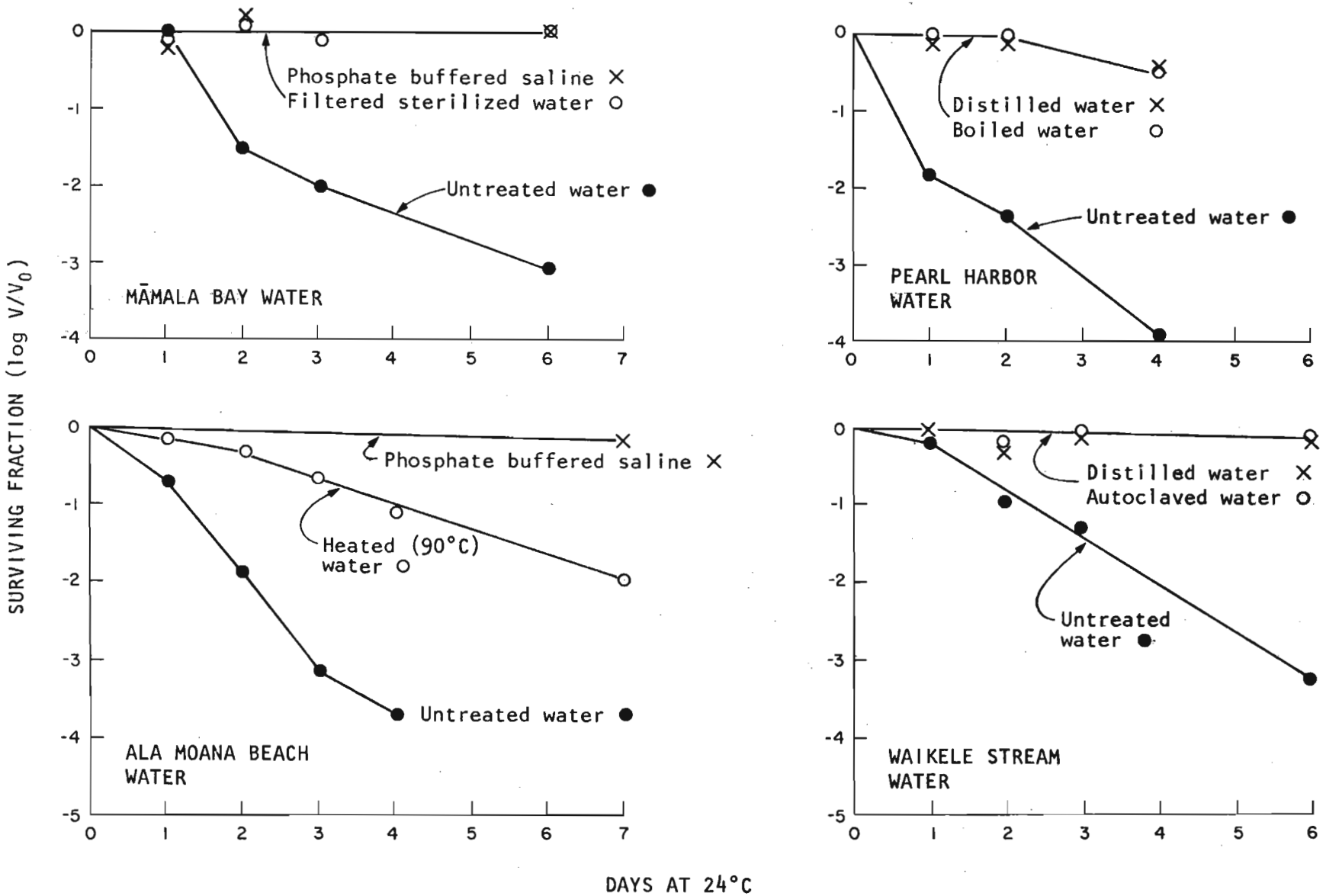


Figure 8. Survivability of poliovirus type 1 in natural waters, O'ahu, Hawai'i

the bacterial density of Pearl Harbor water, after which time the inactivation rates for both water samples were similar.

Biological, Physical, and Chemical Properties (Table 8).

a. Size. MAVAs were filtrable through 1 000-nm pore size filters, but not through 450- and 220-nm membrane filters; and were nondialyzable through standard dialyzing membranes, which suggests that some kind of close contact is necessary between the MAVAs and the virus before inactivation occurs.

b. pH. The antiviral activity of MAVAs was not affected at pH's between 7 and 8.

c. Temperature. The antiviral activity of MAVAs was active at 24°C, retarded at 10°C, and inactive at 4°C. MAVAs were rapidly inactivated by heating (at 90°C for 1 hr), boiling (1-2 min), and autoclaving (120°C for 15 min) the sea water samples.

d. Phototropism. When Pearl Harbor water containing MAVAs was inoculated into filtered sea water and incubated in the dark, virus inactivation was obtained which suggested the involvement of a nonphototrophic organism(s).

e. Antibiotics. The addition of antibiotics, such as penicillin or streptomycin, to sea water samples interfered with the antiviral activity of the MAVAs.

TABLE 8. PROPERTIES OF MARINE ANTIVIRAL AGENTS IN OCEAN WATERS

1.	Stable at 4°C for weeks
2.	More active at 25°C than at 4°C
3.	Destroyed by heat: boiling for 15 min; autoclaving at 121°C for 15 min
4.	Active at natural pH of sea water (pH 8.0), as well as at pH 7.0
5.	Removed by filtration through membrane filters of 0.22- and 0.45-μm porosities, but not through membranes with 1.0-μm porosity
6.	Nondialyzable
7.	Inhibited by treatment with either penicillin and/or streptomycin
8.	Present in varying amounts in different sea water
9.	Viruses sensitive to agent(s): poliovirus type 1, coxsackievirus B4, echovirus 7

The accumulated data strongly suggest that some kind of marine microorganism is responsible for the virus-inactivating capacity of the sea waters.

Preliminary studies indicated that human enteropathogenic viruses (coxsackievirus B4 and echovirus type 7) were also inactivated by the MAVAs.

Isolation and Identification of MAVAs. In published reports that implicate microorganisms in antiviral activity, the traditional approach was to culture the different microorganisms present in the sea water samples and to examine each of these microorganisms for their antiviral activity. Since this approach is tedious and prolonged, a procedure which has greatly facilitated the approach to the isolation of MAVAs was developed. Antiviral activities of sea water samples were screened by using selected groups of antibiotics which (1) suppressed much of the vast background of microbial flora normally present in sea water, particularly those with no MAVA activity, and (2) permitted the growth of those microorganisms with antiviral acitvity. The antibiotics used were penicillin, streptomycin, rifampin, kanamycin, polymyxin B, and chloramphenicol. This procedure initially isolated several bacterial cultures. The "suspect" bacterial colonies were picked, grown out, and tested for their antiviral activities. Initial studies of 48 morphologically distinct colonies of bacteria isolated from Pearl Harbor and the open Māmala Bay waters indicated that these isolates did not have antiviral activity. Subsequently, two bacterial isolates with different growth characteristics were recovered and both isolates were found to have antiviral activity. Studies are currently being conducted under a new grant from Sea Grant to identify and characterize the properties of the two isolates.

Mechanism of MAVA Activity. Preliminary studies were made on the mechanism by which MAVA inactivates poliovirus by using poliovirus either labeled radioactively in the protein coat or in the nucleic acid. The labeled virus was placed into the sea water and, at various times thereafter, the infectivity as well as the buoyant density of the radioactive-labeled virus determined in a CsCl or sucrose gradient. The preliminary results indicated that the structure of poliovirus was degraded upon incubation in the sea water and suggested that the MAVA alters the structure of the poliovirus, consequently inactivating the virus. Continuing studies on the mechanism of inactivation are being currently pursued under a new grant from Sea Grant.

DISCUSSION AND CONCLUSIONS

The present investigation has demonstrated the feasibility of using the Aquella portable virus concentrator to recover human enteric viruses from 0.08 to 0.76 m^3 (20-200 gal) of sea waters receiving sewage, despite a relatively low recovery efficiency of about 20 to 30%. Typical sewage-borne human enteric viruses, such as poliovirus, coxsackievirus, and echovirus, were recovered from the sewage entering the major outfall at Māmala Bay and from ocean sites near sewage outfalls in Māmala Bay, Pearl Harbor, Kāne'ohe Bay and Kailua Bay. Furthermore, the finding that turbidity, fecal bacterial counts, as well as frequency of virus recovery, were highest within the sewage boil and the plume, and decreased as the distance from the plume increased, strongly indicated that the primary source of these viruses was the sewage outfall.

Attempts to relate the role of tides, winds, and ocean currents to the movement of viruses in Māmala Bay from the sewage outfall were inconclusive for the following probable reasons: (1) samples were taken 0.3 to 0.6 m (1-2 ft) deep from the ocean surface and surface water currents were found to be greatly affected by local conditions and could not always be predetermined by the predicted tides, and (2) the length of the sampling period was generally 1 to 4 hr, during which time the direction and velocity of the wind and surface ocean current at the sampling site varied considerably. Despite these problems, viruses were most frequently recovered from stations when the predictable effects of the tides were transporting water in their direction. Within Māmala Bay, viruses were recovered 804.5 m ($\frac{1}{2}$ mile) south, 1 609 m (1 mile) west, and 3 218 m (2 miles) east of the boil. Significantly, viruses were not recovered 6 436 m (4 miles) east of the boil and 804.5 m off Waikīkī Beach, an extremely popular swimming and recreational area. The viruses recovered from the ocean sites were assumed to originate from ocean sewage outfalls; however, other sources of viruses, such as streams flowing into Māmala Bay, have not been ruled out.

Although currents are responsible for the movement of human enteric viruses in the ocean environment, the extent of virus dispersion appears to be dependent on their stability in the ocean environment. The present data indicate that the T_{90} of poliovirus type 1 added to Māmala Bay water was

approximately 48 hr and complete inactivation occurred by 72 to 120 hr. This corresponds very closely to the reported T_{90} for poliovirus added to Mediterranean waters as previously reported by Shuval et al. (1971). Initial studies indicated that virus-inactivating agent(s) of a microbiological nature is present in both the "clean" and sewage polluted waters of Māmala Bay. The virus-inactivating properties of sea water have been reported for water samples taken from the Mediterranean Sea (Shuval et al. 1971), the Baltic and North Seas (Lycke, Magnusson, and Lund 1965), the Gulf of Mexico (Akin et al. 1976), and the Atlantic Ocean (Denis et al. 1977; Lo, Gilbert, and Hetrick 1976). No such reports have been made for the Pacific Ocean. Among several factors that have been implicated are marine microorganisms which have been suggested as being responsible for this antiviral activity. In this respect, it is extremely important to isolate and identify the microorganisms responsible since several beneficial uses of these microorganisms are possible. For example, such antiviral microorganisms may be used in the biological control of contaminating enteric viruses or other viruses in coastal sea waters and other bodies of waters. The isolation and further characterization of the marine antiviral agent(s) in Māmala Bay water are currently in progress.

In contrast to the long survival time for viruses in sea water, the T_{90} for coliform bacteria in Māmala Bay has been reported to be less than 1 hr (City and County of Honolulu 1971). This suggests that coliform bacteria would not serve as an adequate indicator for the absence of human enteric viruses in the ocean environment. Direct support for this conclusion was provided by the recovery of viruses from sea waters which were essentially negative for the coliform indicator bacteria. The absence of coliform indicator bacteria and the presence of human enteric viruses in sea waters have been observed by Berg et al. (1976) and Goyal, Gerba, and Melnick (1978). Moreover, the relative instability of the fecal coliform to fecal streptococus bacteria in the sea water indicates that the significance of the FC to FS ratio established in the freshwater environment may not be valid in the ocean water environment. A thorough and necessary evaluation of the use of bacterial indicators to monitor human sewage pollution in the ocean environment is currently in progress.

The failure to isolate human enteric viruses outside the prescribed zone of mixing from the new sewage outfall area off Sand Island indicated

that the outfall design essentially fulfilled the planned objectives. These results, in conjunction with other related projects, have provided the necessary baseline data to the Department of Public Works of the City and County of Honolulu to seek a waiver from the Environmental Protection Agency (EPA) for secondary treatment of O'ahu municipal waste water for ocean disposal.

The recovery of human enteropathogenic viruses from several sea water sites receiving sewage discharge in the current study indicates that precautionary measures must be practiced for sewage disposal, especially untreated sewage into shallow ocean waters. It should be stressed that the potential health hazards of sewage-borne human enteric viruses in sea waters are not known and, other than infectious hepatitis, are difficult to document because of the unknown nature of these viruses.

Recently, a paper by Goyal, Gerba, and Melnick (1978) reported on the recovery of high concentrations of enteric viruses from recreational coastal waters which were receiving secondarily treated waste water effluent in Galveston County, Texas. They also reported that the coliform indicator bacteria cannot serve as unequivocal indicators of the virological quality of sea water. Human enteric viruses were isolated from water samples which were negative for fecal coliforms.

APPLICATION OF RESULTS

The data from this project have provided (1) information on the survivability and the effect of transmittal of human enteropathogenic viruses in the ocean environment; (2) information on the interactions of other microorganisms with human enteropathogenic viruses in the ocean environment; (3) guidelines for the proper methods of disinfection and disposal of sewage presently discharged into the ocean; (4) means for the prevention of contamination and/or destruction of marine resources in the sea water of Hawai'i; (5) provision for alternate methods for the disinfection of sewage wastes and sewage-contaminated waters; (6) methods for the routine surveillance of human enteric viruses in sea water contaminated with sewage; (7) assistance to agencies, such as the Hawaii State Department of Health, City and County of Honolulu Department of Public Works, and the Environmental Protection Agency, in properly assessing the health hazards of sewage-borne viruses in

the ocean and in safeguarding the marine environment; and (8) assistance to the Division of Fish and Game in evaluating the effect of viral pathogens on marine life.

The Department of Public Works, City and County of Honolulu, has received data on pathogenic enteric viruses and bacteria and their peripheral spread in marine waters. Bimonthly meetings of the Department of Public Works and Water Resources Research Center were held for data and information dissemination of the sampling findings. The Hawaii State Department of Health has also received data and information on human enteric viruses in marine waters.

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APPENDICES

APPENDIX A. METHODOLOGY

Field Investigations

Field studies were conducted primarily with a 5.8 m (19 ft) Glaspar boat from the City and County of Honolulu Wastewater Division. 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 site sampling when the City and County boat was not used.

The two largest and heaviest pieces of equipment consisted of the self-contained Aquella virus concentrator (Figs. 5, 6) 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 × 0.91 m (1.5 × 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 (6.6 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, space was extremely limited and movement of personnel on board was restricted because of the bottles of $AlCl_3$, test equipment, gasoline, and spare filters.

VIRUS FILTRATION AND SAMPLE PREPARATION. At the study site, water was pumped through the filtration by means of a centrifugal 19.05 mm (0.75 in.) rubber impellor Teel pump driven by a 1/3 hp AC motor. The intake line was suspended over the side of the boat, usually at a 0.3-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 large, pore size prefilter was used, thus lessening the clogging of the other filters. However, these runs were limited to 1 hr or less with a throughput rate of 0.08 to 0.15 m^3 (20-40 gal). At the clear-water sites, 52.6 × 10^{-6} m^3/s (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 adsorbing filters is lowered to 3.5 to 3.8 by injecting 0.03 *M* $AlCl_3$ and HCl through a Johanson proportional pump. 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. Excess water was first removed from the adsorbing filters (Filterite and No. 4 filter) by blowing out with air from the compressor and then rinsing 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 except 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 cross-sighting with a Davis handbearing compass for visible landmarks on shore. For sampling sites in the vicinity of the old and new Sand Island Outfall, bearings were taken of Aloha Tower, Tripler Hospital, the Dole pineapple water tower, the KGU radio tower, and the land end of Diamond Head. At the Ala Moana Beach and Waikīkī Beach area, the Channel 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 with a YSI Model 33 salinity, temperature, and conductivity meter. Measurements were taken at various sampling depths during intervals of the run for an average of the water quality entering the filter system. Direction and velocity of water movement at the sampling site was measured with a pygmy-type current meter. Because of the noise of the generator, revolutions of the meter were counted by observation instead of utilizing the clicks heard with earphones. Current direction during the run was 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. In the Aquella unit, 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 bac-

terial samples. The Millipore method was used for all bacteria work. Media for total and fecal coliforms, and fecal streptococci were preprepared in the Water Resources Research Center laboratory and refrigerated in an ice chest before sampling. Samples appropriately diluted with buffered water were filtrated with 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 media used for each bacterial type were for total coliform, M-Endo Agar LES; for fecal coliform, m-FC Agar with rosolic acid; and for fecal streptococcus, M-Enterococcus Agar. During the bacterial analysis, 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.

APPENDIX B. PUBLICATIONS AND PRESENTATIONS

1976

Fujioka, R.S., and Loh, P.C. 1976. "Pathogenic human viruses in the Hawaiian ocean environment." Paper presented to the American Society of Microbiology meeting, Hawaii Branch, Honolulu, Hawaii.

1978

Fujioka, R.S.; Lau, L.S.; and Loh, P.C. 1978. "Characterization of virucidal agent(s) in the ocean waters off Hawaii." Paper presented to the 78th Annual Meeting of the American Society of Microbiology, Las Vegas, Nevada.

Fujioka, R.S.; Lau, L.S.; and Loh, P.C. 1978. "Characterization of the marine antiviral agent(s) naturally present in the ocean waters off Oahu." Paper presented to the American Society of Microbiology meeting, Hawaii Branch, Honolulu, Hawaii.

Lau, R.K.T.; Fujioka, R.S.; and Loh, P.C. 1978. "Attempted isolation of a marine antiviral agent using selected antibiotics." Paper presented to the American Society of Microbiology meeting, Hawaii Branch, Honolulu, Hawaii.

1979

Loh, P.C.; Fujioka, R.S.; and Lau, L.S. 1979. Recovery, dissemination, and survival of human enteric viruses in the Hawa ian ocean environment. *Water, Air, Soil Pollut.* 12:197-217.

Loh, P.C.; Lau, L.S.; and Fujioka, R.S. 1979. The dissemination of pathogenic enteric viruses in the marine environment from an ocean sewage outfall. In *Toxic Materials and Their Effects on Marine Resources*, Sea Grant Symposium at University of Wisconsin, Madison, p. 17.

1980

Fujioka, R.S.; Loh, P.C.; and Lau, L.S. 1980. Survival of human enteric viruses in the Hawaiian ocean environment: Evidence for virus-inactivating microorganisms. *Appl. Environ. Microbiol.* 39(6):1105-110.