MAMALA BAY STUDY

COLIPHAGE AND INDIGENOUS PHAGE IN MAMALA BAY

PROJECT MB-7

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1 EXECUTIVE SUMMARY

A study of the distribution of bacterial viruses (bacteriophages) in Mamala Bay was undertaken in conjunction with the Microbiological group, MB-7. Coliphages, or viruses which infect the bacterium *E. coli* and are indicators of fecal pollution, were enumerated along with total viral direct counts, and phage 16-like vibriophages. The latter are a type of marine bacteriophage that we have found abundant in the coastal waters of Florida. Samples were collected approximately quarterly over a thirteen month period. Coliphage were found during each quarterly sampling along an offshore transect to the Sand Island Outfall. High concentrations were also found in three of four samplings in the Manoa Stream near the University of Hawaii campus. During the storm event sampling in February 1994, the nonpoint coastal stations (Pearl Harbor, Ala Wai Canal, and Hanauma Bay) had high levels of coliphages, but much lower or none when sampled during dry weather. Coliphages were absent at all samplings at Waikiki Beach and at the control station off Diamond Head. Viral direct counts were highest in sewage and in the Manoa Stream samples (10^{11} and 10^{10}, respectively). Eutrophic coastal stations (Pearl Harbor, Ke'ehi Lagoon, Ala Moana Beach, and Ala Wai canal) averaged 10^{9}/L. Offshore stations ranged from $9x10^{7}$ to $1x10^{9}$ viruses/L, values similar to those for other marine environments. Phage 16-like vibriophages were found mainly in eutrophic coastal environments (Ala Wai Canal, Pearl Harbor, and Ke'ehi Lagoon) and in the Sand Island Transect stations D1 and D2. The greatest abundance was found during the storm event (February) sampling.

Collectively these results indicate that the fecal indicator phages (coliphages) were abundant in the Sand Island transect during all quarterly samplings, were prevalent in the coastal stations during storm events, and were never found on Waikiki Beach and offshore control sites. Thus, the major input of indicators into the coastal zone and

beaches seems to be from land runoff entering via non-point sources. Thus, better treatment of stormwater is recommended.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

2 INTRODUCTION

2.1 Scope of Work

Mamala Bay is a complex system that encompasses the entire south shore of the island of Oahu. This system includes estuarine environments (Pearl Harbor, Ke'ehi Lagoon, Honolulu Harbor), beaches used for tourism (Waikiki, Ala Moana Park), major shipping and port activity, numerous freshwater and stormwater inputs, and wastewater effluent inputs (Honouliuli outfall, Sand Island outfall, and Pearl Harbor outfall). The Mamala Bay Study Commission has brought together a suite of investigators studying the physical, biological, and public health aspects of this system yielding an integrated coastal management plan for Mamala Bay. Our project is an important component of this total picture, the coliphage and indigenous phage in Mamala Bay. We have worked closely with the other microbiologists in the MB-7 program to coordinate sampling and share results. Our data also gives a complete picture of the bacteriophage distribution in this system, and can "stand alone" as a separate publication from this study.

Coliphage as indicators of water quality. Coliphage abundance has become an acceptable indicator of water quality. Coliphage are viruses of the bacterium *Escherichia coli*, a fecal coliform. Because of viral stability compared to its host, coliphage appear to be better indicators of marine water quality and are in fact found to be more persistent in the environment (Simkova et al., 1981). Recent microbiological reports state that coliphage are the best indicators, to date, of human enteric viruses in polluted waters (Sobsey, 1989) and based on the isolation of phage at various bathing beaches, should be used for continuing evaluation of recreational waters (Palmateer et al., 1991). These bacteriophages have not yet been detected in unpolluted waters (IAWPRC Study on Health Related Water Microbiology, 1991). In addition, coliphage are easily and inexpensively isolated in the laboratory. A specific example of a potential indicator

coliphage is the MS2 virus. It is an F-specific RNA phage with an *E. coli* host. Since MS2 can be found anywhere its bacterial host is found, it is capable of indicating fecal contamination. MS2 has been recommended for use as a surrogate for the enteric viruses by the Environmental Protection Agency (EPA, 1989). More recently the MS2 coliphage has been used as a microbial tracer in a full-scale wastewater treatment facility to evaluate disinfection efficacy and transport. The coliphage tracer was found to represent the fate of human enteric pathogens through the concurrent monitoring of indigenous enteroviruses in this study (Rose and Carnahan, 1992). Furthermore, the MS2 virus has been used in disinfection studies and documented as being highly resistant when compared to bacterial indicators (Bonde, 1975). Coliphage may therefore still be present in chlorinated sewage after bacterial indicators cannot be detected.

The use of these indicator systems has focused on the sanitary quality of water. There has been no attempt to correlate the occurrence of these indicators with the level of urbanization and magnitude of human activities impacting the marine environment in a spatial monitoring program in the water column.

The Abundance of Viruses in the Marine Environment. Viruses are now known to be the most numerically abundant form of life in the surface waters of our planet. This revelation has come to light relatively recently, because of the development of methods to enumerate viruses in water samples directly by transmission electron microscopy, termed viral direct counts (VDC; Bergh et al., 1989; Proctor and Fuhrman, 1990; Paul et al., 1991). These viruses are believed to be primarily bacteriophages, although viruses capable of lysing phytoplankton are also common in surface waters (Suttle et al., 1990, Waterbury et al., 1993). The role of viruses in water column trophodynamics are not completely understood, but it is thought that they can cause 10-50% of the bacterial mortality (Jiang and Paul, 1995). In general, the highest viral direct counts are associated with eutrophic environments, such as estuaries, and polluted lakes (Bergh et al., 1989; Paul et al., 1991; Boehme et al., 1993). Thus, viral direct counts can be an indicator of

relative trophic status of surface waters, as bacterial direct counts and chlorophyll a are often used. Thus, we felt that it was important to measure these in the Mamala Bay system.

Vibriophages. Historically, the abundance of phages in seawater was determined by plaque titer (Moebus, 1980). The problem with this approach is that it drastically underestimated the number of viruses present, because only a few hosts could be used. We have become interested in the distribution of viruses which infect a marine host we have isolated termed host #16, tentatively identified as *Vibrio parahaemolyticus* strain 16. We have isolated phages on this host from the Gulf of Mexico, the Dry Tortugas, Key Largo, and Tampa Bay. We are embarking on a study of the genetic diversity of the phages which infect this host (Paul et al., 1993; Kellogg et al., 1995). As part of this study, we measured the abundance of such vibriophages in the waters around Mamala Bay.

2.2 Objectives

The study described below was undertaken to understand the distribution and dynamics of coliphage and indigenous phage in Mamala Bay. These data will be helpful in assessing the impact of the Sand Island sewage outfall on the health of Mamala Bay and the users of its marine resources.

Objective 1. To enumerate viruses by in water column samples by direct counts.

Objective 2. To enumerate coliphages by plaque agar overlays.

Objective 3. To enumerate vibriophages by plaque titer overlay.

Objective 4. Using PCR technology, to detect coliphages and vibriophages by amplification in select samples.

2.3 Project Organization

Dr. John Paul was the principal investigator, in charge of field operations, viral direct counts, and vibriophage studies. Dr. Joan Rose, co-PI was in charge of coliphage analysis. A series of graduate students participated in sampling and sample analysis, including Sunny Jiang, Chris Kellogg, Pam London, Xingting Xhou, and Jordan Brown.

3 METHODS

3.1 Task Summary

The major tasks were to concentrate samples collected in the quarterly sampling for viral direct counts, coliphage enumeration, and vibriophage enumeration. Certain samples were selected for further analysis by PCR.

3.2 Task Methodology

Sampling sites. A description of the sampling sites appears in Table 1 and the approximate locations can be seen in the data figures 1-13. Sampling occurred over four quarters in 1993-1994: October 25-29, 1993; February 14-17, 1994; June 20-24, 1994; and November 14-18, 1994. It rained heavily on Oct. 25, and during the whole week of the February sampling.

Sampling procedures. Beach and shore-based sampling was accomplished by immersion of a sterilized 20 l carboy in the water to be sampled. For offshore samplings, a gasoline driven impeller pump was used. This was fitted with up to 75 m sample hose (1" id) for sampling depths up to 75 m. All samples were returned to the lab and processed usually within 4 h of collection, but always within 8 h of collection.

Concentration of Samples for Phage Analysis. 5 liter or 20 liter samples were concentrated by vortex flow filtration using a Membrex Rotary Biofiltration device (Membrex, Inc, Garfield, NJ) equipped with a 400 cm2, 100 kd filter (Paul et al., 1991; Jiang et al., 1992). The systems were run at 1500 RPM and 8-12 PSI, and the final retentate volume was 45-65 ml. This procedure has been shown to be 70-99% efficient for the concentration of phage and microbial populations (Paul et al., 1991; Jiang et al., 1992). Sand Island sewage effluent (S1) samples were not concentrated.

Coliphage analysis. For coliphage analysis, one and 0.1 ml samples in triplicate of the retentate or S1 effluent were plated by top agar overlay using the coliphage hosts *E. coli* ATCC 15597 and/or *E. coli* Cabelli. Hosts were grown to mid stationary phase on trypticase-soy broth (TSB; Difco) medium immediately prior to top agar overlay.

Vibriophage/indigenous phage isolation. Two marine bacterial hosts were used for phage isolation in this study: host #16, tentatively identified as a *Vibrio parahaemolyticus* strain, and HSIC, an unidentified marine bacterium isolated from Ke'ehi Lagoon. The latter was used only in the first two quarterly samplings. Hosts were grown into log phase on ASWJP medium (Paul, 1982) at room temperature (24-26°C) with shaking. One ml and 0.1 ml aliquots of the retentate from each station were assayed for the presence of these phages by top agar overlay.

Viral Direct Counts. A portion of the Vortex flow filtration retentate (usually 12 ml) was fixed with EM grade glutaraldehyde (Sigma Chemical Co.; 2% final conc.) The sample was further concentrated by ultracentrifugation at 201,000 xG for 90 min in a table top ultracentrifuge (Beckman). The sample was then diluted in DI water to reduce salt content and 1 ml was spotted onto a formvar coated TEM grid. The grids were air dried and stained with uranyl sulfate. The grids were examined at 25,000 to 48,000 X with a Hitachi 5500 TEM or a Hitachi 7100 TEM.

Amplification of MS-2 coliphage. Primers were designed based on the MS-2 replicase gene sequence. The "upstream" or 5' primer sequence (designated #2175) was 5'-CAA GTT GCA(G) GGA TGC AGC GCC-3' and the 3' primer (#2375) as 3'-GCC CGA CGG ACA TTC CTC GG-5'. The retentates were purified prior to reverse transcriptase-linked PCR (RT-PCR) by the method of Abbaszadegan et al. (1993). Briefly, 0.3 ml of the retentate was passed over a mixed bed column consisting of Chelex 100 and Sephadex 100 in a 1.0 ml disposable syringe. Five ml of the purifed retentate was used in a RT reaction containing 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 1 mM each

dNTP, and 5 mM $MgCl_2$. The reaction was heated in a thermal cycler for 5 min at 100°C, followed by cooling at 4°C. RNasin (9 units), reverse transcriptase, and 1 mM of 3' primer. The reaction was incubated for 10 min at 24°C, 42°C for 50 min, 99°C for 5 min, and 4°C for 5 min. The entire RT reaction (30 ml) was added to a PCR reaction mixture containing 1x PCR buffer, 0.3 mM each primer (3' and 5'), 2.5 mM $MgCl_2$, and 2.5 units Taq Polymerase in a total volume of 100 ml. The first cycle of amplification consisted of denaturation at 95°C for 2 min, annealing at 50°C for 30 s, and extension at 72°C for 45 s, followed by 30 cycles of heating at 95°C for 1 min, annealing at 45°C for 45 s, and extension at 72° for 45 s. The final extension (last cycle) was at 72°C for 7 min.

Detection of Vibriophage by amplification. Samples were further concentrated for PCR by the method of Tsai et al., 1991. Fifteen ml retentate was concentrated to ~4.0 ml by centrifugation using an Amicon Centriprep (100 kd filter). The concentrated sample (~4 ml) was extracted with an equal volume of Chloroform:isoamyl alcohol, centrifuged for 5 min at 800 rpm for 5 min. The supernatant was transferred to an Amicon Centricon and concentrated to 300-500 ml. The concentrated extract was precipitated in two volumes of ethanol at -20°C until processing. The ethanol precipitates were collected and washed with 70% ethanol, dried, and dissolved in 300 µl sterile DI. The samples were then further purified by passage over mixed bed columns of Chelex 100 and Sephadex G-100 as described by Abbaszadegan et al. (1993). The eluates were ethanol precipitated and redissolved in DI water.

Genetic analysis of vibriophages. Representative plaques from various samplings were selected for further study by genetic analysis. Plaques were isolated and repurified by top agar overlay. The DNA was purified by the method of Maniatis et al. (1982) as modified by Kellogg and Paul (1995). A 488 bp fragment was cloned from these phages into M13 after PCR, and the fragment sequenced using the Sequenase kit (US Biochemicals). The sequences were compared to other vibriophage isolates from the Gulf of Mexico using CLUSTAL and maximum likelihood analysis.

4 RESULTS

Detection of Coliphage. The overall seasonal average coliphage data appears in Fig. 1 and seasonal values appear in Figs 2 to 5. The highest concentration of coliphage was found, not surprisingly, in the Sand Island sewage effluent, ranging from 1.4 to 2.78 x 10^6/L. Coliphage were found in every seasonal sampling at certain stations along the Sand Island transect. The highest concentrations of coliphage were found consistently in the surface waters of station D1, which was located shoreward of the sewage outfall D2, Levels of coliphage showed little variation with season in the Sand Island transect, with the exception of the November sampling (Fig. 5), when only 3 of 7 samples in this transect were positive.

The Manoa Stream samples at the University of Hawaii campus contained high levels of coliphage in three of four quarterly samplings, with none detected in the February sampling (Fig. 3). Stations located in Ala Wai canal contained coliphage in two of four samplings (October and February; Figs. 2 and 3). These sampling times were also characterized by rain, especially the February sampling (Fig. 3). Stations in Pearl Harbor were positive for coliphage only in February and June. Hanauma Bay, Sand Island Beach, and Ala Moana Beach only had one positive each, and two of these occurred in the February sampling (Fig 3).

No coliphages were ever detected at the Diamond Head Control station at all depths, nor were any found at the Waikiki Beach samples.

The non-point sources of input into Mamala Bay (i.e. Pearl Harbor, Ala Wai Canal, Hanauma Bay and Ala Moana Beach) seemed to have the highest coliphages during the storm event sampling. This is consistent with land runoff (storm sewers, culverts) into coastal waters. However, this input source did not apparently influence Waikiki Beach, which had no coliphages at any sampling. Even though the Sand Island

transect contained coliphage during all samplings, these apparently were not influencing Waikiki Beach.

Detection of coliphages by PCR. Table 2 shows the results of the detection of coliphage by PCR in comparison to plaque titers. There was agreement in the results obtained by the two methods in 58 of 71 samples (81.6%). For the samples that did not agree, 2 of 71 (2.8%) were positive by PCR but negative by plaque titer, and the remainder (11 of 71; 15.5%) were negative by PCR but positive by plaque titer. In some of the latter samples, it may be that there were coliphage that were present that were not MS-2, for which the primers were designed.

Viral Direct Counts (VDC). Fig. 6 shows the means of all seasonal samplings for viral direct counts, and figures 7 to 11 show the individual quarterly data. As with the coliphage date, VDC were greatest in the raw sewage, averaging $>10^{11}$/L. The freshwater environment Manoa Stream had the second greatest concentrations, in excess of 10^{10}/L. The eutrophic estuarine stations (Pearl Harbor, Ke'ehi Lagoon, Ala Moana Beach, and the Ala Wai Canal (AW1) averaged in excess of 10^{9}/L.

At most stations, viral direct counts were lower in June and November Fig. 9 and 10), two samplings with little or no rain, compared to October and February, the wetter samplings. We hypothesize that the general increase in runoff during the latter two samplings resulted in greater nutrients and production in the coastal waters, resulting in higher viral titers.

Vibriophage. The use of the Hawaiian host HSIC resulted in no plaques from any samples during the first two quarterly samplings. We therefore discontinued use of this host. Plaques were found only in samples from nearshore estuarine samples (Ala Wai Canal, Pearl Harbor, Ke'ehi Lagoon, and the Sand Island transect stations D1 and D2; Fig. 11). Unlike the other viruses determined in this study, vibriophage were totally absent from sewage or freshwater samples (Manoa Stream). As with coliphage in coastal

environments, Vibriophage were most abundant during the rainy (February 94; Fig 12) sampling. The lowest abundance was in the October 93 sampling, when these phages were only detected in two samples (data not shown).

Detection of Vibriophage by PCR. Several samples were examined for vibriophage detection by PCR. None of these resulted in positive amplification. It may be that the concentration of phages were sufficiently low that amplification did not occur, or that interfering substances inhibited PCR.

Genetic Analysis of Vibriophages. Figure 14 shows the result of clustal analysis of the 488 bp cryptic fragment of the Vibriophage genome. The one Hawaiian isolate thus far sequenced is most closely related to F16, an isolate from Tampa Bay.

5 CONCLUSIONS

Our data clearly shows a localization of coliphage, indicators of fecal pollution, around the Sand Island Outfall transect. Coliphage were also abundant in coastal areas such as Ala Wai Canal, Pearl Harbor, Ala Moana Beach, Ke'ehi Lagoon, but only during the wet (February) sampling. Coliphage were not found nearshore or offshore at Waikiki Beach or at the control station off Diamond Head, E4. From these observations we conclude that non-point sources have a greater input of coliphage in the coastal zone than the Sand Island sewage outfall, particularly during periods of heavy rainfall. These inputs could therefore influence water quality at the beaches. However, we only measured one indicator of fecal pollution, and we understand that some pathogenic viruses have been isolated from Waikiki Beach (K. Reynolds, University of Arizona, personal communication). Thus, before conclusions can be made as to the impact of the Sand Island Outfall on the water quality an integrated coastal management plan must first be devised.

Our measurements of viral direct counts are the first to be made in Hawaiian waters. The fact that most oceanic measurements were 108/ml illustrates the oligotrophic nature of the waters around Oahu, and are consistent for oligotrophic waters in other parts of the world (Boehme et al., 1993; Berg et al., 1990). The estuarine waters contained values similar to other coastal waters (Jiang et al, 1995).

The occurrence of Vibriophage (i.e. those capable of forming plaques on *V. parahaemolyticus* strain 16) in coastal waters has been found by us in the Key Largo and other environments previously (Paul et al., 1992; Kellogg et al. 1995). The genetic similarity of these viruses with those of the Florida waters is surprising (Kellogg et al., 1995). These data show that there are circumglobal populations of closely related bacteriophages in the marine environment.

6 RECOMMENDATIONS

6.1 Recommended Actions

Our study suggests that even though the waters around the Sand Island Outfall show indications of fecal contamination, this is not influencing the beaches of Oahu. Rather, nonpoint sources, with input from storm drains and canals, seem to have a greater input of indicator microorganisms into the coastal zone. Thus, it seems that better treatment of the nonpoint sources might be more valuable in maintaining water quality in the coastal zone. Such treatment might include construction of retention ponds for storm drain treatment, aeration of canals, and other technologies available for treatment of nonpoint sources and stormwater.

6.2 Future Studies

A regular monitoring program of the waters of Mamala Bay for fecal indicators is advised. Such a plan may already be in existence for the island of Oahu. After the recommendations are carried out for modification of stormwater and other nonpoint inputs has occurred, a second seasonal study of the distribution of indicators is recommended, to include a storm event sampling. Such a study would determine if an improvement in nonpoint discharge had been made.

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8 APPENDICES

Table 1. Description of Stations Sampled

Site	Station Designation	Quarters Sampled 10/93	2/94	6/94	1/94
Manoa Stream, UH Campus	MS	Y	Y	Y	Y
Ala Wai Canal at Ala Moana Bridge	AW1	Y	Y	Y	Y
Ala Wai Canal Mouth, at Green Marker #3	AW2	Y	Y	Y	Y
Waikiki Beach near Police Station	W1	Y	Y	Y	Y
Waikiki Beach offshore 21°13.28 N, 157°48.58W	W2	Y	Y	Y	Y
Ke'ehi Lagoon, first station in Sand Island transect D0		Y	Y	Y	Y
Sand Island Transect, Inshore Surface Water 21°17.15N, 157°54.35W	D1S	Y	Y	Y	Y
Sand Island Transect, Inshore Bottom Water 21°17.15N, 157°54.35W	D1B	Y	Y	Y	Y
Sand Island Outfall, Surface Water 21°16.82N, 157°54.43	D2S	Y	Y	Y	Y
Sand Island Outfall, Mid-depth Water 21°16.82N, 157°54.43	D2M	N	Y	Y	Y

Table 1. Description of Stations Sampled (cont.)

Site	Station Designation	Quarters Sampled 10/93	2/94	6/94	1/94
Sand Island Outfall, Bottom Water 21°16.82N, 157°54.43	D2B	Y	Y	Y	Y
Sand Island Transect Offshore Surface Water 21°16.40N, 157°55.56	D3S	Y	Y	Y	Y
Sand Island Transect Offshore Bottom Water 21°16.40N, 157°55.56	D3B	Y	Y	Y	Y
Diamond Head Control Site, Surface Water 21°13.28N, 157°48.58	E4S	Y	Y	Y	Y
Diamond Head Control Site, Mid-depth Water 21°13.28N, 157°48.58	E4M	Y	Y	Y	Y
Diamond Head Control Site, Bottom Water 21°13.28N, 157°48.58	E4B	Y	Y	Y	Y
Upper Pearl Harbor Waipio Pt.	C1	Y	Y	Y	Y
Mid Pearl Harbor Bishop's Pt.	C2	Y	Y	Y	Y

Table 1. Description of Stations Sampled (cont.)

Site	Station Designation	Quarters Sampled 10/93	2/94	6/94	1/94
Mouth of Pearl Harbor	C3	Y	Y	Y	Y
Hanauma Bay	HB	Y	Y	Y	Y
Ala Moana Beach	AM	N	Y	Y	Y
Sand Island Effluent	S1	Y	Y	Y	Y
Sand Island Beach	SB1	N	N	Y	Y
Honouliuli Outfall, Surface Water 21°17.00N, 158°01.50	HU1	N	N	Y	Y
Barbers Point Surface Water 21°16.50N, 158°06.40W	BP1	N	N	Y	Y
Honolulu Harbor Surface water 21°17.31, 157°42.27	HH1	N	N	N	Y
Ewa Beach (Iroquois Beach)	EW1	N	N	N	Y

Table 2. Results of RT-PCR detection of MS-2 coliphage: Comparison to plaque titers

STATION	October, 1993 (1st quarter)		February, 1994 (2nd quarter)		June, 1994 (3rd quarter)		December, 1994 (4th quarter)	
	PFU/L[1]	RT-PCR[2]	PFU/L	RT-PCR	PFU/L	RT-PCR	PFU/L	RT-PCR
AM1	N.D.[3]	N.D.	39.3	+	<2.6	-	<2.5	N.D.
AW1	TNTC[4]	+	150	-	<2.2	-	<2.9	-
AW2	2.88	+	$1.30x10^4$	+	<2.8	-	<3.2	-
BP1	N.D.	N.D.	N.D.	N.D.	<2.9	N.D.	<2.3	N.D.
C1	<2.7	N.D.	193.5	+	6.3	-	<2.8	-
C2	<3.2	N.D.	14.1	+	<3.0	-	<3.1	-
C3	<2.5	N.D.	<2.29	-	<3.5	-	<3.2	-
D1S	19.0	-	$1.2x10^3$	+	$1.3 x10^3$	+	3.2	+
D1B	<2.75	N.D.	14.7	+	$1.1x10^3$	+	2.1	N.D.
D2S	<2.5	-	24.7	-	544	+	10.6	-
D2M	N.D.	N.D.	77.7	-	957	+	<3.2	N.D.
D2B	377	N.D.	9.6	+	$6.62X10^3$	+	<3.0	N.D.
D3S	5.52	-	<2.6	+	36	+	<3.3	-
D3B	12.2	N.D.	9.2	-	5.4	-	<3.2	N.D.
DOS	<2.6	-	4.6	-	<2.8	-	<2.4	-
E4S	<2.5	-	<2.4	-	<2.2	-	<2.9	-
E4M	<2.7	N.D.	<2.3	-	<2.6	-	<3.2	-
E4B	<2.7	N.D.	<2.3	-	<3.6	-	<2.9	-
EW1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<2.9	N.D.
HB1	<2.6	N.D.	270.0	+	<3.0	+	<2.8	N.D.
HH1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<2.7	N.D.

Table 2. Results of RT-PCR detection of MS-2 coliphage: Comparison to plaque titers (continued)

STATION	October, 1993 (1st quarter)		February, 1994 (2nd quarter)		June, 1994 (3rd quarter)		December, 1994 (4th quarter)	
	PFU/L[1]	RT-PCR[2]	PFU/L	RT-PCR	PFU/L	RT-PCR	PFU/L	RT-PCR
HU1	N.D.	N.D.	N.D.	N.D.	<3.5	N.D.	<3.1	N.D.
MS	$8.4x10^3$	+	<13.3	+	785	+	800	+
SB1	N.D.	N.D.	N.D.	N.D.	2.67	N.D.	<2.9	N.D.
SI	$1.34x10^6$	+	$2.4x10^6$	-	$2.78x10^6$	+	$1.65x10^6$	+
W1	<1.9	-	<1.9	-	<2.8	-	<3.1	-
W2	<2.6	-	<2.5	-	<3.0	-	<3.3	-

Notes:
(1) Plaque-forming units per liter
(2) +: detected in polymerase chain reaction. -: not detected in polymerase chain reaction
(3) Not done (4) Too numerous to count

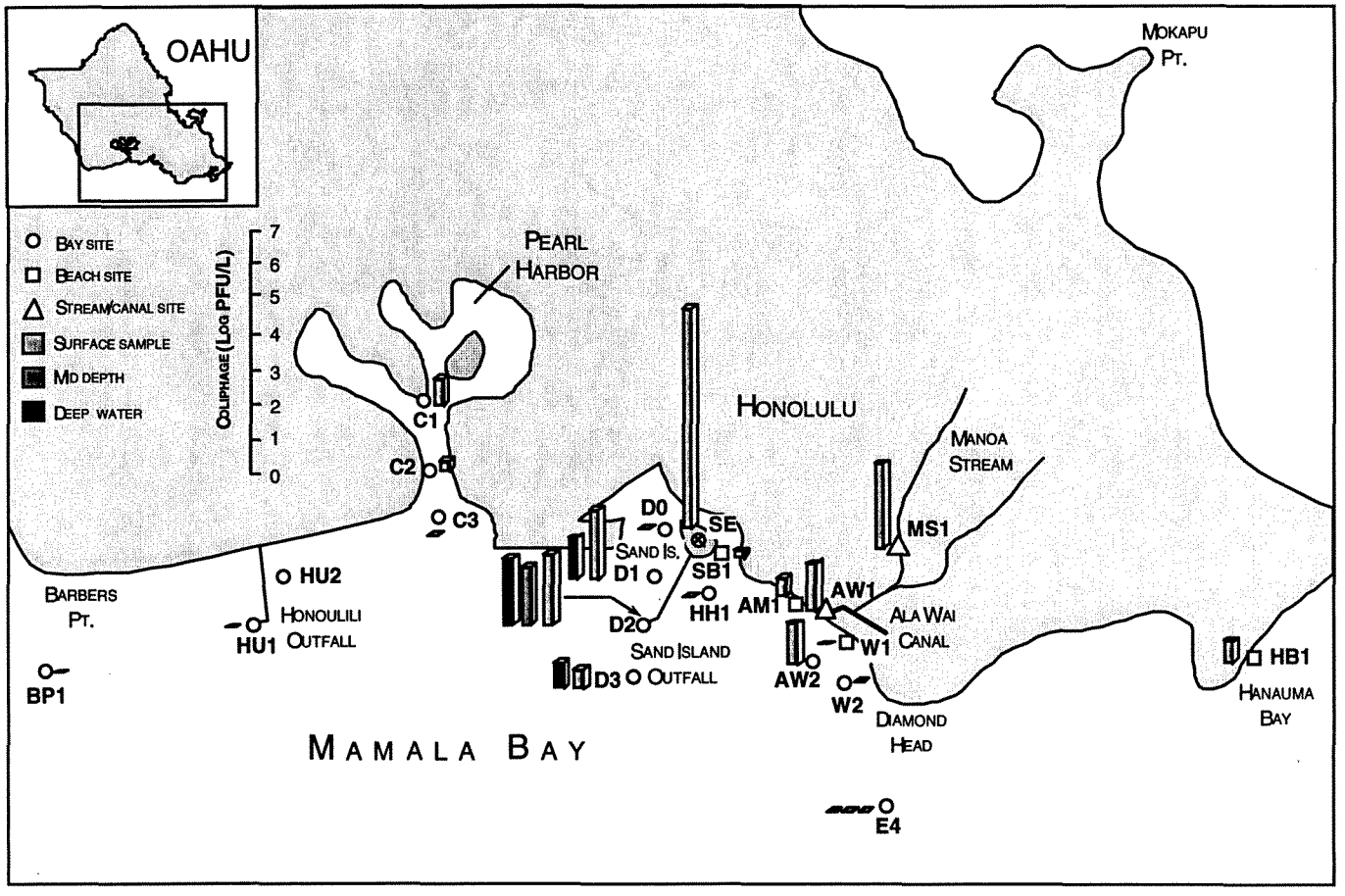

Fig. 1. Coliphage-Means of All Seasonal Samplings

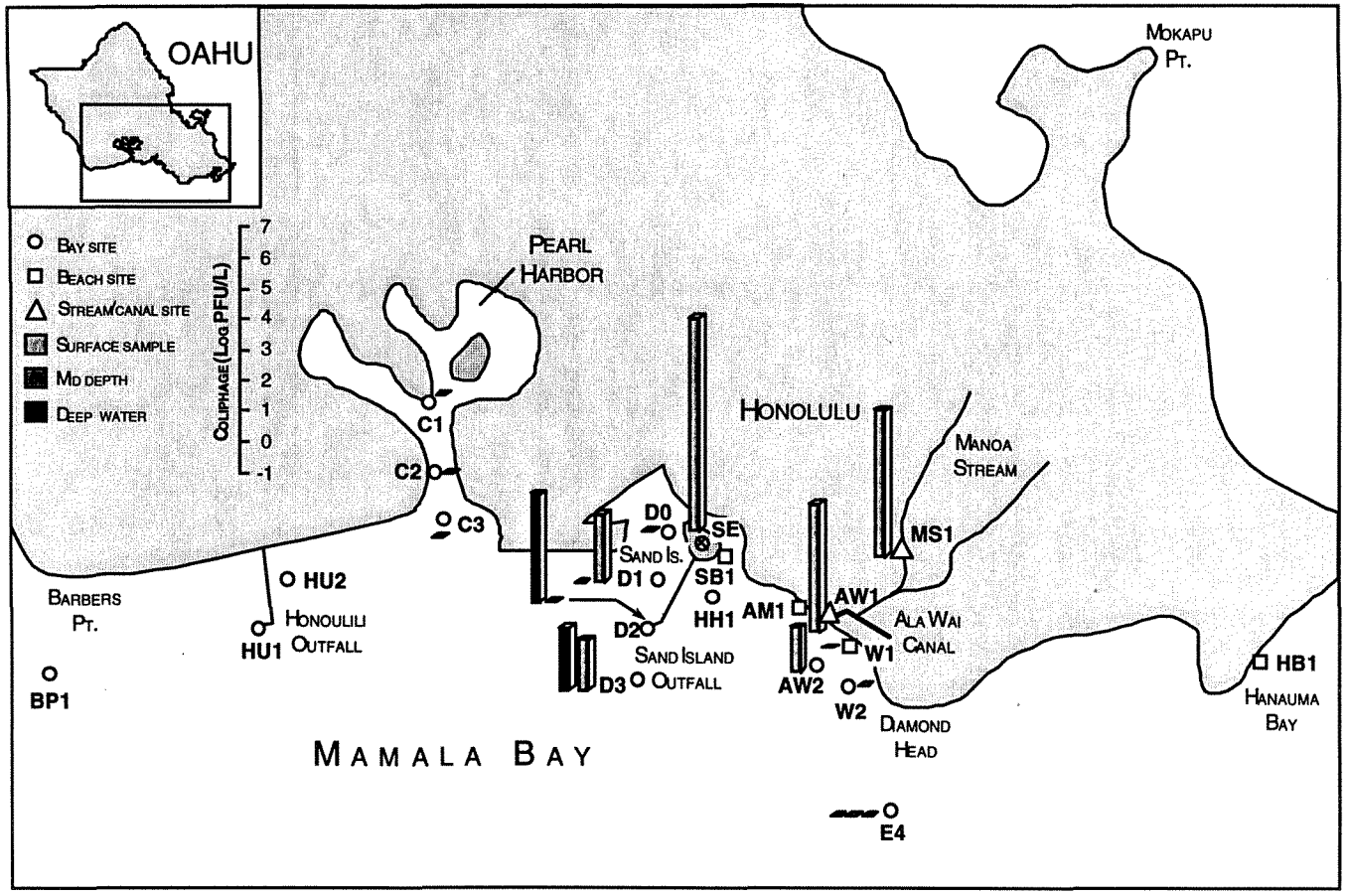

Fig. 2. Coliphage abundance for stations sampled in October 1993

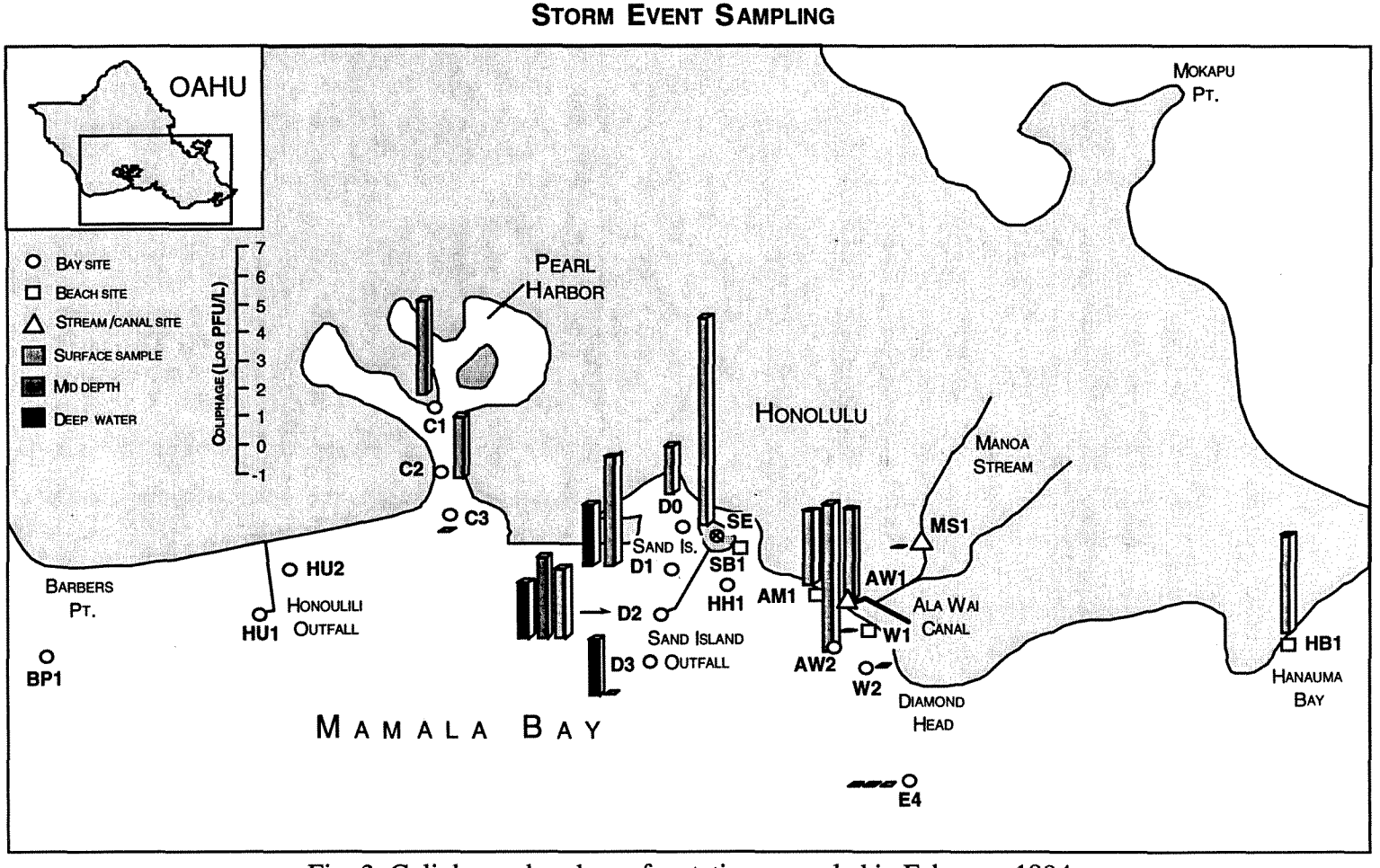

Fig. 3. Coliphage abundance for stations sampled in February 1994

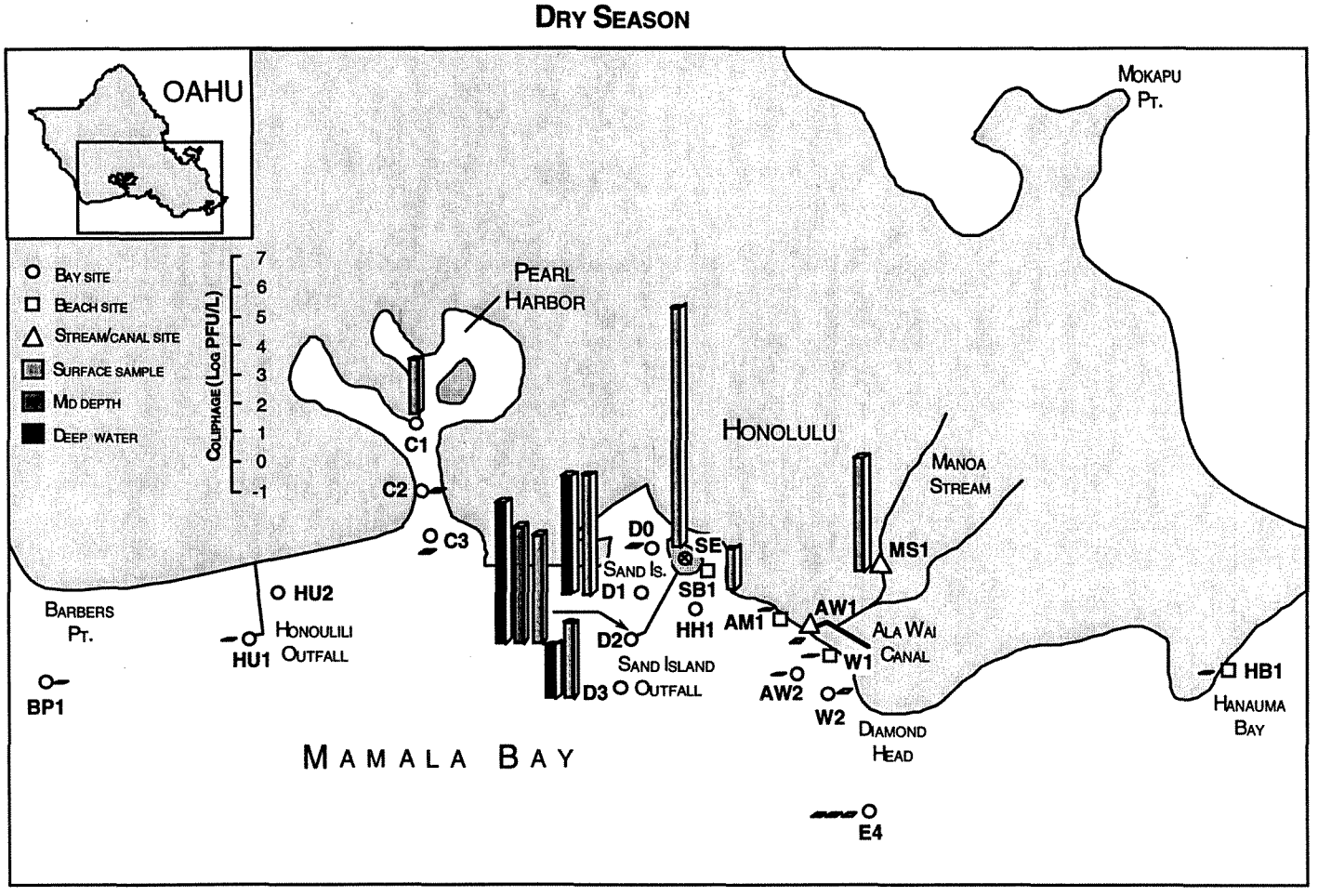

Fig. 4. Coliphage abundance for stations sampled in June 1994

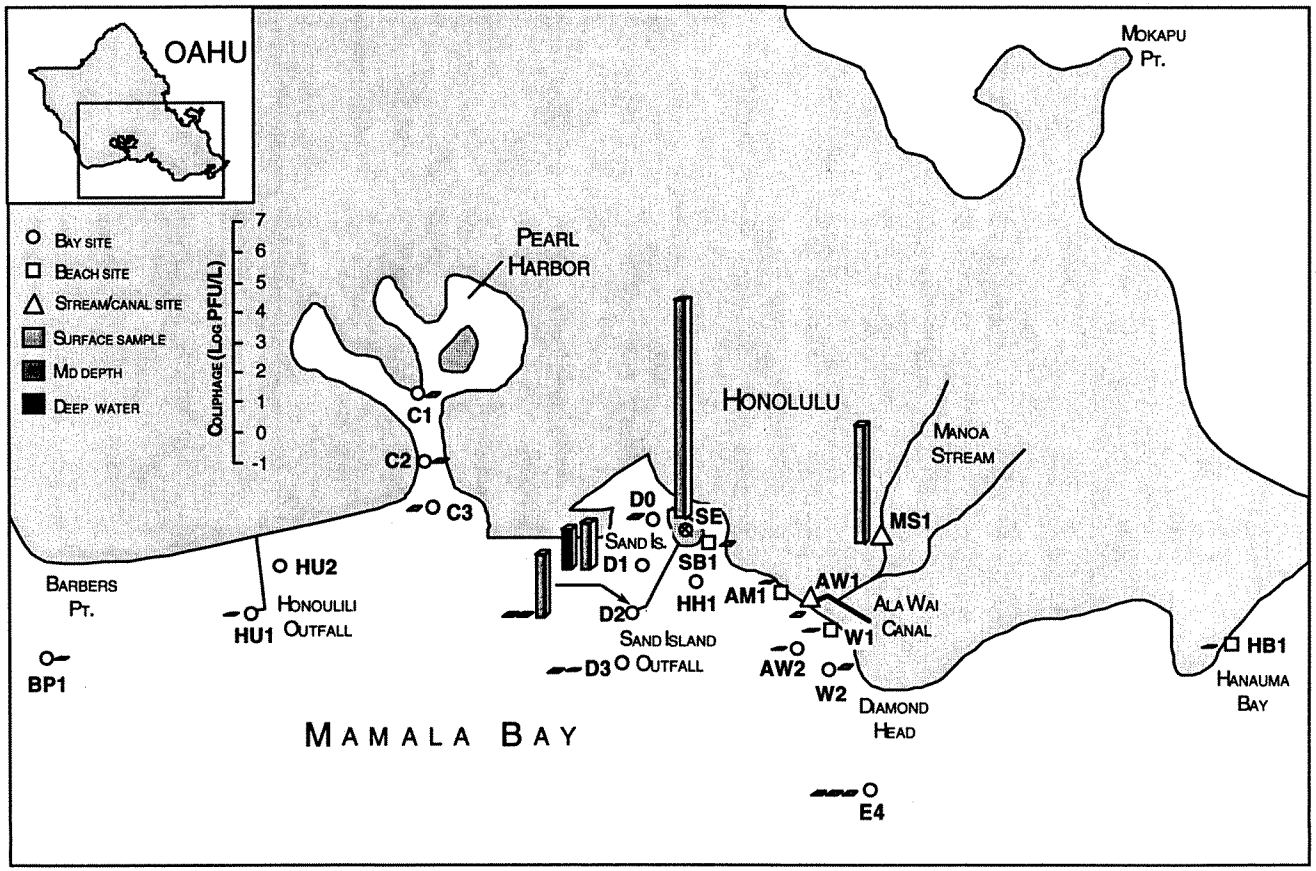

Fig. 5. Coliphage abundance for stations sampled in November 1994

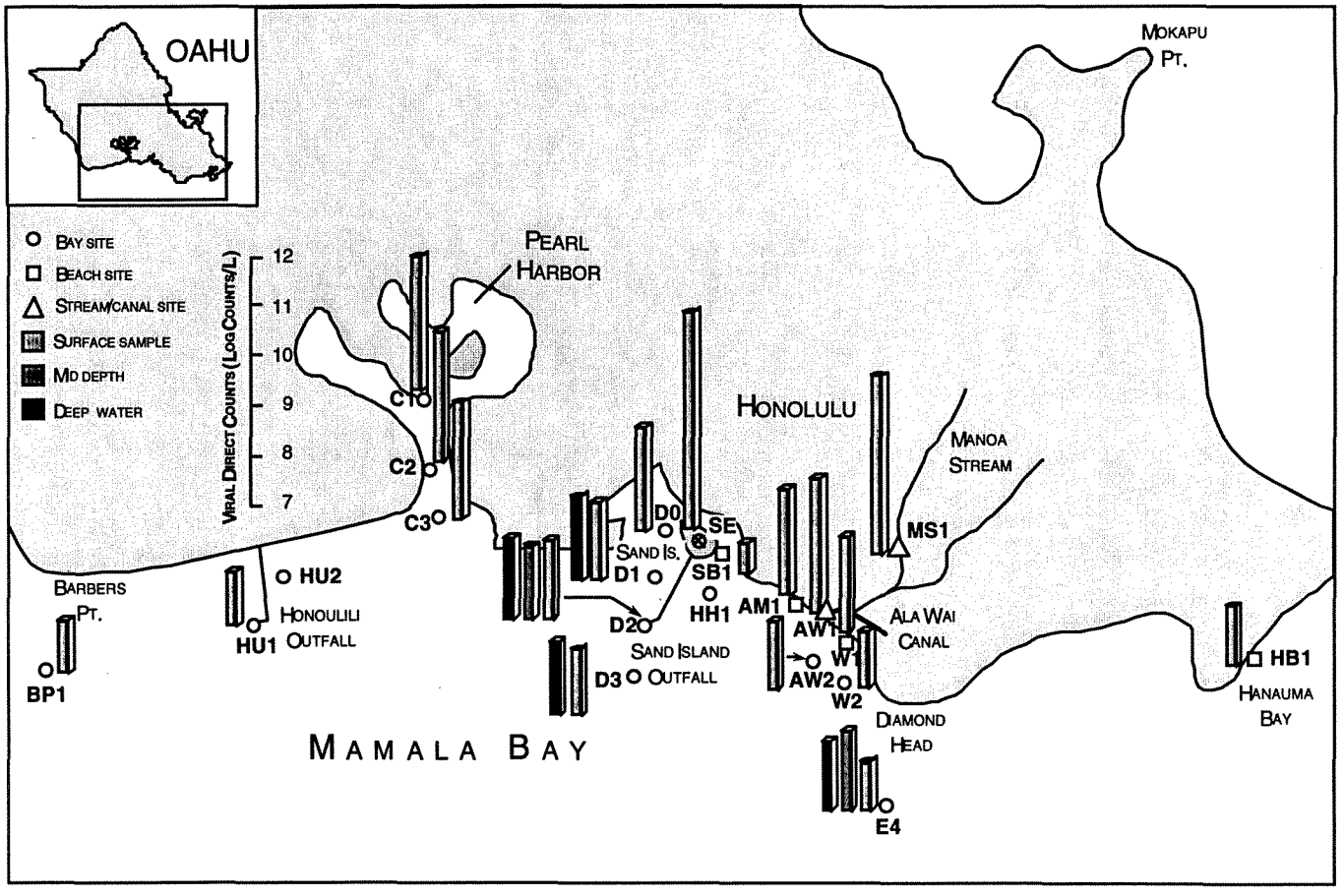

Fig. 6. Viral Direct Counts-Means of All Seasonal Samplings

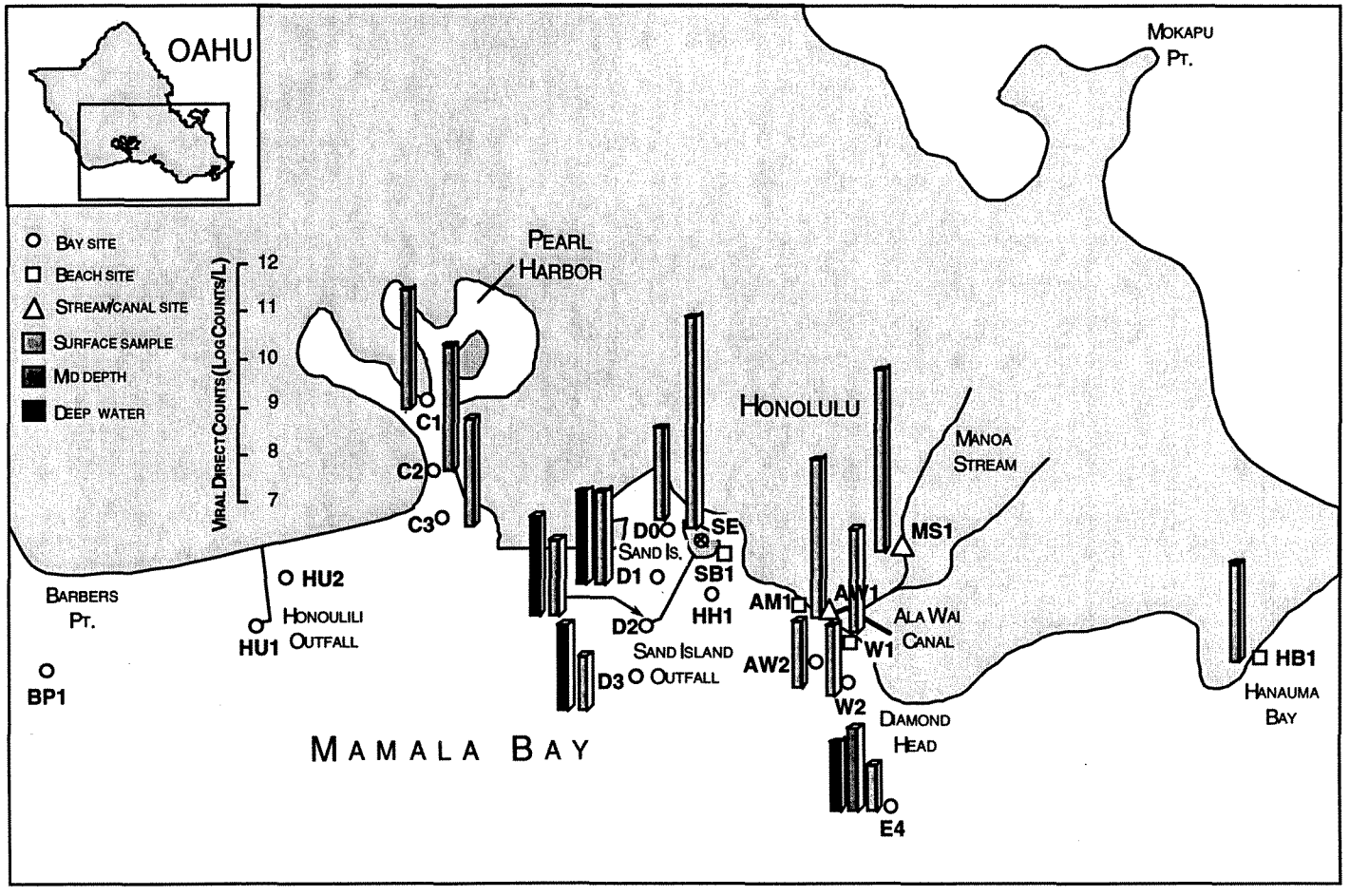

Fig. 7. Viral Direct Counts for stations sampled in October 1993

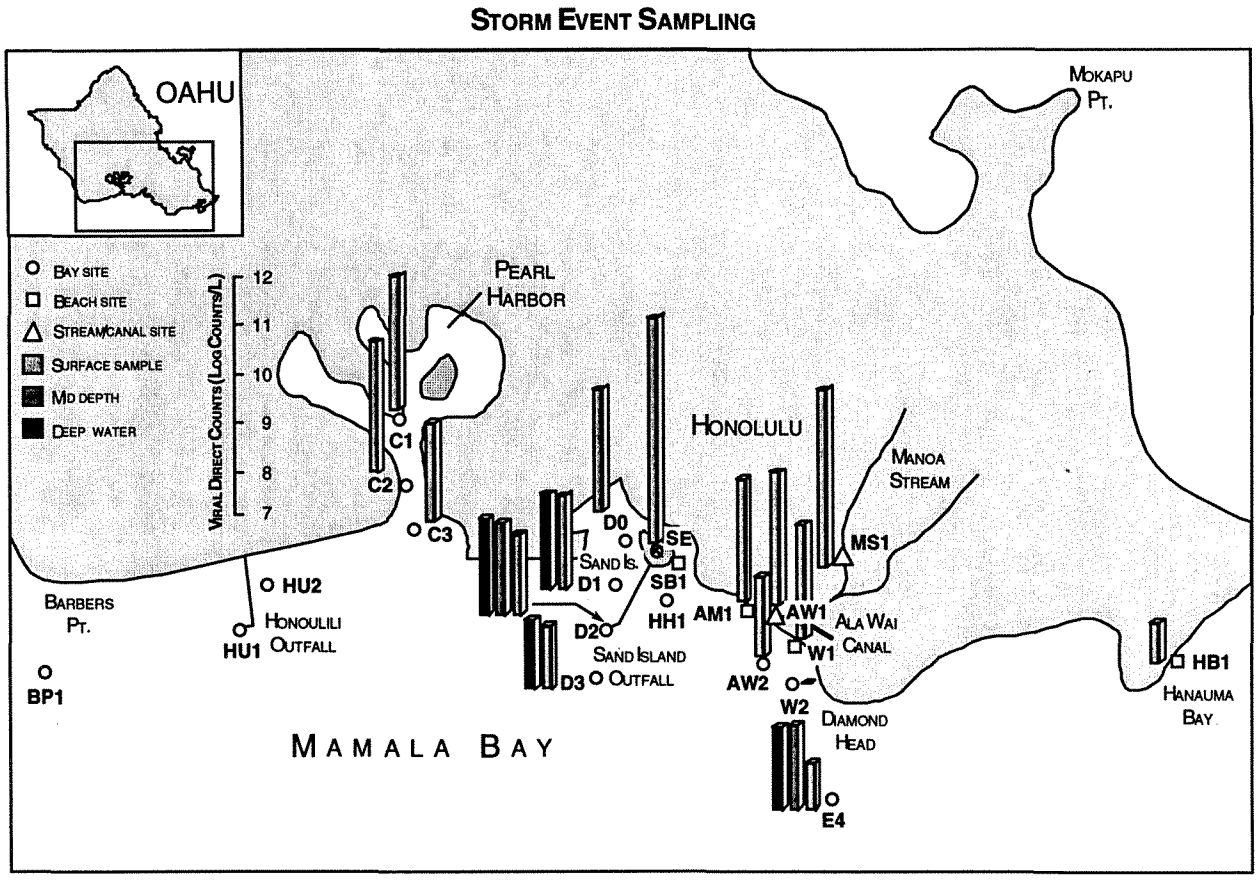

Fig. 8. Viral Direct Counts for stations sampled in February 1994

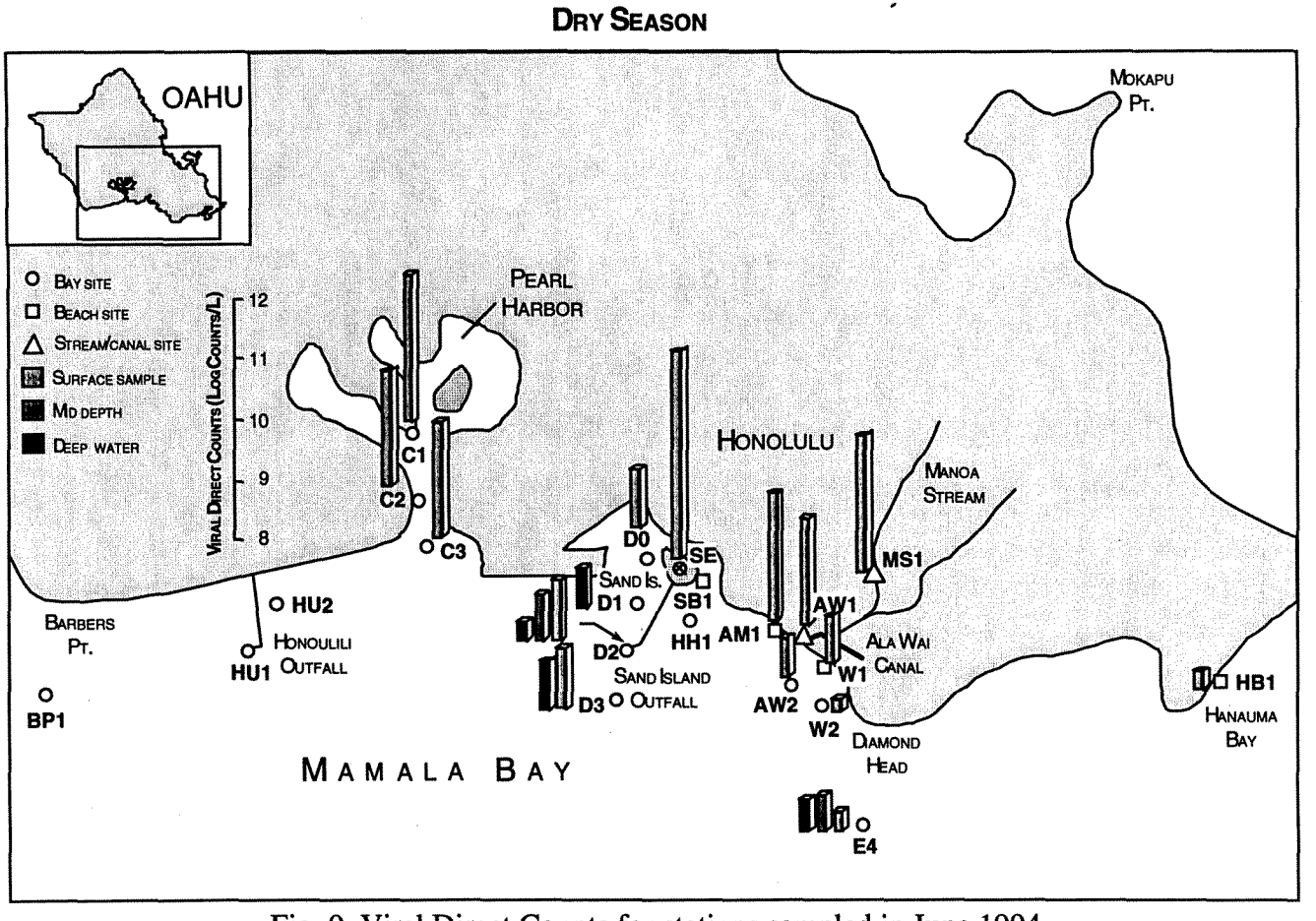

Fig. 9. Viral Direct Counts for stations sampled in June 1994

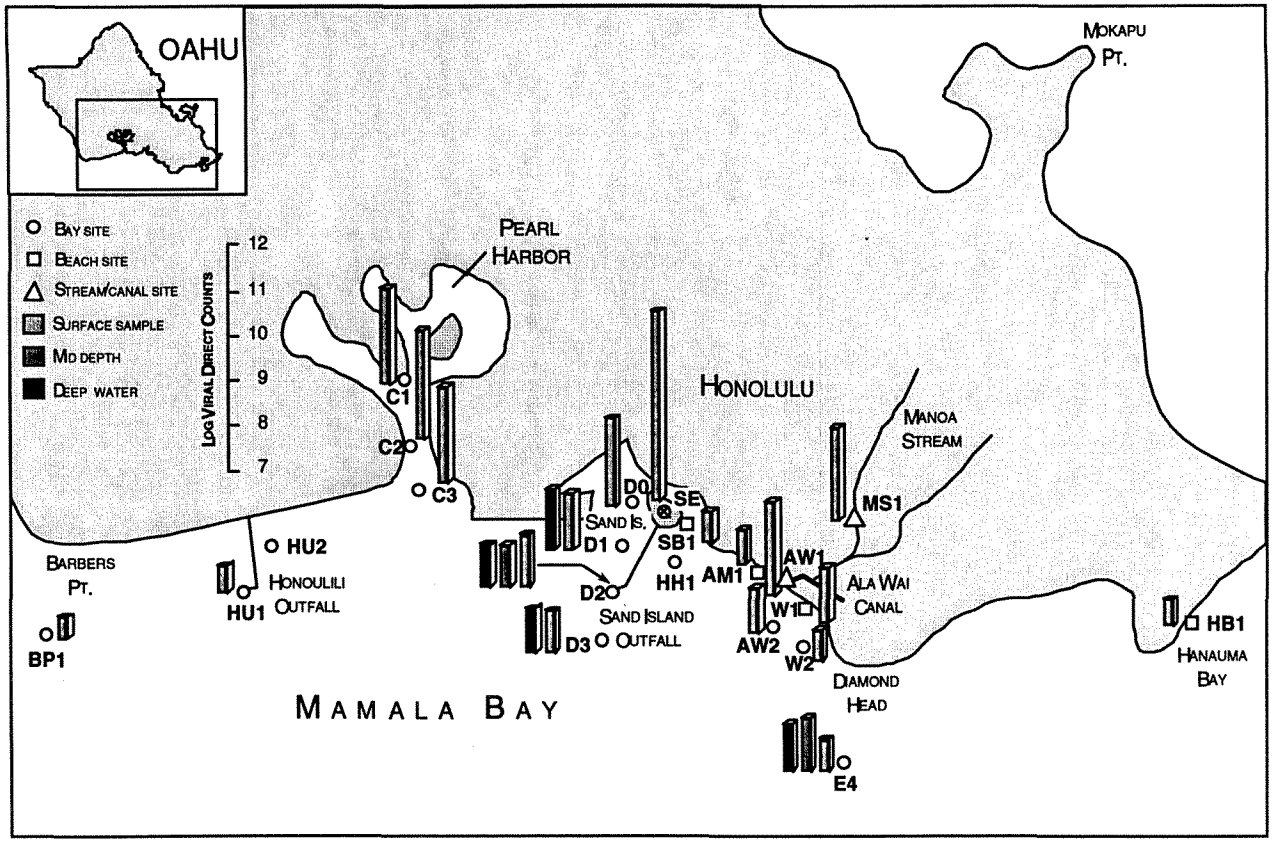

Fig. 10. Viral Direct Counts for stations sampled in November 1994

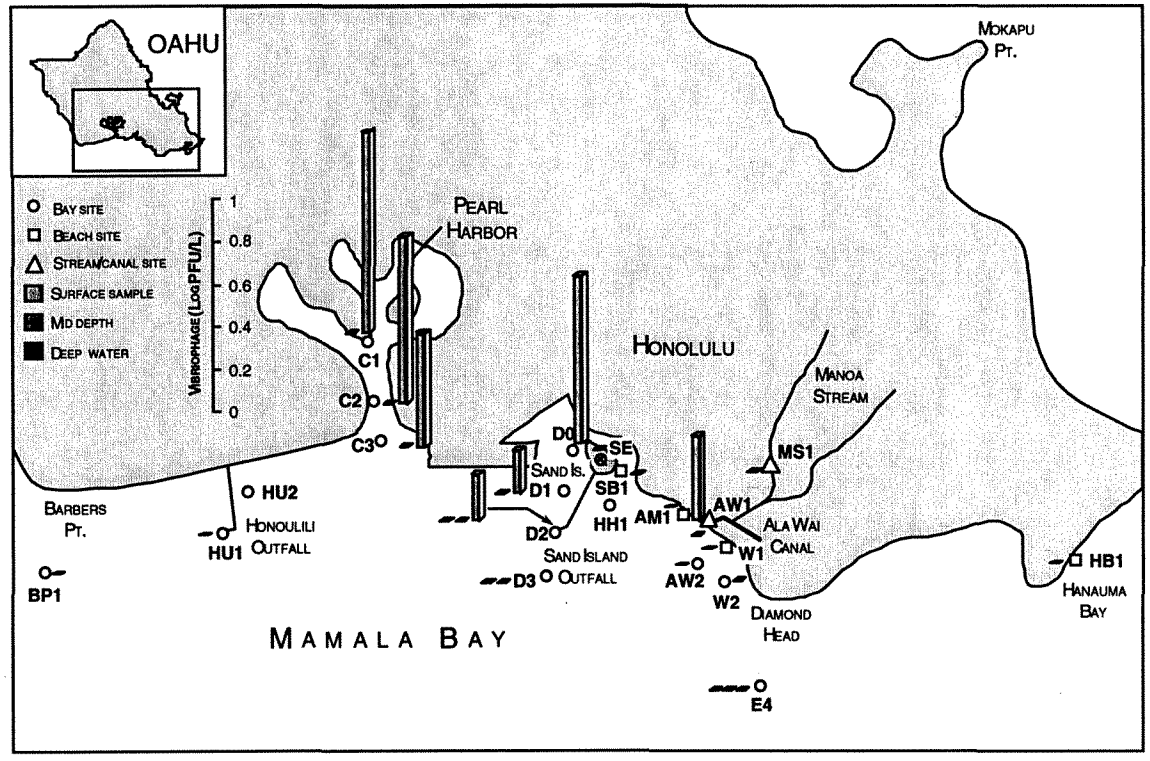

Fig. 11. Vibriophage-Means of All Seasonal Samplings

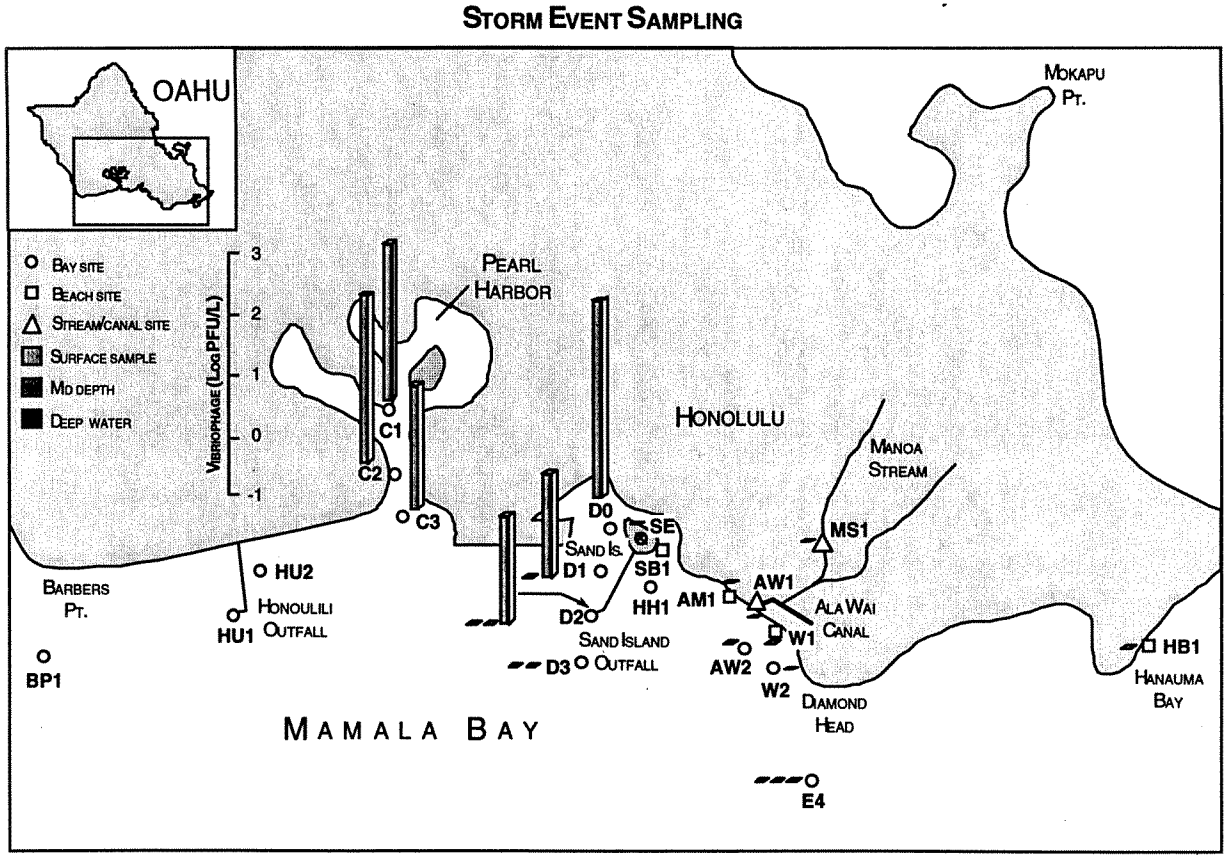

Fig. 12. Vibriophage abundance for stations sampled in February 1994

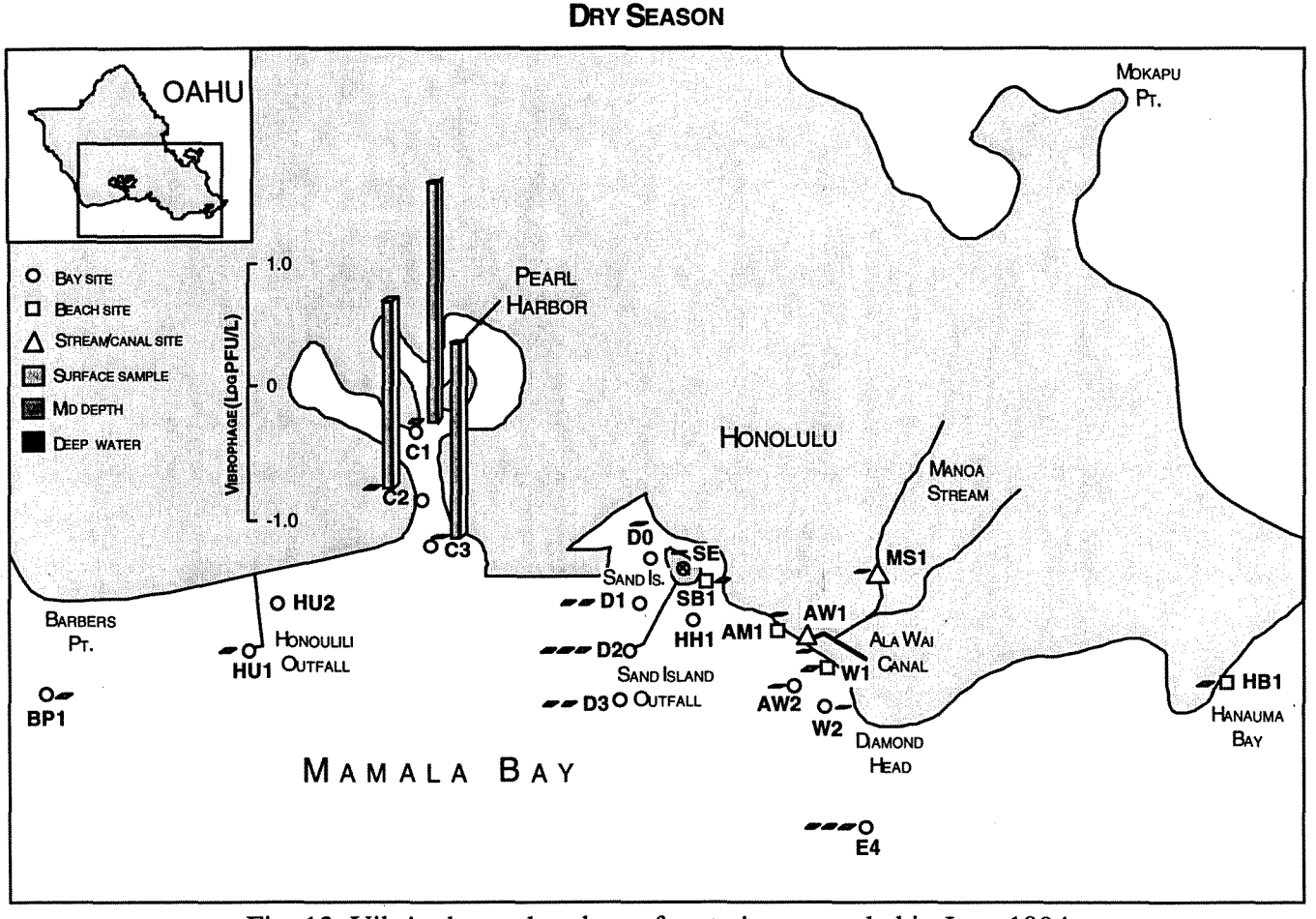

Fig. 13. Vibriophage abundance for stations sampled in June 1994

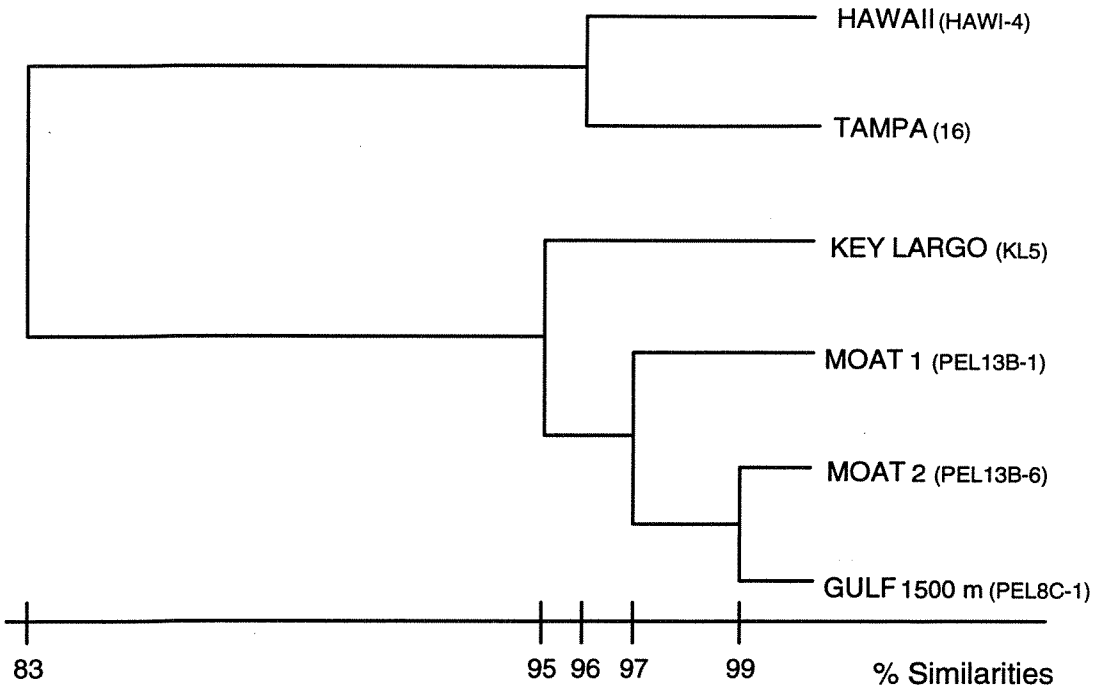

Fig. 14. CLUSTAL Analysis of Cryptic Gene Sequence from Related Vibriophages

CLUSTAL alignment of Vibriophage strains isolated based on sequence analysis of a cryptic gene fragment. The phage from Mamala Bay (HAWA-4) was most closely related to F16, the isolate from Tampa Bay.