# AN INNOVATIVE APPROACH TO ASSESS AND MONITOR THE QUALITY OF COASTAL WATERS

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## SUMMARY

An exploratory study was conducted under Sea Grant Mini-Grant Program to test the hypothesis that by implementing an innovative monitoring strategy of analyzing waters for three complementary tests, the combined test results can more reliably determine whether that environmental water is contaminated with point source pollution (sewage) or non-point source pollution (stream, storm drain). These three selected tests were used to analyze the quality of water samples obtained from streams, coastal waters used for swimming as well as shoreline, nearshore and offshore ocean sites near three of the ocean sewage outfalls (Waianae, Mokapu and Sand Island) operated by the City and County of Honolulu. The monitoring strategy involved the use of the following three tests:

1. Viable assay for enterococci bacteria. This is a relatively simple test and is the only test, which can be used to determine whether the water meets the EPA water quality standard for swimming based on 35 enterococci/100 ml. This test was able to enumerate enterococci bacteria primarily in water samples from coastal waters and in ocean water samples near the sewage ocean outfalls. However, this assay has three disadvantages. First, the test results take so long (48 hours) that it is not applicable to address the risk to swimmers using that water on that given day. Second, the enterococci bacteria are less stable in marine waters than many sewage-borne pathogens such as viruses and therefore absence or low levels of enterococci in water samples, may provide a false sense of security. Third, in Hawaii, there are environmental sources (soil, stream, storm drain) of enterococci, which are not directly related to sewage contamination, and therefore the presence of these bacteria in environmental waters may provide a false sense of risk to swimmers.

2. Enzymatic assay for ATP. This test measures total microbial activity and indirectly the levels of nutrients as a class of pollutant in a given water sample. Nutrient, as a source of pollution, may be from point or non-point sources of pollution. Therefore, the test results may be ambiguous by itself, but are useful when combined with other water quality data. An advantage of this test is that it can provide results rapidly. Some tests can provide results in minutes although the test used in this study took approximately 6 hours. Use of this test can provide results within minutes so conclusions about water quality can be made quickly and actions can be taken on that same day. The results of this assay can characterize the quality of water at different marine sites. For example it can differentiate between coastal sites with high microbial activity (high nutrient load) and those with low microbial activity (low nutrient load).

3. Gene probe test using polymerase chain reaction (PCR) assay for *Bacterioides fragilis* group (BFG) bacteria in water samples. This group of bacteria is present in human feces and sewage at concentrations approximately 10,000 greater than viable concentrations of enterococci. Since the PCR test detects both dead and alive bacteria, this is a very sensitive and conservative test for the presence of sewage in the water. The advantage of this test is its sensitivity. The disadvantage of this test is that it measures the presence of dead as well as live microorganisms

and therefore its public health significance is difficult to interpret unless there are other types of data. The detection of BFG in marine waters indicates that the water is contaminated with sewage. Significantly, BFG was generally not detected in stream water samples, which contain high concentrations of enterococci bacteria from non-point sources. Thus, by analyzing environmental waters for BFG bacteria, data is available to more reliably determine whether that water is contaminated with sewage.

In summary, evidence was obtained to support the hypothesis that by implementing an innovative monitoring strategy of analyzing water samples for three different and complementary tests, more reliable results were obtained to assess the sources (point versus non-point) and kinds (sewage versus environmental) of pollution in that water. The basis of this approach is that each test has advantages and disadvantages and by itself cannot provide sufficient information. However, by using a combination of tests, the disadvantages of individual tests can be addressed by other test results and the advantages of the three tests can be used. Based on these results, the use of this innovative monitoring strategy appears to be promising. Since the data is more reliable, risks to people and to the other aquatic animals using these marine waters can be determined faster and with greater reliability using this approach.

# I. INTRODUCTION TO THE PROBLEM

**A. Value of Clean, Safe and Productive Coastal Waters.** The value and many uses of coastal waters are borne out by the fact that people in the US prefer to live near coastal waters and those who live inland will travel thousands of miles to vacation in areas near coastal areas. Moreover, industry, businesses and cities have been built near coastal waters due to many uses of coastal waters such as shipping, fishing, other marine water-related businesses as well as for recreation and for tourism. In island nations located long distances from continents, the dependence on the use of coastal waters is much greater. For example, in Hawaii, coastal waters have traditionally been a primary source of food, for transportation, for recreation, for esthetic appeal and have become interwoven in the culture and religion of people who live there. In summary, coastal waters are valuable assets to coastal cities in terms of their economy, the quality of life as well as culture of the people who live in that environment (Fujioka, 1992).

Due to the popularity and many uses of coastal waters, there has been a dramatic and corresponding increase in construction (hotels, restaurants, roads, parks, airports), in services (water lines, sewer lines, fishing piers, marine recreation) and in other related activities (harbor, mariculture) to meet the needs of all people using coastal waters. These kinds of growth come at the inevitable expense of polluting coastal waters and the corresponding implementation of regulations to protect the quality of coastal waters (Fujioka, 1992). Pollution of coastal waters by pathogens, nutrients, sediments, as well as pesticides and toxic chemicals restrict the many uses of coastal waters and devalues the resources provided by coastal waters.

**B. Problems in Assessing Water Pollution.** Although EPA has published and mandated the use of prescribed methods to analyze coastal waters for pollutants, the application of these prescribed methods often does not result in easily interpretable results for two basic reasons. First, the approved methods to assess the hygienic quality of water measure for the presence of fecal indicator bacteria (eg. enterococci) rather than the sewage-borne pathogens. It is well known that the concentrations of fecal indicator bacteria are not directly related to levels of pathogens. Another limitation is that the fecal indicator bacteria (enterococci) dies off much faster in ocean water conditions than pathogens such as viruses (Fujioka, 1997). Thus, absence of enterococci in ocean waters does not guarantee that pathogens are absent. A serious problem occurs when this test and EPA standards are applied to Hawaii because in Hawaii, the fecal indicator bacteria, including enterococci, are naturally present in the soil environment (Hardina and Fujioka, 1991). Rainfall washes these soil-bound fecal indicator bacteria from the soil and they are transported to streams, storm drains and into marine receiving waters (estuary, harbor, beach sites) as non-point source pollution. Results from our laboratory (Fujioka and Byappanahalli, 1998), have documented that these fecal indicator bacteria are able to multiply in the soil environment of Hawaii, and have become a part of the natural micro flora of soil. Under these conditions, since the source of these fecal bacteria is environmental (soil) and is not feces, the health risks associated with concentrations of these environmental sources of fecal indicator in environmental waters are misleading.

Determining the nutrient contamination of coastal water is also important but current methods to assess total nutrient content of natural water is based on analyzing the water for the many specific nutrients. This approach is unreliable because it is not clear which of the many nutrients (phosphorus, nitrogen, carbohydrates, potassium, etc.) is responsible for causing eutrophication and as a result, it is not clear which nutrient should be measured. An alternative approach is to measure for total microbial activity because total nutrient load determines total microbial activity. For years, ecologists have used an enzymatic test to assay for ATP as means of measuring total microbial activity. From this measurement, total bacteria can be estimated.

**C. A Hypothesis to Use an Innovative Monitoring Strategy to Measure Pollutants and Pollutant Sources.** The EPA approach of selecting a single indicator test to assess the quality of coastal water results in data, which are ambiguous and difficult to interpret in tropical environments such as Hawaii, Guam and Puerto Rico. To address the limitations in the current monitoring strategy, we propose the hypothesis that by using an innovative monitoring strategy of analyzing marine waters by three different kinds of methods (culture, gene probe, enzymatic), more reliable data will be obtained to more confidently measure the level of pollutants, the specific types of pollutants and the source of pollutant. These kinds of data will enable one to better define the health risk to humans and the ecosystem. The three kinds of methods to be used in the proposed monitoring strategy are as follows:

1. Viability Assay for Concentrations of Bacteria. The usefulness of this method is that it can enumerate and isolate for further testing, a specific group of bacteria by using selective growth medium. This assay method is used by EPA to assess the hygienic quality of waters and to set water quality standards. EPA has established the standard for marine waters designated for swimming at 35 enterococci/100 ml. The limitation of this method is that it cannot be used to measure for most pathogenic bacteria and this method is not suitable for detecting viruses or protozoa. So, it is an indirect measurement for the presence of sewage borne pathogens. For ecological assessment, this method is not reliable because this method has been shown to recover less than 1% of all bacteria present in most environmental waters (Fujioka, *et al.*, 1996).

2. Polymerase Chain Reaction (PCR), a Gene Probe Test for Specific Microorganisms. This is a relatively new method, which can detect for the presence of microorganisms in water by selecting for a specific gene sequence and amplifying that gene. It is a very specific test and is very rapid because there is usually no need to have the target cell multiply. This method has not been approved by EPA for monitoring water because this test gives positive or negative measurements and does not enumerate the numbers of microorganisms. Moreover, since this method detects both dead and live cells, it is difficult to assess public health risks because only live microorganisms can infect humans and other animals. The major advantage of the PCR test is that it can detect microorganisms such as pathogens, which cannot be detected by culture methods. In this study, we will use the PCR test to assay for the presence of *Bacteriodes fragilis* group of bacteria (BFG) because this group of bacteria is specific to human feces and at concentrations 100 to 10,000 greater than *E. coli* and enterococci assayed by culture methods. Thus, the assay for BFG is a very specific and highly sensitive marker of sewage. Culture asssay for BFG is unreliable because this group of bacteria is strictly anaerobic, does not form spores and therefore dies as soon as it is discharged into the oxygen-rich environment. The detection of BFG bacteria by PCR and fecal bacteria by culture provides convincing data that the source of

fecal bacteria is from a point-source (sewage) pollution. The absence of BFG by PCR and the presence of fecal bacteria by culture indicate that the source of fecal bacteria is from non-point source (soil), a common situation in Hawaii.

3. An enzymatic Assay for ATP for Total Microbial Biomass. This is a rapid enzymatic assay to measure for total particulate-associated, adenosine triphosphate (ATP) levels in water as an accurate measurement of total microbial biomass. ATP is an essential compound required by all living cells. Ecologists use this test because ATP is produced by every viable cell, and this measurement can be used to determine the total microbial biomass in that water sample. The usefulness of this test is at least two fold. First, culturable test cannot be used to assay for total microbial biomass because culture methods measure less than 1% of the viable microbial population in natural water sample. Second, it is the growth of all microorganisms in natural water, which affect the survival of fecal indicator bacteria (enterococci) and sewage-borne pathogen (human virus), which pollute the marine environment. The ATP assay measures total microbial biomass in a body of water and it is well known that biomass is a direct response to nutrient load. Thus, bodies of water can be classified into nutrient load categories based on biomass determination. In this regard, nutrient loading is one of the common stress factors affecting the health of the ecosystems.

## II. PROJECT GOAL AND METHODOLOGY

**A. Project Goal.** The goal of this six-month mini-grant was to obtain preliminary data to test the hypothesis that by using an innovative monitoring strategy of analyzing marine waters for three different methods (culture, gene probe, enzymatic) more reliable data will be obtained to more confidently measure the level of pollutants, the specific types of pollutants and the source of the pollution. These kinds of data will enable one to better define the health risk to humans and the ecosystem. The ultimate objective of this monitoring strategy is to obtain reliable data for the following purposes: a) To determine when marine waters are contaminated with fecal bacteria from point source (sewage) pollution. Under these conditions, people who use these waters are at high risk to contract sewage borne pathogens. b) To determine when marine waters are contaminated with fecal bacteria from non-point source (soil) pollution, a common situation in Hawaii. Under these conditions, people who use these waters are at much lower risk for sewage borne diseases. c) To develop a more sensitive way to determine when ocean water is contaminated with sewage as a means to assess the movement of sewage from ocean outfalls. d) To rapidly determine the nutrient load at various coastal water sites.

**B. Sampling Sites**. Water samples from sites, which had been previously characterized, were selected as sampling sites. The sites include shoreline beaches on the southern shores of Oahu, which are heavily used for swimming (Ala Moana, Waikiki) and other sites, which are more natural and not used for swimming (Blow-Hole, Blackpoint). Also, stream waters which discharge into ocean sites. Samples of ocean water were obtained from offshore ocean stations near three of the ocean sewage outfalls (Waianae, Mokapu and Sand Island) operated by the City

and County of Honolulu. The ocean water samples were collected by the City and County of Honolulu. All samples were collected into sterile sample containers and immediately stored in a cooled iced-chest. These samples were then, transported back to the laboratory where the assay was initiated within 8 hours of collecting the samples.

**C. Monitoring Strategy.** The overall monitoring strategy using this innovative approach to analyze water is outlined in Fig 1.

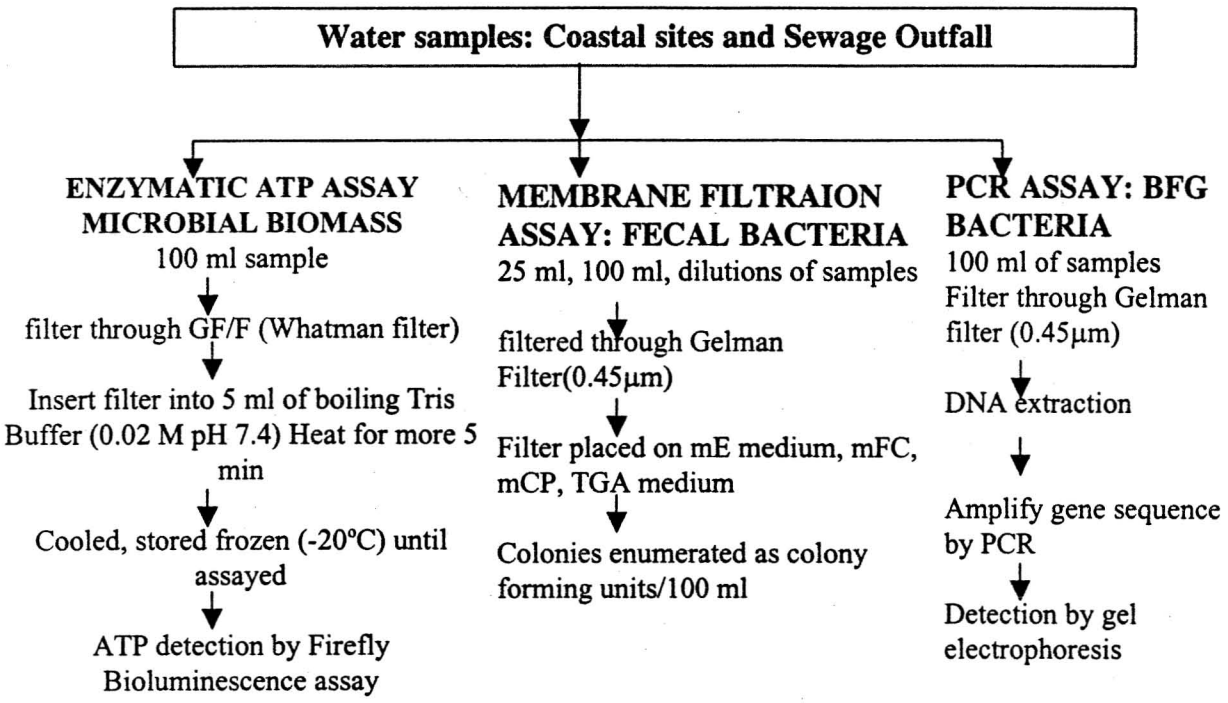


Fig. 1 Schematic representation of the experimental design of this study using three water quality tests.

**D. Culture Methods.** The membrane filtration method as described in Standard Methods (APHA, 1995) was used to measure the concentrations of enterococci bacteria in water samples because this is the fecal indicator bacteria in water used to establish recreational water quality standards.

**E. ATP Assay**. The ATP assay method as originally described by Strehler (1968) and modified by Karl (1993) was used. This method uses a firefly luciferase enzyme reaction and has been reported to be very rapid, sensitive, and reproducible. ATP is a measurement of total microbial biomass, which is defined as the total amount of living cellular material and generally expressed as mg $C/m^3$ for water samples, (Karl, 1980; Karl, 1993). The ATP assay is based on four assumptions (Karl, 1980): 1) all living organisms contain ATP, 2) ATP is easily extracted from microbial assemblages and can be precisely measured, 3) ATP is not associated with dead cells nor absorbed onto detritus material, 4) there exists a constant ratio of ATP to total cell carbon for all microbial taxa independent of metabolic activity.

**F. PCR Assay**. The PCR method was established in our laboratory as a result of a collaborative study with the Environmental Sciences Laboratory of the Orange County Sanitation Districts of California and the Dept. of Environmental Sciences, University of North Carolina (Palmer et al, 1995). The PCR method involves a series of steps as outlined in Fig 1 to recover DNA from cells and to test this DNA for specific genes. Initially, the sample water is filtered through a membrane with pores small enough to retain (concentrate) the bacteria on the surface of the membrane. The DNA from the bacteria on the membrane is extracted using the method as described by Ausubel *et al.*, (1992). Briefly, the DNA is released from the cells by placing the membrane filter into a 2 ml tube containing 560 ul of TE buffer, adding SDS (40 µl), Proteinase K (4 µl) followed by vigorous mixing for 30 seconds. The mixture was then incubated at 37°C for 1 hour followed by the addition of CTAB (hexadecyltrimethyl-ammoniumbromide) and NaCl and incubation at 65°C for 10 min. The DNA was then recovered by treatment with phenol:chloroform:isoamyl alcohol and chloroform:isoamyl alcohol mixture and then precipitated with isopropanol. The purified DNA was then resuspended in TE buffer. Approximately, 10 µl of this DNA was used as template for the PCR reaction by mixing with the proper primers, which had been selected to specifically attach to ends of specific gene sequences of your target microorganism. This DNA is then added to a thermal cycler with appropriate reagents to make many copies of the gene sequence. If the correct genes have been copied, it will be seen as a band with the correct molecular weight on a gel electrophoresis separation pattern.

For *Bacteroides fragilis* Group the following primers were selected to detect their sequences in the (16s rRNA gene):

| Primers | Sequence 5' – 3' | Fragment size |
|---|---|---|
| BFG410 | GTGAAGGATGAAGGCTCTAT | 410 bp |
| BFG800 | CGTTTACTGTGTGGACTACC | |

## III. RESULTS AND CONCLUSIONS (Tables 1,2,3)

**A. ATP Assay for microbial Biomass in Marine Environment.** ATP measures the enzymatic activity of microbial growth in a given water sample. This measurement indirectly determines the concentrations of total nutrient load in a given water because nutrient load determines the concentrations of microbial biomass. The results of ATP measurements show that relatively higher concentrations were measured in surface ocean water samples near coastal areas such as at Ala Moana Beach, Magic Island, and other commonly used beaches on Oahu. These results are consistent with the knowledge that these coastal ocean water sites are impacted by man-made activities, which add nutrients to these bodies of water. Moreover, these swimming sites are characterized by calmer waters, which have slower rates of exchange with open ocean water. In contrast lower concentrations of ATP were measured in offshore ocean sites and generally decreased with increasing depth of ocean water where better circulation of water occurs. These results show that deep ocean sites are not measurably impacted by nutrient loading from land-based activities. Elevated levels of ATP were detected in some samples near zone of mixing sites where sewage is directly discharged into the ocean. However, most of the water samples near the ocean sewage outfall site did not contain elevated levels of ATP, indicating that the sewage effluent was being mixed well and was efficiently being transported away to the open ocean. These ATP results provide a conservative approach to determine the relative quality of ocean water. The results indicate that this method can differentiate between coastal sites with high microbial activity and those with low microbial activity. These data can provide information on nutrient loading at specific coastal water sites and is especially useful in identifying these sites. Testing marine water for ATP is simple and provides rapid measurement of total microbial biomass. The ATP assay is the only test, which enables quick processing of samples so that the results can be obtained within the same day. Although our test procedure generally required 4 to 6 hours, newer methods can obtain ATP measurements within a few minutes.

**B. Assay for Viable Concentrations of Fecal Indicator Bacteria (Enterococci) in the Marine Environment.** The shoreline sites contained the greatest number of viable fecal bacteria (enterococci). As determined previously, the source of fecal indicator bacteria in shoreline sites is primarily from non-point source run-off such as from streams and storm drains and other human activities. Most nearshore and open ocean sites contain low levels of enterococci because these waters are not impacted by sources of point or non-point sources of fecal bacteria. However, enterococci were occasionally recovered from ocean sites near or in the zone of mixing area where sewage is directly discharged into the ocean. Thus, there was a general correlation between the presence of enterococci and elevated levels of ATP because point source pollution such as sewage and non-point source pollution such as streams and storm drains are also sources of nutrients. In summary, the value of analyzing water for concentrations of enterococci is that it determines when the quality of that water exceeds the water quality standard and legal action can be taken when this level is exceeded

**C. PCR Assay for *Bacteriodes fragilis* Group in the Marine Environment.** The PCR assay for BFG is the most sensitive assay for the presence of sewage. Significantly, many stream water samples, which contain non-point sources (soil) of fecal bacteria, including enterococci were

negative for BFG. These results provide independent evidence that sewage is not the source of fecal indicator bacteria in streams. In this regard, sewage samples could be diluted with sterile water to levels where enterococci could not be detected but BFG was still detectable. These results show that the BFG assay is specific to sewage and the PCR test is much more sensitive test for the presence of sewage than the enterococci test (Bonilla *et al*, 1998). The samples immediately near the sewage outfall were positive for BFG at all depth locations for nearly all sites of the three ocean sewage outfalls. Most of these samples were negative for enterococci. The trend was to detect less incidence of BFG as samples were taken closer to the shore. These results show that sewage discharged from the outfall does not appear to flow toward the shoreline. In summary, the PCR measurement for *Bacterioides fragilis Group* bacteria in water samples is a sensitive and conservative test for the presence of sewage in any water sample. By analyzing water samples for BFG and for enterococci one can determine whether the source of enterococci is from sewage (high public health risk) or from soil (low public health risk). In ocean water samples near the sewage outfall site, the BFG measurement appears to be the most sensitive test to track the movement of sewage.

**D. Conclusions.** Most test procedures used have advantages and disadvantages. Thus the results of a single test, often provides insufficient information to draw reliable conclusions regarding the source and type of pollution in the water sample. By analyzing the water sample for three selected tests, the weakness of individual tests is supported by the strength of the other two tests. In this study, the three complementary tests to determine the quality of water included a rapid, enzymatic test for ATP (nutrients), enumeration for viable concentrations of a fecal indicator bacteria (enterococci) and a gene probe test for BFG bacteria which is the most sensitive test for the presence of sewage in a water sample. Taken together, the results provide the necessary information so that the source (point vs non-point) and kinds of pollutants (nutrient, sewage, environmental) in the water can be reliably measured. The results of this exploratory study provide evidence that the proposed innovative monitoring strategy is feasible and will provide data more rapidly and with greater reliability.

Table 1. Distribution of ATP, biomass of carbon, viable enterococci bacteria, and *Bacteroides fragilis* Group (BFG) by polymerase chain reaction (PCR) in marine water samples from shoreline, nearshore and offshore sites in the vicinity of Waianae sewage outfall.

| Monitoring Stations | ATP | Biomass | Enterococci | PCR:BFG |
|---|---|---|---|---|
| **Waianae Shoreline** | **(ng/l)** | **mg C/m3** | **CFU/100 ml** | **(+/-)** |
| ws1 surface (0 m) | 62.9 | 15.7 | <1 | + |
| ws2 surface (0 m) | 12.7 | 3.2 | <1 | - |
| ws3 surface (0 m) | 35.5 | 8.9 | <1 | - |
| ws4 surface (0 m) | 22.5 | 5.6 | <1 | - |
| ws5 surface (0 m) | 23.4 | 5.9 | <1 | - |
| **Waianae Nearshore** | | | | |
| wn1s surface (0 m) | 21.7 | 5.4 | <1 | - |
| wn2s surface (0 m) | 9.3 | 2.3 | <1 | + |
| wn3s surface (0 m) | 8.7 | 2.2 | <1 | + |
| wn4s surface (0 m) | 13.7 | 3.4 | <1 | + |
| wn5s surface (0 m) | 7.1 | 1.8 | <1 | - |
| wn1b bottom (10 m) | 5.5 | 1.4 | 2 | + |
| wn2b bottom (9 m) | 2.8 | 0.7 | <1 | + |
| wn3b bottom (8 m) | 2.5 | 0.6 | <1 | + |
| wn4b bottom (9 m) | 1.2 | 0.3 | <1 | + |
| wn5b bottom (9 m) | 2.0 | 0.5 | <1 | + |
| w1s surface (0 m) | 5.8 | 1.5 | <1 | - |
| w2s surface (0 m) | 5.3 | 1.3 | <1 | + |
| w10s surface (0 m) | 1.4 | 0.3 | <1 | - |
| w1m | 4.4 | 1.1 | <1 | - |
| w2m | 1.3 | 0.3 | <1 | + |
| w10m | 1.6 | 0.4 | <1 | + |
| w1b | 1.0 | 0.2 | <1 | + |
| w2b | 1.2 | 0.3 | <1 | + |
| w10b | 1.0 | 0.2 | <1 | + |
| **Waianae Offshore** | | | | |
| w4s surface (0 m) | 11.5 | 2.9 | <1 | + |
| w5s surface (0 m) | 5.2 | 1.3 | <1 | + |
| w6s surface (0 m) | 2.5 | 0.6 | <1 | + |
| w7s surface (0 m) | 2.5 | 0.6 | <1 | + |
| w4m | 1.0 | 0.2 | <1 | + |
| w5m | 4.5 | 1.1 | <1 | + |
| w6m | 9.2 | 2.3 | <1 | + |
| w7m | 2.0 | 0.5 | <1 | - |
| w4b | 1.0 | 0.2 | <1 | + |
| w5b | 1.0 | 0.2 | <1 | + |
| w6b | 1.0 | 0.2 | <1 | + |
| w7b | 1.0 | 0.2 | <1 | + |

Table 2. Distribution of ATP, biomass of carbon, viable enterococci bacteria, and *Bacteroides fragilis* Group (BFG) by polymerase chain reaction (PCR) in marine water samples from shoreline, nearshore and offshore sites in the vicinity of Sand Island sewage outfall.

| Monitoring Stations | ATP | Biomass | Enterococci | PCR:BFG |
|---|---|---|---|---|
| **Sand Island Shoreline** | **(ng/l)** | **mg C/m3** | **CFU/100 ml** | **(+/-)** |
| ss1 | 31.8 | 8.0 | 0 | + |
| ss2 | 14.8 | 3.7 | | + |
| ss3 | 14.5 | 3.6 | 1 | + |
| ss4 | 90.9 | 22.7 | | + |
| ss5 | 21.9 | 5.5 | 2 | - |
| **Sand Island Nearshore** | | | | |
| sn1s | 21.4 | 5.3 | 0 | + |
| sn2s | 64.3 | 16.1 | 0 | + |
| sn3s | 18.7 | 4.7 | 0 | + |
| sn4s | 46.0 | 11.5 | 0 | + |
| sn5s | 24.8 | 6.2 | 0 | + |
| sn1b | 21.9 | 5.5 | 0 | + |
| sn2b | 17.1 | 4.3 | 0 | + |
| sn3b | 15.9 | 4.0 | 0 | + |
| sn4b | 21.0 | 5.2 | 1 | - |
| sn5b | 16.6 | 4.1 | 0 | - |
| **Sand Island Offshore** | | | | |
| zm1s | 30.9 | 7.7 | 0 | + |
| zm2s | 20.5 | 5.1 | 0 | + |
| zm3s | 30.9 | 7.7 | 0 | + |
| zm4s | 29.7 | 7.4 | 0 | + |
| zm1b | 16.0 | 4.0 | 21 | + |
| zm2b | 11.8 | 3.0 | 0 | + |
| zm3b | 16.8 | 4.2 | 0 | + |
| zm4b | 35.4 | 8.9 | 0 | + |
| **East Oahu Shoreline** | | | | |
| Blow Hole | 138.6 | 34.6 | 0 | - |
| Waikiki | 74.7 | 18.7 | 4 | - |
| Ala Moana | 194.4 | 48.6 | 1 | - |
| Black Point | 129.4 | 32.4 | 0 | - |
| Kaimana | 115.0 | 28.8 | 0 | - |

Table 3. Distribution of ATP, biomass of carbon, viable enterococci bacteria, and *Bacteroides fragilis* Group (BFG) by polymerase chain reaction (PCR) in shoreline, nearshore and offshore sites in Mokapu area in the vicinity of the Kailua sewage outfall.

| Monitoring Stations | ATP | Biomass | Enterococci | PCR:BFG |
|---|---|---|---|---|
| **Mokapu Shoreline** | **(ng/l)** | **mg C/m3** | **CFU/100 ml** | **(+/-)** |
| MS1 | 49.8 | 12.5 | 0 | + |
| MS2 | 60.2 | 15.0 | 55 | - |
| MS4 | 119.9 | 30.0 | 0 | + |
| **Mokapu Beach** | | | | |
| KAI beach | 117.0 | 29.2 | 0 | - |
| KC | 144.0 | 36.0 | 290 | - |
| KS | 498.6 | 124.6 | 10600 | - |
| PYR | 44.9 | 11.2 | 0 | - |
| KALAMA | 114.5 | 28.6 | 0 | - |
| NORTH | 43.7 | 10.9 | 0 | - |
| ONE | 126.2 | 31.5 | 0 | - |
| **Mokapu Nearshore** | | | | |
| MN1S | 48.2 | 12.1 | 0 | - |
| MN1B | 46.7 | 11.7 | 0 | + |
| MN2S | 61.0 | 15.2 | 1 | + |
| MN2B | 41.8 | 10.5 | 0 | + |
| MN3S | 52.5 | 13.1 | 0 | - |
| MN3B | 48.4 | 12.1 | 0 | + |
| MN4S | 52.7 | 13.2 | 0 | - |
| MN4B | 51.3 | 12.8 | 0 | + |
| **Mokapu Offshore** | | | | |
| M1S | 45.0 | 11.2 | <1 | + |
| M1M | 42.1 | 10.5 | 2 | + |
| M1B | 43.3 | 10.8 | <1 | + |
| M2S | 44.4 | 11.1 | <1 | - |
| M2M | 43.8 | 11.0 | <1 | + |
| M2B | 39.3 | 9.8 | 2 | + |
| M3S | 43.1 | 10.8 | 2 | + |
| M3M | 39.2 | 9.8 | 2 | + |
| M3B | 41.6 | 10.4 | <1 | + |
| M4S | 41.6 | 10.4 | 0 | + |
| M4M | 42.9 | 10.7 | 6 | + |
| M4B | 38.9 | 9.7 | 0 | + |
| M5S | 39.8 | 9.9 | 0 | + |
| M5M | 48.4 | 12.1 | 3 | + |
| M5B | 41.3 | 10.3 | 4 | + |
| M6S | 42.2 | 10.5 | 0 | + |
| M6M | 43.9 | 11.0 | 0 | + |
| M6B | 43.3 | 10.8 | 0 | + |

| | | | | |
|---|---|---|---|---|
| **Mokapu Nearshore** | | | | |
| MN1S | 48.2 | 12.1 | 0 | + |
| MN1B | 46.7 | 11.7 | 0 | + |
| MN2S | 61.0 | 15.2 | 1 | + |
| MN2B | 41.8 | 10.5 | 0 | + |
| MN3S | 52.5 | 13.1 | 0 | - |
| MN3B | 48.4 | 12.1 | 0 | + |
| MN4S | 52.7 | 13.2 | 0 | - |
| MN4B | 51.3 | 12.8 | 0 | + |

Sample code: s - surface, m - middle, b - bottom

zm - sewage outfall site (zone of mixing)

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