Application of Innovative Methods and Strategies to Differentiate Sewage from Non-Point Source Pollution in Hawaii

Problem and Research Objectives

The basic water quality problem in the state of Hawaii is related to the fact that fecal indicator bacteria (fecal coliform, *E. coli*, and enterococci) are naturally present in all streams and consistently exceed the U.S. Environmental Protection Agency-recommended recreational water quality standards (R.S. Fujioka, K. Tenno, and S. Kansako, 1988, Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams, *Toxicity Assessment* 3:613–630). In establishing and implementing recreational water quality standards, USEPA provides the following four guidelines. First, the natural habitat of fecal indicator bacteria is the intestines of mammals and there are no significant environmental sources of these fecal indicator bacteria. Second, these fecal indicator bacteria cannot multiply in the environment. Third, the concentrations of fecal indicator bacteria in environmental waters are directly related to the amount or degree of fecal or sewage contamination and the probability that sewage-borne pathogens are present. Fourth, when *Escherichia coli* and enterococci exceed the current USEPA recreational water quality standards, a predictable and unacceptable number of people who use bodies of water (streams, lakes, coastal beaches) for recreational purposes (swimming, wading) will become ill with diseases associated with diarrhea symptoms. In establishing these guidelines, USEPA relied on data obtained exclusively from the U.S. mainland and supported by data from other temperate regions of the world. USEPA then applied these water quality standards equally to all U.S. jurisdictions, including areas in the tropical and subtropical region of the world (Hawaii, Guam, Samoa, Puerto Rico, Virgin Islands, south Florida). A recent review (R.S. Fujioka and M.N. Byappanahalli, 2003, Tropical Water Quality Indicator Workshop: Proceedings and Report, Special Report SR-2004-01, Water Resources Research Center, University of Hawaii, 95 pp.) of all monitoring data has shown that the four USEPA assumptions are not applicable in these tropical environments where the ambient concentrations of USEPA-recommended fecal indicator bacteria (*E. coli*, enterococci) exceed the recreational water quality standards. These standards are not useful in these tropical environments because the USEPA-recommended fecal indicator bacteria are able to grow and become established in tropical soil environments due to the consistently warm temperature, high humidity, and available nutrients that allow them to become part of the natural microflora (R.S. Fujioka and M.N. Byappanahalli, 2001, Microbial ecology controls the establishment of fecal bacteria in tropical soil environment, *Advances in Water and Wastewater Treatment Technologies*, ed. T. Matsuo, K. Hanaki, S. Takizawa, and H. Satoh, 273–283, Elsevier Science, Amsterdam). In Hawaii (C.M. Hardina and R.S. Fujioka, 1991, Soil: The environmental source of *E. coli* and enterococci in Hawaii's streams, Environ. Toxicol. Water Quality 6:185–195) and Guam (R. Fujioka, C. Sian–Denton, M. Borja, J. Castro, and K. Morphew, 1999, Soil: The environmental source of *Escherichia coli* and enterococci in Guam's streams, J. Appl. Microbiol. 85:83S–89S), rain is the natural mechanism by which these fecal bacteria in soil are transported to streams at concentrations that exceed recreational water standards (in the absence of fecal contamination). Since most fecal-borne pathogens (human enteric viruses, protozoa), cannot multiply in the environment, the concentrations of fecal bacteria in the streams of Hawaii are no longer related to concentrations of sewage-borne pathogens. As a result, because the USEPA-recommended fecal indicators and recreational water quality standards are not reliable in areas such as Hawaii, Guam, and most likely other tropical Pacific islands, there is a need to develop more reliable fecal indicators and water quality standards specifically for these tropical regions.

One solution to the water quality problem in Hawaii was solved by R.S. Fujioka and L.K. Shizumura (1985, Clostridium perfringens, a reliable indicator of stream water quality, *Journal of the Water Pollution Control Federation* 57:986–992) when they showed that monitoring streams of Hawaii for *Clostridium perfringens* rather than the USEPA-recommended fecal indicator bacteria could be used to determine when streams are contaminated with sewage. Several additional studies were conducted using *Clostridium perfringens*, and we (R. Fujioka, B. Roll, and M. Byappanahalli, 1997, Appropriate recreational water quality standards for Hawaii and other tropical regions based on concentrations of Clostridium perfringens, *Proceedings of the Water Environment Federation 70th Annual Conference and Exposition*, Chicago, Illinois, 405–411) concluded that it was superior to any of the USEPA-recommended fecal indicator bacteria in fulfilling six criteria to select an ideal fecal indicator in Hawaii (R. Fujioka, 1997, Indicators of marine recreational water quality, *Manual of Environmental Microbiology*, ed. Hurst, Knudsen, McInerny, Stetezenbach, and Walter, 176–183, American Society for Microbiology Press). Based on ambient concentrations of *C. perfringens*, we (Fujioka et al., 1997) proposed two alternative recreational water quality standards for Hawaii based on geometric mean concentrations. For freshwater, the proposed standard was a geometric mean of 50 CFU/100 ml, whereas for coastal marine waters, the proposed standard was a geometric mean of 5 CFU/100 ml. These standards have proved to be reliable and have been adopted by the Hawaii Department of Health to determine when streams and coastal waters are contaminated with sewage. Some of the reasons why *C.*

perfringens can be reliably used in Hawaii are as follows. First, this bacterium is present in sewage at moderate concentrations (10^4 CFU/100 ml). Second *C. perfringens* is anaerobic and cannot multiply in the soil environment. Third, the method of assay is reliable and feasible. Fourth, the spores of *C. perfringens* are very stable in the environment, making it a more reliable indicator than the USEPA-recommended indicator bacteria, which are inactivated in environmental waters at a faster rate than sewage-borne pathogens. Although monitoring for *C. perfringens* can be reliably used to determine when most streams in Hawaii are contaminated with sewage, there are some conditions when this bacterium may not be reliable indicator of sewage contamination. One condition is when streams and storm drains merge and there is sediment build up in the stream/storm drain system. In this situation, non-sewage sources of *C. perfringens* such as feces of human and animals are discharged from storm drains and the spores accumulate and survive in the muddy sediment. The *C. perfringens* in the sediment are periodically re-suspended and the concentrations in the water column may at times exceed 50 CFU/100 ml. Feces of some animals such as dogs have higher concentrations of *C. perfringens* than human feces. Moreover, cattle and pigs discharge large volumes of feces into the environment. Feces from ducks are a primary aquatic source of *C. perfringens* because ducks live in streams where their feces are directly discharged. Although USEPA has been informed that their recreational water quality standards are not reliable in Hawaii and that the *C. perfringens* standards are much more reliable, USEPA continues to insist that Hawaii monitor its waters for their fecal indicators. The *C. perfringens* standards used in Hawaii has not been approved by USEPA.

The county wastewater departments in Hawaii are most vulnerable to the application of USEPA-recommended recreational water quality standards because whenever concentrations of fecal bacteria in streams or coastal waters exceed the recommended standards, sewage discharge from the wastewater facilities is blamed as the source of contamination. In this regard, the City and County of Honolulu is the agency with greatest vulnerability because it has the largest sewage treatment facilities and serves the greatest population in Hawaii.

The identified need for this study is to develop a monitoring scheme to determine when streams and other environments (stream, stream sediment, ocean, ocean sediment, wastewater) are contaminated with sewage under all conditions. In this regard, the assessment of experts is that no single indicator can be reliable for all conditions. Therefore, the strategy is to develop tests based on measuring other fecal indicators, in addition to *C. perfringens*, to reliably answer the following three basic questions regarding sewage contamination: (1) Is it really sewage contamination? (2) What is the extent of sewage contamination? (3) How recent or how long ago was the sewage contamination? If the answers to three questions can be obtained, all agencies as well as the general public in Hawaii will benefit because it will definitely be an improvement over the current situation, since the current fecal indicators and current recreational water quality standards are unreliable. The wastewater agencies in Hawaii will be a direct beneficiary because they can be assured when a pollution event is their responsibility and can take appropriate action. The regulatory agencies (Hawaii Department of Health, USEPA) will also be major benefactors because it is their responsibility to inform the public whether water is safe or unsafe for use, and these new tests will provide the necessary information to determine the risk to the public. The data obtained can be used to determine real risks from perceived risks to human health. This kind of information will lead to appropriate and effective management decisions.

The objectives of this study is to assess the reliability of monitoring environmental waters in Hawaii for *C. perfringens* in combination with other alternative fecal indicators (F-RNA coliphages, sorbitol-fermenting *Bifidobacteria*, fecal sterols) for the purpose of developing the best combination of test method and environmental monitoring plan to reliably determine when any environment (streams, storm drain, ocean, sediments, soil) is contaminated with sewage and whether the source of contamination is environmental (soil) or due to fecal wastes from non-human sources (e.g., birds, fowl, cattle, dog, cat, and chicken). The strategy of this proposed study is based on the fact that monitoring stream and ocean waters for *C. perfringens* can usually determine when sewage contamination has occurred. However, there are some conditions when the information provided by monitoring alone does not provide enough information to make conclusive decisions, especially regarding the time of contamination and associated health risks to humans. The scope of this research is to evaluate the reliability of determining when sewage contamination has occurred under a variety of conditions by analyzing samples of water or sediments for *C. perfringens* and two other potential alternative fecal indicators (sorbitol-fermenting *Bifidobacteria*, fecal sterols) in situations identified by the City and County of Honolulu as requiring more conclusive information.

Methodology

The design of this study is to monitor streams and coastal waters for one of the USEPA-recommended fecal indicator (enterococci, *E. coli*, fecal coliform), for *C. perfringens*, and for the two new alternative fecal indicators (sorbitol-fermenting *Bifidobacteria* and fecal sterols)

Sorbitol-fermenting *Bifidobacteria*. A recent study indicated that sorbitol-fermenting *Bifidobacteria* are specific to human feces, and although these bacteria are anaerobic, they survive long enough to be cultured (S.C. Long, E.J. Mahar, R. Pei, C. Arango, E. Shafer, and T.H. Schoenberg, 2002, Development of Source-Specific Indicator Organisms for Drinking Water, Technical report, American Water Works Association Research Foundation, Denver, Colorado). Thus, when these bacteria are recovered by a culture method, the results indicate very recent contamination. We will use the methods published by Long et al. (2002) to recover and enumerate sorbitol-fermenting *Bifidobacteria* using the Human Bifid Sorbitol Agar as well as to identify and confirm the presence of these specific bacteria. In this regard, Dr. Long has consented to assist us. Briefly, fecal, sewage, sediment, or water samples are collected in sterile bottles and transported to the laboratory. Using a sterile technique, various concentrations of water are filtered through a membrane. The membranes are then incubated anaerobically at 37ºC for 48 hours. Colonies that appear yellow, domed, and mucoid are presumptive sorbitol-fermenting *Bifidobacteria*. A representative number of presumptive positive colonies are transferred onto two Reinforced Clostridial Agar plates and tested for strict anaerobiosis by incubating one plate anaerobically and one plate aerobically at 37ºC for 48 hours. Strict anaerobes are tested by Gram stain, glucose, lactose and sorbitol fermentation, nitrate reduction, motility, catalase, fructose-6-phosphate phosphoketolase (F6PPK) activity, and nitrate reduction tests for confirmation as sorbitol-fermenting *Bifidobacteria*.

Fecal sterols. Fecal sterols are a class of metabolic by-products of mammals that end up in feces. Since the food and metabolism of animals differ, the fecal sterols differ. The study by P.D. Nichols, R. Leeming, M.S. Rayner, and V. Lathan (1993, Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia, *Journal of Chromatography* 643:189–195) indicated that monitoring environmental waters for different kinds of fecal sterols can be used to determine whether the source of contamination is human or animal feces. Fecal sterols are measured using gas chromatography (GC) to measure coprostanol as a source of human feces. Water or sediment samples are collected and filtered in a manner similar to Standard Methods 9222A. Lipid extraction and fractionation samples are extracted quantitatively using $CHCL_3$ MeOH. After phase separation, the lipids are recovered in the lower $CHCl_3$ layer and are made up to a known volume and stored sealed under nitrogen at -20ºC. Total lipid sterols are obtained following alkaline saponification of an aliquot (10%) of the total lipids. Products are extracted into hexane-$CHCl_3$ and stored at -20ºC. Sterols are converted to their corresponding trimethylsilyl ethers by treatment with bis(trimethylsilyl)trifluoroacetamide. The samples are analyzed by GC using a flame ionization detector and a split/splitless injector.

Principal Findings and Significance

The sorbitol-fermenting *Bifidobacteria* test was evaluated by applying this method to assay sewage, streams and streams contaminated with sewage. The bifidobacteria concentrations were high in sewage but very low in most environmental water samples such as streams. A major limitation of this method is the difficulty in identifying the bacterial colonies as *Bifidobacteria*. As a result, the yellow, presumptive colonies had to be tested using many methods to confirm that the colonies were truly sorbitol-fermenting *Bifidobacteria*. Moreover, these bacteria quickly inactivated in water samples, and therefore their usefulness was limited to only very recent contamination. Based on the low recovery rate, the instability of sorbitol-fermenting *Bifidobacteria* in environmental waters, and the number of tests required to confirm their presence, it was concluded that this test was not feasible or reliable for monitoring environmental waters for fecal contamination.

The fecal sterol test for coprostanol was evaluated for its use in assaying sewage, streams, and streams contaminated with sewage. Using this method, it was shown that sewage contained high concentrations of coprostanol (280,000 ng/L) and high concentrations of fecal indicator bacteria (*E. coli*, enterococci) as well as *C. perfringens*.

When ambient streams were assayed, the concentrations of coprostanol were low (<5 ng/L) but the concentrations of *E. coli* and enterococci exceeded EPA standards, whereas the concentrations of *C. perfringens* was lower than the Hawaii standard of 50 CFU/100 ml. These results confirmed that the source of high concentrations of

the USEPA- recommended fecal indicator bacteria (*E. coli*, enterococci) was not from sewage but from environmental soil. The usefulness of this method was shown during a known spillage of sewage into a stream. When tested, the concentrations of coprostanol was high (18,000 ng/L) after 24 hours, but this level dropped to 60 ng/L in 72 hours and down to <10 ng/L after 4 days. These results showed that the chemical assay for fecal sterol in the form of coprostanol was a reliable and feasible independent assay for the presence or absence of sewage contamination in Hawaii stream waters.

Results of this research have been presented at two venues. Kathleen Brostrom presented a poster, entitled "A possible alternative chemical indicator of human waste contamination for Hawaii," at the Hawaii Water Environment Association annual meeting, held in 2005 at the Hawaii Convention Center, Honolulu, Hawaii. She was awarded the second prize of $75. At the 2005 American Society for Microbiology, Hawaii Branch, annual spring meeting at the East–West Center, Honolulu, Hawaii, Kathleen Brostrom and Roger Fujioka's "Are fecal sterols an alternative of human waste contamination in Hawaiian recreation al waters?" was awarded $150 for second place (tied).