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INDICATORS FOR DETERMINING THE SOURCES AND EXTENT OF FECAL CONTAMINATION IN COASTAL WATERS: AN ANNOTATED BIBLIOGRAPHY

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INDICATORS FOR DETERMINING THE SOURCES AND EXTENT OF FECAL CONTAMINATION IN COASTAL WATERS: AN ANNOTATED BIBLIOGRAPHY

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For the:

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Water Resources Division

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EXECUTIVE SUMMARY

PURPOSE OF STUDY

This initial literature review was designed to expand the current information available to the Broward County Department of Natural Resource Protection (DNRP) in the area of fecal pollution indicators and their use for distinguishing human from nonhuman sources of fecal contamination in both fresh and marine surface waters. Without knowledge of the specific sources of contaminants, efforts to control microbial contaminants are difficult. This paper is divided into specific indicator sections with annotated bibliographies containing methods, observations, and conclusions. Annotations concern only new or expanded materials.

BACTERIAL INDICATORS OF FECAL CONTAMINATION

Coliform bacteria, particularly the fecal subgroup have been used for fifty years as an indicator of fecal contamination in fresh waters for known point sources such as sewage treatment plants. It is now known that the use of the Coliform group as indicators of the presence of feces-derived pathogenic bacteria and viruses has shortcomings which can make them untrustworthy indicators in a variety of situations. They exhibit **rapid die-off** in marine waters and are adversely affected by sunlight and other environmental factors. They enter the water from sewage, liveaboard boats, septic tanks, **storm drains** and runoff as well as from non-human mammal and bird feces.

False positives for fecal coliform may be caused by a variety of different organisms including *Klebsiella* and nonfecal coliform. In addition, coliforms have been isolated from pristine areas and may survive and **multiply** in tropical environments. Thus, the reliability of fecal coliform as an indicator of fecal contamination has been questioned in tropical waters.

Fecal Streptococci are also inadequate indicators in marine waters and the use of fecal coliform/fecal streptococci ratios to differentiate human and non-human sources is highly suspect, due to variable die off rates of the two groups. Bacteria of the genus *Aeromonas* behave similarly to *E. coli* in polluted waters, but deactivate more rapidly in seawater. The *Bifidobacteria*, *Campylobacteria*, *Pseudomonas* and *Salmonella* groups seem to offer no advantages over the coliforms and indicators of fecal contamination.

Bacteria of the genus *Staphylococcus* survive in sea water and may provide an index of the health risks of swimming in polluted waters, but the group also includes species not associated with fecal pollution. Species of *Bacteroides* and their phage have the potential to serve as indicators of recent fecal contamination, and may be specific to humans.

VIRUS

The coliphage group has received the most attention as a superior indicator to the coliforms in a variety of marine and fresh waters. They seem to have better survival and dispersal characteristics, and may serve as superior indicators of pathogenic fecal-derived viruses. They are also found in avian feces, but possibly at lower concentrations (dry weight basis) than in raw sewage. It is unclear if marine mammal feces are coliphage sources. None were found in fresh dolphin and sea lion feces, but they are also usually undetectable in fresh human stool samples. This may be because the host bacteria have not yet lysed to release the lytic phage particles. This lysis must occur after defecation, because coliphage are always detectable in raw sewage. This delay may provide a means to differentiate a release of fresh human feces from pollution from leaking sewage mains or septic tanks. Bacteriophage of *Bacteroides sp.* may also be useful. Although their concentrations are usually lower than for coliphage in marine waters (making them less valuable as indicators), their presence may indicate human fecal contamination, because they were not found in animal feces or in water polluted with animal waste.

NONCULTURABLE PATHOGENS

No nonculturable pathogens were found that are reliable indicators of fecal pollution.

GENE PROBES

The use of gene probes coupled with polymerase chain reaction amplification of bacterial DNA can distinguish specific strains of bacteria and may have the potential to distinguish bacteria from human and non-human sources. The method is relatively rapid and comparable with standard plate counting. More work is required to overcome interference and to establish a database of genetic fingerprints.

LIPID BIOMARKERS/COPROSTANOL

The fecal sterol coprostanol is found in the feces of humans through the bacterial metabolism of cholesterol in the intestinal tract. It is also excreted by a variety of other mammals, and has been found in Antarctic sediments, where it originates from marine mammals. Coprostanol is degraded relatively rapidly after excretion, usually disappearing in twenty to twenty-five days. It has been used to monitor sewage sludge and to detect fecal pollution in live-aboard marinas. In the absence of large marine mammal populations or livestock herds, high concentrations in sediments probably indicates recent human fecal pollution.

CAFFEINE

While caffeine has been investigated as a water quality indicator of human population, no documentation was available.

OPTICAL BRIGHTENERS

Optical brighteners and detergents are not specific indicators of fecal pollution.

OTHER FECAL-RELATED BIOCHEMICALS

Human secretory immunoglobin alpha (IgA) is secreted by the mucosa of the gut and can be detected in natural waters. It is specific to humans, but the analysis is expensive and reproducible results are difficult to achieve. Urobilins are breakdown products of bilirubin and are present in mammalian feces and urine. The analytical method is simple but it is unclear if the method is specific to human urobilins. Both of these methods have potential, but are not yet fully developed or tested.

RECOMMENDATION FOR HUMAN-SPECIFIC FECAL POLLUTION INDICATORS

In the opinion of the authors, the following offer the best prospects as human-specific fecal pollution indicators.

- *Bacteroides sp.* may be specific to the human gut, but they seem to be rapidly inactivated in the external environment.

- *Bacteroides* phage appears to be human specific and do not seem to multiply in waters or sediments.

- Molecular biological methods using gene probes and PCR technologies have the potential to quantify specific strains of bacteria. Human specific strains need to be identified and genetic fingerprint libraries must be established. Some methodological interference need to be overcome.

- Human secretory immunoglobin is probably specific to humans, but more developmental work is necessary.

- Future research may show that the development of human sterol profile may be used to distinguish human sources.

Fecal coliform continues to be used as a regulatory standard for surface water quality by Broward County (Broward County Code 1994) and the state of Florida (Florida Administrative Code 1995). In addition, high levels can be used to identify areas of concern. A suite of appropriate indicators can then be applied to narrow the range of possible sources leading the way to more efficient and immediate solutions.

I. INTRODUCTION

A. PATHOGENS VS. INDICATORS

Pathogenic (disease producing) microorganisms can be either opportunistic or highly invasive. Opportunistic pathogens present a health hazard only under certain conditions. Such as when an individual's normal defense mechanisms are compromised, such as by a break in the skin or by immunosuppressive agents, or when pathogens are present in elevated numbers. Highly invasive pathogens can infect less compromised individuals at lower numbers. This last group is less common and can cause epidemics. Normal routes of pathogen uptake are via ingestion, inhalation and breaks in the skin. Infectious dosages vary widely depending on species of microorganism and status of individual. In general, 10-100 viruses, 1-10,000 parasites and 100-1,000,000 bacteria are required for infection.

Pathogens from fecal matter (enteric pathogens) enter the aqueous environment from a number of sources including raw sewage, septic seepage, treated sewage, boats, stormwater, agricultural waste and run-off via estuaries and rivers. These pathogens can be freely suspended or associated with particulate matter. Pathogens associated with particulate material are protected from die-off and predation and may cause problems in enumeration. Predicting die-off is difficult due to the many factors involved. One must use caution in interpreting and comparing quantitative results presented in the literature.

It is impossible to attempt to identify all enteric pathogens present in a water supply. An indicator organism that is always present in fecal material and is readily identified is needed. There are three requirements necessary for a reliable indicator. First they should be native to the intestinal tracts of man and enter the water with fecal discharge. While relatively harmless, themselves, they are found in the company of other enteric pathogens. Secondly they should normally survive longer than their disease producing companions. Thus, once they have died-off, the danger is normally past. Thirdly they should be relatively easy to isolate and identify because of specific biochemical or culturable reactions.

Chemical indicators that are natural byproducts of human metabolism or activity may be beneficial. Also specific chemicals or microorganisms can be used as tracers to indicate sources or routes of contamination.

B. PURPOSE OF STUDY

This initial literature review was designed to expand the current information available to the Broward County Department of Natural Resource Protection (DNRP) in the area of fecal pollution indicators and their use for distinguishing human from nonhuman sources of fecal contamination in both fresh and marine surface waters. Without knowledge of the specific sources of contaminants, efforts to control microbial contaminants are

difficult. This paper is divided into specific indicator sections with annotated bibliographies containing methods, observations and conclusions. Annotations concern only new or expanded materials.

II. TRADITIONAL INDICATORS

A. TOTAL AND FECAL COLIFORM

Originally total coliform bacteria were used as an indicator of fecal pollution. This group includes all the aerobic and facultative anaerobic, gram negative, nonspore-forming, rod shaped bacteria that ferment lactose in 24 hr at 35° C. The definition includes the genera: *Escherichia, Citrobacter, Enterobacter* and *Klebsiella*. They are useful indicators for the following reasons. They are always present in human feces and survive longer than pathogens outside the body. They are easy to identify since they ferment lactose and produce an acid aldehyde that combines with fuchsin dye to produce an iridescent green coating over the growing colonies.

Later studies demonstrated that the feces of cold blooded animals contain certain bacteria that are in the coliform group. Soil run-off contains non-fecal bacteria that are also part of the coliform group. A sub-group of the total coliforms termed "fecal coliforms" grow mainly in the intestines of warm blooded animals including man. These are predominantly *Escherichia coli* and *E. coli* varieties. The fecal coliform test makes use of a key trait of these organisms, their tolerance for higher temperatures. When a mixed coliform culture is incubated on a selective nutrient at precisely 44.5 C (+/- 0.2 C), only the fecal coliform organisms will grow into visible colonies.

Total coliform is the indicator of choice for determining the potability of drinking water, while fecal coliform levels are used for the evaluation of environmental samples. A description of various methods for coliform bacteria is found in Standard Methods for the Examination of Water and Wastewater, 18 edition, (American Public Health Association [APHA] 1992). All species of coliform bacteria may occur in feces and their significance in water is subject of considerable study. *E. coli* is nearly always found in fresh contamination from the feces of warm blooded animals. Other coliform organisms may be found in fresh pollution in the absence of *E. coli*. Not all coliforms originate from sewage, some are free-living saprophytes and can multiply in anaerobic environmental conditions (APHA 1992).

In the marine environment, *E. coli* does not conform to the concept of an indicator because it is rapidly killed or inactivated by seawater (1, 9). Sampling near wastewater outfalls often yields concentrations of *E. coli*, which are far less than can be explained by dilution alone. Once an enteric pathogen of human origin enters a marine or estuarine environment, there are various factors affecting its survival. These include sedimentation, predation, parasitism, inactivation by sunlight, temperature, osmotic

pressure, and toxic chemicals (12). Cooler waters and lack of sunshine increase survival. Release of subsurface sewage plumes may reduce harmful light effects and allow for adaptation to higher salt concentrations with less cell stress. This may significantly increase survivability by tens of hours for bacteria (43) and many months for enteric viruses (25).

For all the above reasons additional methods are needed to truly identify the source and degree of contamination of human feces in surface waters. The following annotated bibliography will address the current developments in achieving this goal.

1. **Abdel, G.A., L. El-Attar, F. El-Sharkawi and S. Molazem.** 1989. Some environmental factors affecting survival of fecal pathogens and indicator organisms in seawater. Water Sci. Technol. **21**:115-120.

It was found that survival time of *Salmonella*, *Shigella flexneri* and *E. coli* were longer in fresh water than seawater at temperatures between 30 and 35 C. Daylight (sunlight) had a lethal effect on all test organisms. Survival time being shorter when exposed to daylight than in the dark in different types of waters, being up to a maximum of 24 hours as compared to several days in the dark.

2. **Alhajjar, B., S. Stramer, D. Cliver and J. Harkin.** 1988. Transport modeling of biological tracers from septic systems. Water Res. **22**:907-915.

Indicator bacteria in septic tank effluent are not transported to local groundwater but are removed by soil. As distance of monitoring wells increased from the drain field, virus counts in ground water also increased. Viruses appear to adsorb to the sediments of the unconfined aquifer closer to the septic system due to high ionic strength, and to desorb farther away from the drainfield as ionic strength is reduced. Indicator bacteria (TC, FC and FS) are not indicators for the presence of viruses. Virus contamination of ground water can occur from a well functioning system.

3. **Avila, M.J., M. A. Morinigo, R. Cornax, P. Romero and J. Borrego.** 1989. Comparative study of coliform-enumeration media from seawater samples. J. Microbiol. Methods **9**:175-193.

Selectivity, recovery efficiency and precision of various media was studied. The highest specificity for coliform was obtained with MacConkey and mFC media.

4. **Barzily, A. and Y. Kott.** 1991. Survival of pathogenic bacteria in an adverse environment. Health Related Water Microbiology, Water Sci. Technol. **24**:395-400.

Pathogenic bacteria are sensitive to changes in environmental conditions. This applies to Enterobacteriaceae as well, where the optimum temperature is 37 C. Wastewater of

various qualities are also hostile environment. This study investigated the influence of temperature on the die-off rate of enteric bacteria in waste stabilization ponds. Survival of indicator organisms at the control pond was higher than increased temperature ponds. The number of salmonella and shigella decreased by a few orders of magnitude within days depending on temperature.

5. **Barbe, D.E. and J. Francis.** 1995. An analysis of seasonal fecal coliform levels in the Tchefuncte River. Water Res. Bull. **31**: 141-146.

The climate of the area is characterized by different precipitation/runoff mechanisms for the summer and winter season. Runoff is higher in the winter resulting in higher fecal coliform counts. Statistical analysis revealed a statistically significant relationship between fecal coliform concentration and discharge for each season.

6. **Barnhart, H. M. and O. C. Pancorbo.** 1992. Microbial pathogens and indicators in estuarine environments and shellfish: critical need for better indicators of human specific fecal pollution. J. Environ. Health **54**: 57-63.

A review of the major microbial diseases associated with shellfish, and existing literature on the fate of bacteria and viruses in the estuarine environment is presented. Included are new potential indicators of human specific fecal pollution, such as *Bacteroides fragilis* bacteriophages and other phages belonging to gastroenteric organisms.

7. **Bartlett, M.S., J. H. Dorsey and J. A. Lemay.** 1989. Microbiological monitoring of recreational waters in Santa Monica Bay, California, and the effects of storm drain effluents on three bacterial indicators. Oceans '89: The Global Ocean **2**:684-689.

Analyses of data revealed several important trends. Low levels of indicator bacteria at nearshore stations and results of special offshore studies suggest association with storm drain effluents or some other known onshore source. Levels of all three indicators dramatically rose during wet weather, then fell to background levels two to three days after the event.

8. **Bonnefont, J.L., P. Lelong and Y. Martin.** 1984. Experimental study of fecal coliform survival of urban sewage in seawater. Second International Colloquium on Marine Bacteriology. **3**:567-572. Brest, France: 1-5 October.

Numerous investigations have examined the decline of fecal indicators in seawater with varying results. An experimental system was constructed with continuous flow and an increased dilution of a mixture of seawater and urban effluents. The role of sunlight is dominant over the ratio of other parameters, such as residence time and bacteria adaptation phenomenon. This study points out that T_{90} depends on the hour of the sampling and the exposure to sunlight at that time. As a consequence, it would be

hazardous to give values of decreasing averages of enterobacteria discharged in seawater that are adaptable to different situations.

9. Bonnefont, J.L., B. Guiennet and Y. Martin. 1990. Experimental studies on the survival of fecal bacteria from urban sewage in seawater. Water Res. **24**:267-273.

The role of sunlight in the death of fecal bacteria in seawater remains preponderant over the ratio of other parameters, such as residence time and bacteria adaptation phenomenon. The T_{90} depends on the hour of the sampling and the exposure to sunlight at that time.

10. Boone, D. 1996. Survival of Fecal Bacteria in Surface Waters. USF, personal communication.

Developing computer models of the transport and decay in estuaries. He is analyzing genetic fingerprints of baral sources that may contaminate surface waters.

11. Borrego, J.J., D. Castro, R. Cornax, M. Morinigo and E. Martinez-Manzanares. 1990. Viability of *Salmonella spp.* and indicator microorganisms in seawater using membrane diffusion chambers. Antonie van Leeuwenhoek **57**:109-117.

The relationships between indicators and *Salmonella spp.* depended mainly on the source of fecal discharges and on the survival capability of the microorganisms in aquatic environments. The organisms most closely related to *Salmonella spp.* were fecal coliforms and *Clostridium perfringens*, the latter yielding the highest linear regression slope value.

12. Brickler, S.K., J. D. Doyle, R. Kramer, R. Kuehl and B. Tunnicliff. 1992. Instability of fecal coliform populations in waters and bottom sediments at recreational beaches in Arizona. Water Res. **26**:979-988.

Fecal coliform populations in aquatic bottom sediments have been cited as a more stable index of overall water quality than corresponding populations in waters. However, comparative population stability of fecal coliforms in sediments and waters have not been evaluated in water bodies with tidal fluctuations. This was studied at two reservoirs. Both water and sediment fecal coliform populations were highly variable over time and sediment populations were not more stable. Trends in fecal coliform populations in sediments could not be used to predict those in water or vice versa. Factors believed to contribute to the high variability of sediment fecal coliforms included the coarse composition of the beach sediments and fluctuations in reservoir elevations due to hydroelectric power operations.

13. **Buckhouse, J.C., J. R. Miner, J. A. Moore and B. M. Sherer.** 1992. Indicator bacterial survival in stream sediments. J. Environ. Qual. **21**:591-595.

The survival of fecal coliform and fecal streptococci organisms was demonstrated to be significantly longer in sediment laden waters than those without sediment. Survival was longer in the sediment laden waters than in a supernatant from that same sediment suspended in water. Fecal coliform and fecal streptococci revealed half lives from 11 to 30 days and 9 to 17 days, respectively when incubated with sediment. This is longer than when they are similarly incubated without sediment.

14. **Carballo-Cuervo, S., M. C. de la Rosa-Jorge, R. M. Fernandez-Alvarez and J. Rodriguez-de Lecea.** 1991. The influence of agricultural run-off on bacterial populations in a river. J. Appl. Bacteriol. **70**:437-442.

The counts of fecal indicators greatly increased when cattle were allowed to roam free. Counts of enterobacteria and fecal coliform ranged from 1000 to 1,000,000 cfu/100ml. Fecal streptococci counts were smaller <10 cfu/100ml. *E. coli* and *Pseudomonas aeruginosa* were isolated from all samples.

15. **Cooney, J.J. and G. W. Pettibone.** 1986. Effect of organotins on fecal pollution indicator organisms. Appl. Environ. Microbiol. **52**:562-566.

Organotins alone would not be likely to cause reductions in counts of indicator organisms. When combined with other stresses such as chlorine, copper from antifouling paints and toxic organics, all of which may be found in urban harbors and marinas they may have an influence. Organotins such as the butyltins which are components of antifouling paints may contribute to the injury of indicator organisms.

16. **Cox, F., G. Plews and R. Seabloom.** 1990. Effect of sewage discharge from pleasure craft on marine water and shellfish quality. Canadian Waste Management Conference 101-118. 3-5 Oct.

The analysis of data accumulated at a harbor revealed mixed results in terms of the influence of boat wastes on water quality. There was an observed increase in fecal coliform counts both inside and outside the harbor during the Memorial Day weekend as contrasted to the background data taken previously. A deterioration in water quality attributable to boat wastes was suggested by the fact that the fecal coliform concentrations inside the harbor failed to meet water quality standards on three days of the boating weekend, while outside it failed on one day. The data from this study demonstrated an impact by boat fecal wastes on bacteriological water quality, although the influence was considered modest. The sewage discharges from boats must be regarded as a source of fresh fecal pollution. A much greater potential for the presence and survival of pathogens therefore exists.

17. Criswell, C. F. 1994. The effect of the West Indian Manatee (*Trichechus manatus*) on fecal coliform levels in the Indian River Lagoon, Brevard County, Florida. Thesis. Florida Institute of Technology.

Data indicates that manatees are not contributing to high counts of fecal coliform or enterococci.

18. Davis, E., D. Manville, J. Mathewson and G. Meriwether. 1992. Investigation of bacterial populations producing elevated MPN values and false positive fecal coliform counts and applicable disinfectants in an industrial wastewater. Water Sci. Technol. **26**:9-11.

Elevated MPN and false positives principally due to *Klebsiella* of environmental not enteric origin.

19. Ding, M. and F. Huang. 1992. A preliminary analysis of the distribution of fecal coliform bacteria in the near shore waters of the south of Fildes Peninsula, Antarctica. Antarctica Research/Nanji Yanjiu **4**:84-87.

The results indicate that in surface water of the near shore waters the average density of the detection of fecal coliforms is 20/l. The closer to shore the greater the quantity of coliform bacteria which demonstrates their on shore origin.

20. Davies, C., J. Long, M. Donald and N. Ashbolt. 1995. Survival of fecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. **61**:1888-1896.

The survival of culturable fecal coliforms, fecal streptococci and C*lostridium perfringens* in marine sediments near a sewage outfall was examined. Die-off of these organisms to 10% of their initial numbers occurred in both marine and freshwater sediments within 85 days. After 68 days the same proportion of *E. coli* organisms remained culturable, suggesting that sediment provides a favorable, nonstarvation environment for the organisms.

21. Dean, K. S. Hayes, K. Morgan, and L. Newland. 1990. Septic tank and agricultural non-point source pollution within a rural watershed. Toxicol. Environ. Chem. **26**:137-155.

Results indicate that there is significant pollution from agricultural sources and seepage from onsite wastewater disposal system. Excessive fecal coliform and fecal streptcoccus counts (>500 bacteria/100ml) were generally associated with rainfall events and several samples showed values >100,000 bacteria/100ml.

22. De Villiers, J.C., W. O. Grabow and P. Jagals. 1994. Evaluation of indicators for assessment of human and animal fecal pollution of surface run-off. Water Sci. Technol. **31**:235-242.

The value of selected indicators for determination of fecal pollution and the assessment of human or animal origin was investigated. Fecal coliform bacteria, fecal streptococci, sorbitol fermenting bifidobacteria, *Rhodococcus coprophilus* and somatic and male specific coliphages can distinguish by means of a combination of indicators, fecal pollution of human and animal origin.

23. Dosso, M., D. Guiral and A. Kouassi. 1990. Seasonal variations of microbial contamination of an estuarine tropical lagoon urban area. The case of the city of Abidjan (Ivory Coast). Rev. Hydrobiol. Trop. **23**:181-194.

Bacteriological enumerations of *E. coli*, enterococci and *C. perfingens* were made along with the determination of physical characteristics (salinity, pH and temperature) of the water and an estimation of organic pollution. In polluted water of human origin a ratio of *E. coli*/enterococci > 40 was determined. Temporal variations of *E. coli* and enterococci are directly linked to the water salinity variation.

24. Evison, L.M. 1988. Comparative studies on the survival of indicator organisms and pathogens in fresh and sea water. Water and Wastewater Microbiology, Water Sci. Technol. **20**:309-315.

There is a direct relationship between swimming in sewage contaminated waters and contacting gastro-intestinal infections. Enterococci concentration in the water best correlates with the incidence of infection.

25. Gerba, C and S. M. Goyal. 1988. Enteric virus: risk assessment of ocean disposal of sewage sludge. IAWPRC Conference on Water and Wastewater Microbiology. Newport Beach, CA. 8-11 Feb.

A review of the risk of infection, clinical illness and mortality associated with enteroviruses suggest that the presence of these viruses in shellfish and bathing waters presents a significant risk to the consumer.

26. Muniz, I., L. Jimenez, G. Toranzos and T. Hazen. 1989. Survival and activity of *Streptococcus faecalis* and *Escherichia coli* in tropical freshwater. Microb. Ecol. **18**:125-134.

Although the reliability of fecal coliform and streptococci as indicator organisms in temperate waters is acceptable, many questions have been raised in tropical waters.

27. **Howell, J., M. Coyne and P. Cornelius.** 1995. Fecal bacteria in agricultural waters of the bluegrass region of Kentucky. J. Environ. Qual. **24:**411-419.

When fecal bacteria were present, rainfall rapidly moved them from the soil surface to spring and well water. FC/FS ratios did not distinguish between domestic animal and human sources of contamination.

28. **Howington, J.P., G. McFeters and J. Smith.** 1994. Survival, physiological response, and recovery of enteric bacteria exposed to a polar marine environment. Appl. Environ. Microbiol. **60**:2977-2984.

Percentages of respiring *E. coli* and *Salmonella typhimurium* inputs to low temperature marine environments allow for the long term persistence of enteric bacteria in a nonrecoverable state.

29. **Jenkins, A., D. Kay, J. Wilkinson and M. Wyer.** 1995. Modeling fecal coliform dynamics in streams and rivers. Water Res. **29**:847-855.

A model is based on the assumption that the entrainment and deposition of organisms from storage within a stream bed is governed by the relationship between flow and the channel bed. The bacteria are assumed to be associated with particulates of low settling velocity.

30. **Jiang, S., C. Kellogg, J. Paul, J. Rose and E. Shinn.** 1995. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Appl. Environ. Microbiol. **61**:2235-2241.

To determine whether there was potential contamination of the subsurface aquifer and nearby coastal surface waters by waste disposal practices, the presence of fecal coliforms, *Clostridium perfingens* and enterococci were examined. Effluent and waters from onshore shallow monitoring wells contained two or all three of the fecal indicators in all samples taken, whereas deeper wells at these same sites contained few or none. Offshore wells two to six miles from shore showed little sign of contamination. Indicators were also found in surface waters in a canal and in offshore surface waters. These results indicate that fecal contamination of the shallow onshore aquifer, parts of the nearshore aquifer, and certain surface waters results from sewage disposal practices.

31. **Joncas, M., S. Michaud, J. Carmichael and M. Lavoie.** 1985. Detection of false positives among total and fecal coliform counts by factorial analysis of correspondence. Appl. Environ. Microbiol. **49**:229-231.

Coliform, both total and fecal, were isolated by membrane filtration from water and fecal samples. The biochemical characteristics were determined by an API-20E system (Analytab Products, Inc.). Water specific total coliforms were citrate positive, indole

negative, and amygdaline positive. Water specific fecal coliforms were either citrate positive, indole negative, amygdaline positive, and inositol negative or indole negative, amygdaline positive, and inositol positive. Any isolates not fitting the above pattern could be considered of fecal origin. These tests could be used in conjunction with membrane filtration as a confirmatory test for the evaluation of the bacteriological quality of water.

32. Juanico, M., D. Ronen and S. Gedaliah. 1990. The use of non-conservative parameters to trace wastewater effluents in water bodies. Water Res. **24**:1245-50.

A combination of non-conservative parameters was used to label wastewater effluent entering a water body where the use of conservative parameters was not possible. The simultaneous use of several parameters enables a smoothing out of high variables of single non-conservative parameters. Parameters discussed were conductivity, detergents, TDS, PO_4, alkalinity, Na and nutrients.

33. Kim, S.J., D. H. Lee, E. J. Lee, S. H. Lee and S. J. Park. 1995. Spectrofluorometric assay for rapid detection of total and fecal coliforms from surface water. Appl. Environ. Microbiol. **61**:2027-2029.

Using a spectrofluorometer, the length of the incubation time required in the fluorogenic assay was reduced to 12 hours.

34. Kuhn, I., G. Allestam, T. Stenstrom and R. Mollby. 1991. Biochemical fingerprinting of water coliform bacteria, a new method for measuring phenotypic diversity and for comparing different bacterial populations. Appl. Environ. Microbiol. **57**:3171-3177.

An automated microplate system for biochemical characterization of water isolates was used to fingerprint the bacterial flora from various water samples. Mathematical models for calculating the diversities and similarities between bacterial populations are described. The diversity may give information on whether an indigenous or allochthonous flora is present. The system was demonstrated on coliform bacterial populations from various water samples. For unrelated water samples the similarity coefficient (Sp) values were close to 0, whereas repeated samples of the same source showed Sps of 0.64 to 0.74. The Sp values from several water samples were also clustered to form dendrogram, thus indicating the relative similarities between the bacterial population to confirm suspected common sources of pollution.

35. Legendre, P. and M. Troussellier. 1989. Dynamics of fecal coliform and culturable heterotroph densities in an eutrophic ecosystem: Stability of models and evolution of these bacterial groups. Microb. Ecol. **17**: 227-235.

Time series of a population of fecal coliform and heterotrophic bacteria were monitored five and six years after the construction of a sewage treatment lagoon. These time series were used to evaluate previously published models that predicted population dynamics. The fecal coliform abundances displayed an annual cycle with maximum reduction during the summer, which was due to environmental variables such as sunlight and pH. Model predictions were supported by the data.

36. Lechevallier, M., C. Cawthon and R. Lee. 1988. Factors promoting survival of bacteria in chlorinated water supplies. Appl. Environ. Microbiol. **54**:649-654.

Attachment of bacteria to surfaces provided the greatest increase in disinfection resistance.

37. Mates, A. and M. Schaffer. 1992. Quantitative determination of *Escherichia coli* from coliforms and fecal coliforms in sea water. Microbios. **286**:27-32.

A simple rapid method was developed for counting *E. coli* in seawater using the membrane filter method. After filtration the filters were incubated on mFC medium for 24 hours at 44.5 C for determination of fecal coliforms. An in situ test for determination of *E. coli* was performed by transferring the filter to nutrient agar containing 4-methylumbelliferyl-B-D-glucoronide, and incubating for 3 hours at 35 C. *E. coli* colonies were detected by their fluorescence under longwave U.V. light. Extensive biochemical confirmation tests on the isolates showed all the fluorescent colonies were *E. coli*. False negatives amounted to 8.4%.

38. McFeters, G.A. and A. Singh. 1991. Effects of aquatic environmental stress on enteric bacterial pathogens. Pathogens in the Environment. J. Appl. Bact. **70** supplement:115s-120s.

Both indicator and pathogenic microorganisms become injured with sublethal stress in aquatic environments. This results in a number of phenotypic alterations, including greater sensitivity to surface active ingredients in selective media. The importance of using media and methods that detect injured indicator bacteria is supported by the observation that a range of enteropathogenic bacteria are less susceptible to injury than coliforms.

39. Niemi, J.S. and R. Niemi. 1991. Bacterial pollution of waters in pristine and agricultural lands. J. Environ. Qual. **20**:620-627.

Fecal indicators were common in the waters of agricultural areas. Levels exceed swimming water limits (100 cfu/100ml) and occasionally bathing limits (1000 cfu/ml). Highest counts were in the wet period. This demonstrates loading from agricultural areas must be considered as a source of fecal pollution. Fecal indicators were found in one

half of the samples from pristine areas, often at high concentrations. Animal contamination was suspected.

40. Niemi, J.S. and R. M. Niemi. 1990. Monitoring of fecal indicators in rivers on the basis of random sampling and percentiles. Water Air Soil Pollut. **50**:331-342.

The use of random sampling and calculation of percentiles was found to provide a good overall picture of the variability of the river. It is recommended to evaluate the bacterial concentrations separately during wet and dry periods.

41. Palmer, M. 1988. Bacterial loadings from resuspended sediments in recreational beaches. Can. J. Civ. Engin. **15**:450-455.

Bacterial loadings from the resuspension of contaminated sediments were measured in situ at three beaches. Sediment resuspension was determined. The loading due to sediment resuspension varied from 0 to 1410 fecal coliform/sq. m. Sediment loading in excess of 100 fecal coliform/sq. m caused significant increases in the water fecal coliform densities in shallow beach areas.

42. Poggi, R. 1991. Sanitary impacts of microbiological contamination. National Colloquium The Sea and Urban Waste Disposal **11**:115-132.

Human health risk linked to sea shore microbiological pollution is mainly affected by swimming activity and raw shellfish consumption. This study shows that sea water monitoring must be done during the week before human weekend activities. The risk to bathers is essentially linked to enterococcus occurrence.

43. Pommepuy, M., Cormier, M., A. Derrien, E. Dupray, J. F. Guilland and F. Le Guyander. 1992. Enteric bacteria survival factors. Water Management in Coastal Areas, Water Sci. Technol. **25**:93-103.

Bacteria are mixed with turbid waters and are able to survive a very wide range of salinity. Because light penetration is prevented by suspended matter, the solar bactericidal effect is very low.

44. Pourcher, A., L. Devriese, J. Hernandez and J. Delattre. 1991. Enumeration by a miniaturized method of *E. coli*, *S. bovis* and enterococci as indicators of the origin of fecal pollution of waters. J. Appl. Bacteriol. **70**:525-530.

Counts of *E. coli*, fecal streptococci and enterococci were made on fecal samples of human and nonhuman origin and urban raw sewage wastewaters, with microtiter plates containing selective media. *E. coli* was more numerous than fecal streptococci and enterococci in 80% of the samples regardless of the origin. The use of the ratio of *E. coli* to fecal streptococci to distinguish human from nonhuman sources is questionable.

Enterococci faecalis was predominant in human and poultry feces. *S. bovis* was typical of the bovine feces and to a lessor extent pig feces. *Ent. durans*. and *Ent. faecium* did not characterize any fecal source. *Streptococcus bovis* could be distinguished in the microtiter plate by its ability to reduce triphenyl tetrazolium chloride (TTC) in the medium.

45. Rector, D. D. 1977. Densities of indicator microorganisms in surface microlayers of recreational fresh waters. Thesis. Georgia State University.

The survival, persistence and dispersal of bacteria in soil raises serious water quality questions. *E. coli* was added to soil samples under conditions designed to mimic those encountered during field studies. Survival times for *E. coli* were up to 23.3 months under laboratory conditions.

46. Rivera, S., T. Hazen and G. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. Appl. Environ. Microbiol. **54**:513-517.

Samples collected from water accumulated in leaf axilae of bromeliads in a tropical forest were found to harbor fecal coliforms. Raises questions as to the validity of using fecal coliform as an indicator of water quality in the tropics.

47. Rhodes, M. and H. Kator. 1988. Survival of *Escherichia coli* and *Salmonella spp.* in estuarine environments. Appl. Environ. Microbiol. **54**:2902-2907.

E. coli can multiply and survive in the estuarine environment. Their death was attributed primarily to either low water temperature or predation.

48. Sjogren, R. E. 1994. Prolonged survival of an environmental *Escherichia coli* in laboratory soil microcosms. Water Air Soil Pollut. **75**:389-403.

The microcosm data gave maximum survival of a strain of *E. coli* in different soil types of 23.3 and 20.7 months.

49. Wright, R.C. 1989. The survival patterns of selected fecal bacteria in tropical fresh waters. Epidemiol. Infect. **103**:603-611.

Fecal bacteria may persist and reproduce in tropical waters thus limiting their usefulness as indicators of recent pollution events. Survival patterns varied according to source type, but some general observations could be made: a portion of the coliform was capable of substantial regrowth: fecal streptococci died off at a faster rate than coliforms initially, but survived longer; *C. perfringens* died off rapidly; and *Salmonella spp.* survived as long as the other fecal organisms.

50. Zaccone, R., E. Crisafi and G. Caruso. 1995. Evaluation of fecal pollution in coastal Italian waters by immunofluorescence. Aquat. Microb. Ecol. 9:79-85.

The immunofluorescent method showed high specificity for enteropathogenic *E. coli* and proved to be a rapid screening method to distinguish between polluted and nonpolluted water. However, a high threshold of detection sets a lower limit to the possibility of applying this method.

Summary - Coliform bacteria

Many articles have been written about the lower **survival rates** of coliforms caused by a variety of environmental conditions (10). Among the conditions studied were sunlight (8, 9) temperature (4) and salinity (1, 11). Coliform residing in bottom sediment survive longer than those suspended in the water column (12, 20). Contaminated bottom sediments contribute to water column levels of coliform bacteria by resuspension (13, 41). Coliforms can survive for months and years in terrestrial soils (45, 48). Fecal coliform may persist and reproduce in **tropical waters** (47, 49). **Storm drains** are a source of fecal bacteria (7), as well as sediments (13, 20, 45, 48).

Coliform bacteria are transported by a variety of conditions: runoff from rain (5), storm drains (7), animals (14, 39), agricultural sources (21, 27, 39), live-aboard boats (16), septic tanks (2, 30) and sewage (8, 9). They have also been isolated from pristine natural areas (46), In contrast, manatees are not contributing to high counts of coliform bacteria (17).

Indicator bacteria do not readily pass through small pore size or charged soil particles as easily as viruses and therefore, may not indicate viral contamination at any distance from the source (2). Different survival rates and soil transport rates are an additional reason that the fecal coliform/fecal streptococci ratio is not reliable (26, 27). Several new techniques have been investigated to more accurately isolate and differentiate *E. coli*. Comparisons of various media have been undertaken (3, 31, 33, 34, 37, 38) and the use of immunofluorescence (50). Evaluation as to source of contamination being human or animal requires additional indicator systems to be used (22). **False positives** for fecal coliform may be caused by *Klebsiella* (18) and nonfecal coliform (31, 34).

Fecal coliforms can persist and reproduce in pristine **tropical** rain forest (46) and tropical estuarine environments (23, 47, 49, 67). The reliability of fecal coliform and fecal streptococci as indicator organisms has been questioned in tropical waters (26, 55).

B. FECAL STREPTOCOCCUS

Fecal streptococci are less numerous in human feces than the coliform bacilli, thus they are a less sensitive indicator. This group consists of a number of species of streptococci. Several members of this group are sanitarily insignificant. Enterococci are a subgroup

of fecal coliform, much as fecal coliform is a subgroup of total coliform. Two specific species have been suggested for use as fecal indicators, *Streptococcus facelis* and *S. faecium*.

51. Bester, D., C. M. E. de Wet, M. Grundlingh, and B. Louw. 1991. Evaluation of fecal enterococci isolation media to indicate fecal pollution in chlorinated water. Water Sci. Technol. **24**:77-801.

The use of either mEnterococcus or KF streptococcus agar for the identification of fecal pollution in chlorinated water has limited value.

52. Charrel, M., J. F. Collin and D. Zmirou. 1988. Comparison of bacterial indicators and sampling programs for drinking water systems. Appl. Environ. Microbiol. **54**:2073-2077.

Comparisons between bacterial indicators showed that the information obtained from the various indicators was very similar.

53. Fujioka, R., A. Ueno and O. Narikawa. 1990. Unreliability of KF agar to recover fecal streptococcus from tropical waters. J. Water Pollut. Control Fed. **62**:27-33.

The KF membrane filter method has a false-positive rate ranging from 10 to 90% in marine and fresh waters. Therefore, the FC/FS ratio is suspect for indicating human pollution.

54. Marino, R. P. 1990. Survival of fecal coliforms and fecal streptococci in storm drain sediment. Dissertation. The University of Michigan.

This study demonstrates that storm drain sediments function as reservoirs of FC and FS during dry weather periods. Competition/antagonism appears to be a major factor determining fecal bacterial survival in drain sediment with protozoan predation being of lessor but significant importance.

55. Muniz, I., L. Gamines, G. Toranzos and T. Hazen. 1989. Survival and activity of *Streptococcus faecalis* and *Escherichia coli* in tropical freshwater. Microb. Ecol. **18**:125-134.

Streptococci faecalis and *E. coli* are less suitable as indicators of recent fecal contamination in tropical waters as determined by microautoradiography, cell respiration and nucleic acid composition.

56. Yoshpe-Purer, Y. 1989. Evaluation of media for monitoring fecal streptococci in seawater. Appl. Environ. Microbiol. **55**:2041-2045.

KF streptococcus agar allowed growth of non-strep colonies and non-typical streptococci colonies when 234 samples of Mediterranean seawater were examined.

Summary:

Fecal Streptococci are useful indicators of fecal pollution in fresh waters (52) however, in marine waters (53, 55, 56) they are of more limited value. The use of the ratio of fecal coliform to fecal streptococci to predict sources of pollution is suspect in all waters and storm drains (53, 54).

III. OTHER BACTERIAL INDICATORS

A. *ACINETOBACTER*

57. Bifulco, J., J. Shirey and G. Bissonnette. 1989. Detection of *Acinetobacter spp.* in rural drinking water supplies. Appl. Environ. Microbiol. **55**:2214-2219.

Acinetobacter was found in 16% of water supplies that did not contain coliform bacteria, posing concern about the usefulness of total coliform as an indicator of contamination.

B. *AEROMONAS*

58. Araujo, R.M., R. M. Arribas, F. Lucena and R. Pares. 1989. Relation between *Aeromonas* and fecal coliform in fresh waters. J. Appl. Bacteriol. **67**:213-217.

In water free from fecal pollution there was no correlation but in polluted waters there was a significant relationship between the numbers of aeromonads, fecal coliform and the concentration of organic matter measured by BOD.

59. Araujo, R.M., F. Lucena and R. Pares. 1990. The effect of terrestrial effluents on the incidence of *Aeromonas spp.* in coastal waters. J. Appl. Bacteriol. **69**:439-444.

There was a positive correlation between aeromonads and fecal indicators on the shoreline but not at 500m offshore. This reflected their common origin and different survival rates in seawater. When sterile sea water was inoculated with *A. hydrophilla* ATCC 7966, the numbers decreased initially by three orders.

60. Chowdhury, M.A.R., S. Miyoshi, S. Shinoda and H. Yamanaka. 1990. Ecology of mesophilic *Aeromonas spp.* in aquatic environments of a temperate region and relationship with some biotic and abiotic environmental parameters. Zentralbl Hyg Umweltmed **190**:344-356.

Aeromonas spp. were found in all environs with high densities throughout. No significant seasonal variation or any correlation with fecal pollution was observed in most of the areas. A reciprocal relationship was seen with salt concentration in the saline environment.

61. Monfort, P. and B. Baleux. 1991. Distribution and survival of motile *Aeromonas spp.* in brackish water receiving sewage treatment effluent. Appl. Environ. Microbiol. **57**:2459-2467.

Aeromonas spp. and fecal coliform distributions showed seasonal cycles in pond effluents. Survival studies confirmed that *Aeromonas spp.* were more sensitive to saline and marine stress than fecal coliforms.

C. *BACTEROIDES*

62. Cox, M.E., B. A. Dixon and D. A. Wadford. 1992. Techniques for the enhanced recovery of *Bacteroides vulgatus* as an indicator of fecal contamination in shellfish. J. Shellfish Res. **11**:557.

Bacteroides vulgatus is present in higher concentrations in the human intestine, and outnumbers fecal coliforms a thousand fold. Well-established clinical techniques based on the organism's resistance to kanamycin, vancomycin, colisin and bile exist. *B. vulgatus* has the potential to be an indicator organism of recent fecal contamination because it is more aerotolerant than other anaerobes.

63. Kreader, C. 1995. Design and evaluation of *Bacteroides* DNA probes for the specific detection of human fecal pollution. Appl. Environ. Microbiol. **61**:1171-1179.

Simple procedures must be developed to overcome interferences before PCR detection can be used to monitor fecal pollution. *Bacteroides* DNA rapidly disappears after a couple of days.

64. Lucena, F., J. Lasobras, D. McIntosh, M. Forcadell and J. Jofre. 1994. Effect of distance from the polluting focus on relative concentrations of *Bacteroides fragilis* phages and coliphages in mussels. Appl. Environ. Microbiol. **60**:2272-2277.

Bacteriophages that infect *B. fragilis* released into the marine environment resemble that of human viruses more than fecal bacteria, somatic and F-specific coliphages.

65. Tartera, C., F. Lucena and J. Jofre. 1989. Human origin of *Bacteroides fragilis* bacteriophages present in the environment. Appl. Environ. Microbiol. **55**:2696-2701.

Although the number of *B. fragilis* phages present in contaminated waters was lower than the number of coliphages, their presence indicated human fecal pollution.

D. *BIFIDOBACTERIA*

66. Okuofu, C. 1989. Comparative study of *Bifidobacteria*, faecal coliform and faecal streptococci as indicators of water pollution. Water Res. **1**:197-199.

E. coli and fecal streptococci are still more preferable water pollution indicators than bifidobacteria.

67. Opara, A.A. 1993. Assessing anaerobic enteric bacteria as indicators of fecal pollution in tropical waters. Trop. Freshwater Bio. **3**:309-318.

Certain anaerobic non-sporing bacteria, namely bifidobacterium, bacteroides and anaerobic gram positive cocci have been found suitable as alternative indicators of fecal pollution in tropical waters. The results in this study confirm the applicability of these anaerobes as indicators of fecal pollution in warm climates. The anaerobes were consistently recovered from tropical waters alongside the conventional indicators. The survival studies of bifidobacterium and *E. coli* showed that both organisms survived in stored river waters, at least up to 48 hours.

E. *CAMPYLOBACTERIA*

68. Hoeller, C. 1988. Long-term study of occurrence, distribution, and reduction of *Campylobacter sp.* in the sewage system and wastewater treatment plant of a big town. Water and Wastewater Technology,Water Sci. Technol. **20**:529-531.

Sewage can be heavily contaminated by *Campylobacter spp.*, however wastewater treatment processes can reduce counts considerably. The most frequently isolated species was *C. coli*.

69. Korhonen, L. and P. Martikainen. 1991. Comparison of the survival of *Campylobacter jejuni* and *Campylobacter coli* in culturable form in surface water. Can. J. Microbiol. **37**:530-533.

Predation and competition for nutrients affect the survival of both campylobacter species in the aquatic environment, with *C. jejuni* surviving longer. This is why *C. jejuni* is generally isolated more frequently from surface waters.

70. Korhonen, L.K., T. U. Kosunen and P. J. Martikainen. 1990. Occurrence of thermophilic campylobacters in rural and urban surface waters in central Finland. Water Res. **24**:91-96.

Bacteriological parameters indicated higher fecal contamination in campylobacter positive waters than in negative ones. Urban sites showed the same levels of fecal indicator bacteria as the rural sites, the isolation frequency of *Campylobacter* in the urban sites was lower than the rural sites.

F. *PSEUDOMONAS*

71. Codina, J.C., A. de Vicente and P. Romero. 1991. Relationship between *Pseudomonas aeruginosa* and bacterial indicators in polluted natural waters. Health Related Water Microbiol. **24**:121-124.

Results confirm that domestic sewage was the major source of *P. aeruginosa* in river and seawater, being isolated from sewage at concentrations about 100,000 cfu/100ml. There was a close correlation between the *P. aeruginosa* concentration and the densities of the three fecal indicators in both river and marine waters. A significant correlation was not observed in sewage and polluted natural waters were generally 3-4 log lower than the total coliform densities and 2 log lower than fecal coliform and fecal streptococci concentrations.

72. Ripp, S. and R. Miller. 1995. Effects of suspended particulates on the frequency of transduction among *Pseudomonas aeruginosa* in a freshwater environment. Appl. Environ. Microbiol. **61**:1214-1219.

Results indicate that aggregations of bacteriophages and bacterial cells are stimulated by the presence of suspended particulates. This aggregation increases the probability of progeny phages and transduction.

G. *SALMONELLA*

73. Alonso, J.L., L. Amoros, M. Botella and A. Rambach. 1992. *Salmonella* detection in marine waters using a short standard method. Water Res. **26**:973-978.

A combination of enrichment broths, isolation and identification systems allowed the detection of *Salmonella* from marine waters: with low or high levels of fecal contamination, in 48 hours.

74. Borrego, J.J., R. Cornax, E. Martinez-Manzanares, M. Morinigo and M. A. Munoz. 1992. Presence of indicators and Salmonella in natural waters affected by outfall wastewater discharges. Marine Disposal Systems, Water Sci. Technol. **25**:1-8.

It was concluded that fecal streptococci and *E. coli* bacteriophages are the most appropriate indicators of the remote pollution in marine areas.

75. Borrego, J. J., R. Cornax, M. A. Morinigo and P. Romero. 1990. Survival of pathogenic microorganisms in seawater. Curr. Microbiol. **20**:293-298.

The influence of different marine self purifying factors on the survival of several indicator and pathogenic microorganisms under control laboratory conditions was studied. Pathogens showed inactivation rates similar to those obtained by indicators under the same conditions. Visible light and biotic components of seawater were the most important inactivating factors.

76. Derrien, A. and E. Dupray. 1995. Influence of the previous stay of *Escherichia coli* and *Salmonella spp.* in waste waters on their survival in seawater. Water Res. **29**:1005-1011.

This study showed that enterobacteriaceae could adapt to seawater stress during their stay in sewage and sewage treatment plants. The actual mechanism is not clear, it may involve osmoprotection, modification of cellular metabolism or other. It appears that adaptation to drastic conditions occurs during the stay in wastewater allowing for better survival in more drastic seawater conditions.

77. Kator, H. and M. Rhodes. 1988. Survival of *Escherichia coli* and *Salmonella spp.* in estuarine environments. Appl. Environ. Microbiol. **54**:2902-2907.

Salmonella spp. can multiply and survive in the estuarine environment. Their death was attributed primarily to either low water temperature or predation.

78. Natarajan, R., T. Ramamurthy and S. Ramesh. 1989. Incidence of *Salmonella* in coastal environment of Porto-Novo (South East India). Eighth International Ocean Disposal Symposium. Dubrovnik, Yugoslavia.

MPN counts of total coliforms, fecal coliforms and *Salmonella* showed minimum during summer and maximum during monsoon season. Incidence of *Salmonella* was registered through the year in water and sediment.

H. *STAPHYLOCOCCUS*

79. Ahtiainen, J., M. Niemi and H. Jousimies-Somer. 1991. Staphylococci in polluted waters and in waters of uninhabited areas. Water Sci. Tech. **24**:103-108.

Compared the ratios of fecal indicator bacteria with staphylococci in a variety of surface waters. The proportion of confirmed staphylococci was higher in uninhabited areas than polluted areas. Therefore the origin of staphylococcal contamination and the evaluation of the indicator value of staphylococci require further study.

80. Cheung, W., K. Chang and R. Hung. 1991. Variations in microbial indicator densities in beach waters and health related assessment of bathing water quality. Epidemiol. Infect. **106**:329-344.

Staphylococci may serve as an indicator of bather density and the risk of microbial cross infection.

81. de Araujo, M.A., V. F. Guimaraes, L. Mendonca-Hagler and A. N. Hagler. 1990. *Staphylococcus aureus* and fecal streptococci in fresh and marine surface waters of Rio de Janeiro, Brazil. Rev. Microbiol. **21**:141-147.

S. aureus was common in bathing areas including sites not meeting coliform standards. Fecal streptococci had higher counts than fecal coliform in clean waters, but included species not associated with fecal pollution. The results indicate that for clean and chlorinated waters the fecal indicators were not efficient to predict *S. aureus* contamination. The confirmed presumptive count of *S. aureus* was a good method to complement counts of fecal indicators.

82. Krstulovic, N. and M. Solic. 1994. Presence and survival of *Staphylococcus aureus* in the coastal area of Split (Adriatic Sea). Mar. Pollut. Bull. **28**:696-700.

Survival of *S. aureus* was statistically significant longer than that of fecal pollution indicators under both light and dark conditions. Strong correlation between *S. aureus* and indicators was established in polluted areas.

83. Seyfried, P., R. Tobin, N. Brown and P. Ness. 1985. A prospective study of swimming related illness. II. Morbidity and the microbiological quality of water. Am. J. Publ. Health **75**:1071-1075.

In this study morbidity among swimmers was related to staphylococcal counts. Total staphylococci appeared to be consistent indicators for predicting total morbidity rates among swimmers.

Summary - Other Bacteria

There has been much work to determine other bacterial indicators that would be more specific than *E. coli*. *Aeromonas sp.* showed a positive correlation with *E. coli* in polluted waters (58, 61) but, proved to be more susceptible to inactivation in saline waters (59, 60, 61).

Bacteroides are normal inhabitants of the mouth, intestinal tract, and genital organs of humans. *Bacteroides vulgatus* outnumbers *E. coli* in human feces (62). Isolation techniques and differentiation media must be developed to examine environmental samples as opposed to clinical. The use of PCR technology may be a productive area

of research to monitor *Bacteroides sp.* (63). Bacteriophage of *Bacteroides*, although found in lower numbers than coliphage (65), should be pursued as a useful indicator (64).

Organisms of the genus *Bifidobacterium* are nonpathogenic inhabitants of human and animal intestinal tracts and survive similarly to *E. coli* in tropical waters (67). They appear to have no advantage over *E. coli* as a pollution indicator (66).

Campylobacteria inhabit the intestinal tracts of humans and animals and have been found in polluted waters (68, 69). Their survival rates and specificity to humans are no better than *E. coli* in water samples.

Pseudomonas bacteria are found in polluted waters along with fecal coliforms, but their numbers were considerable lower than other traditional indicators (70, 71).

Salmonella was also isolated from polluted waters (73, 75, 76, 77, 78), however did not show significant levels or survival rates as compared with traditional indicators.

Staphylococci bacteria show a high correlation with health related risks in bathing waters (80, 81, 83). This may be because Staphylococci have a high survival rate in saline waters (82). In natural waters the levels of Staphylococci vary considerably and their relation to fecal contamination is unclear (79).

IV. BACTERIOPHAGES/COLIPHAGES AND VIRUS

Coliphage are bacteriophages (virus that infects bacteria) that infect and replicate in *Escherichia coli* bacteria. They are present wherever coliform bacteria are present. Correlations between coliphage and coliform bacteria in fresh water generally may be used to indicate the sanitary quality of water (Borrego et al. 1987). Coliphage are more resistant to inactivation by physico-chemical factors than coliform bacteria in marine waters. Borrego et al. (1987) concluded that coliphage appeared to be an attractive indicator of marine pollution.

The most common assay method as described in Standard Methods (APHA 1992):

Add one milliliter of host *E. coli* culture and five milliliters of water sample to five milliliters of melted (45 C) tryptic soy agar. Mix thoroughly and pour into a 100 x 15 mm petri dish and incubate at 35 C. Count plaques at 4 to 6 hours and after 24 hours. Report the number of plaques per 100 milliliters.

84. Armon, R. and Y. Kott. 1995. Distribution comparison between coliphages and phages of anaerobic bacteria (*Bacteroides fragilis*) in water sources, and their reliability as fecal pollution indicators in drinking water. Water Sci. Technol. **31**:215-222.

Supply sources showed increased bacteriophage presence as follows: well > lake > spring. Mixture of the three water supplies revealed different contamination frequency. Bacteriophages' presence in drinking water presumably points to contamination of these sources. Results of a different study on groundwater aquifer recharge with reused water, and retained for more than 20 months before use showed a decline in the presence of the phage. Following additional studies, use of suggested *E. coli* bacteriophages and *B. fragilis* bacteriophages as indicators, in addition to traditional indicators is suggested.

85. Balebona, M.C., D. Castro, R. Cornax and M. A. Morinigo. 1991. Significance of several bacteriophage groups as indicators of sewage pollution in marine waters. Water Res. **25**:673-678.

Seawater samples were collected from two beaches with different levels of pollution and analyzed for classically and newly proposed fecal indicators. Total and fecal coliform showed lower survival rates in seawater than fecal streptococci, F-specific bacteriophages and coliphages. The low concentrations in which F-specific and *B. fragilis* bacteriophages were detected in marine waters compared to the *E. coli* bacteriophage levels, is an important shortcoming for the general use of the former as universal indicators of fecal pollution. The study concluded that fecal streptococci and coliphage are the most appropriate indicators of the remote pollution in marine waters.

86. Borrego, J., R. Cornax, A. de Vincente, M. Morinigo and P. Romero. 1987. Coliphages as an indicator of fecal pollution in water. Its relationship with indicator and pathogenic microorganisms. Water Res. **21**:1473-1480.

87. Borrego, J., R. Cornax, E. Martinez-Manzanares, M. Morinigo and P. Romero. 1990. Coliphages as an indicator of fecal pollution in water. Their survival and productive infectivity in natural aquatic environments. Water Res. **24**:111-117.

Coliphage show similar inactivation rates as salmonella. Their capability to infect *E. coli* depends on the physiological characteristics of the host bacteria, which generally cannot grow optimally in these conditions. Therefore, coliphage may be considered good indicators of fecal pollution in natural waters.

88. Bosch, A., F. Lucena, R. Girones and J. Jofre. 1988. Occurrence of enteroviruses in marine sediment along the coast of Barcelona Spain. Can. J. Microbiol. **34**:921-924.

In samples from recent deposition of fecal contamination correlation between enteroviruses and bacteria was demonstrated. Samples of older depositions and at greater distances did show correlation. This was due to the different decay rates between viruses and bacteria.

89. Chung, H. 1993. F-specific coliphages and their serogroups, and *Bacteroides fragilis* phages as indicators of estuarine water and shellfish quality (fecal contamination). Dissertation. University of North Carolina at Chapel Hill.

90. Cliver, D. O. and J. Snowdonl. 1989. Coliphages as indicators of human enteric viruses in groundwater. Crit. Rev. Environ. Control **19**:231-249.

91. Cornax, R., M. Morinigo, I. Paez, M. Munoz and J. Borrego. 1990. Application of direct plaque assay for detection and enumeration of bacteriophages of *Bacteroides fragilis* from contaminated water samples. Appl. Environ. Microbiol. **56**:3170-3173.

The direct double agar layer plaque assay for the detection and enumeration of specific bacteriophages of *Bacteroides fragilis* from contaminated water samples was performed. Several factors that effect the methods, such as conditions of the bacterial culture, composition of the assay medium, addition of divalent cations, and decontamination technique applied to sample, were evaluated. The results show that the direct assay technique proved to be more efficient than the most probable number technique. A higher recovery of bacteriophages was obtained from 17 of 24 samples with the direct assay. The two methods only showed similar results from samples with a low degree of pollution.

92. Dutka, B.J., E. M. Janzen and G. A. Palmateer. 1991. Coliphage and bacteriophage as indicators of recreational water quality. Water Res. **25**:355-357.

Five beaches in Canada report the association of coliphage and bacteriophage with enterviruses in surface waters, and the consistent occurrence of both coliphage and bacteriophage. The health risk related to bathers should involve virological analyses including the enumeration of coliphage and bacteriophage.

93. Grabow, W., T. Neubrech, C. Holtzman and J. Jofre. 1994. *Bacteroides fragilis* and *E. coli* bacteriophages: excretion by humans and animals. The 17th Biennial Conference of the International Association on Water Quality. Budapest. 24-30 July.

The qualitative presence of phages was determined by an enrichment procedure followed by a plaque spot test. *Bacteroides fragilis* phages were detected in 13% of human stool samples, but not in any animal feces. The results confirm earlier observations that *B. fragilis* phages can be used to distinguish between fecal pollution of human and animal origin.

94. Green, J. and B. O'Keefe. 1989. Coliphages as indicators of fecal pollution at three recreational beaches on the Firth of Forth. Water Res. **23:**1027-1030.

95. Havelaar, A., M. Van Olphen and Y. Drost. 1993. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. Appl. Environ. Microbiol. **59**:2956-2962.

The concentrations of thermotolerant coliforms and fecal streptococci were significantly correlated with virus concentration in river water and coagulated secondary effluent, but were low in disinfected effluents and high in surface water open to nonhuman fecal pollution. Data supports the possibility that enteric virus concentration can be predicted from F-RNA phage data.

96. Ijzerman, M. M. 1993. Development and Evaluation of a colorimetric coliphage assay detection system (coliphage). Dissertation. Virginia Polytechnic Institute and State University.

An improved method for coliphage detection based on the induction of beta-galactosidase in *E. coli* is described. Upon infection by coliphages, the cells are lysed and a stable indolyl product that is dark blue becomes visible within each plaque.

97. Kone, K. 1994. Detection and enumeration of bacteriophages of *Bacteroides fragilis* HSP40 in food and sewage samples by novel procedures. Dissertation. Kansas State University.

98. Krikelis, V. 1991. Research on enteric viruses in aquatic environments. Development and testing of sampling and analytical techniques for monitoring of marine pollutants (Activity A). Final reports on selected microbiological projects., Map. Tech. Rep. Ser. **54:**23-29.

The degree of viral pollution in seawater depends on the viral loads received and the distance from the polluting source. A large number of different serotypes are found in domestic sewage, and these can be detected in seawater as well. Non-sewage receiving coastal waters were practically free of viruses.

99. Lewis, G.D. 1995. F-specific bacteriophage as an indicator of human viruses in natural waters and sewage effluents in northern New Zealand. Water Science and Technology: J. Internat. Assoc. Water Poll. Res. **31:**5-6.

To assess the F-specific bacteriophage as an indicator of pathogenic viruses, a comparative study was made of the occurrence of F-phage and human enteroviruses in sewage and the marine environment. Although F-phage seemed in several respects to match pathogen behavior, its low abundance in bathing beach water, uncertainty as to its source and other detection irregularities make its use as an indicator problematical.

100. Loh, C.L., W. J. Robertson, S. A. Sattar and V. S. Springthorpe. 1993. In situ survival of indicator bacteria, MS-2 phage and human pathogenic viruses in

river water. Health-Related Water Microbiology, Water Sci. Technol. **27**:413-420.

Survival patterns of *E. coli* in nutrient rich river water were highly variable. A ten fold or greater increase in numbers of *E. coli* was sometimes observed in both laboratory and field tests. No regrowth was ever observed with *E. durans* and its titer declined faster than that of *E. coli*. Over the first 24 hours, the phage survival was similar to that of the human pathogenic viruses. The observation regarding the variable survival of *E. coli* and its potential for regrowth raise questions about its suitability as an indicator.

101. Loh, C., H. Zain, Y. Ngeow, C. Wang and Y. Ho. 1990. Assessment of standard methods for the detection of faecal contamination in the monitoring of streams in an equatorial rain forest. Malayan Nat. J. **44**:77-83.

Fecal coliform, fecal streptococci, hydrogen sulfide producing bacteria and coliphage were detected in the waters of the rivers and springs tested. With the exception of the coliphage test, the other tests did not appear to be suitable for the detection of human contamination of pristine waters.

102. McCorquodale, D. S. 1987. Coliphage as an indicator of fecal pollution in marine waters: assay, validation, and application. Dissertation. Nova Southeastern University.

Field validation studies in fresh and brackish water (<10 ppt) in Biscayne Bay compared coliphage with total and fecal coliforms ($n = 53$) and gave correlation coefficients of 0.98 and 0.91 respectively. The combined total/coliphage relationship at 68 saltwater (>10 ppt) stations yielded a correlation coefficient of 0.45. Coliphage are a logical choice for a fecal indicator in marine waters since their titers are closely related to total and fecal coliform in freshwater, survive much better than coliforms in seawater, and they can be enumerated by a simple method which is not subject to salinity artifacts. Monitoring of sanitary water quality in Bell Channel Bay, Bahamas, during repair of a sewer plant showed that following chlorination and diversion of the effluent to a deep well, total coliform declined rapidly below detection limits. Coliphage remained easily detectable ten days later.

Two canals and two marinas on Biscayne Bay were assayed for coliphage to compare sanitary water quality related to point and non-point source pollution. The Biscayne Canal was impacted by periodic upstream sewage spills, while the Little River displayed chronic contamination along its length by liveaboard boats or sewer leaks. Coliphage persisted six days longer than coliform after a sewage spill was tracked down the canal. The liveaboard Dinner Key marina displayed low-level, spotty contamination with no seasonal pattern. Kings Bay marina was free of detectable fecal contamination during the study.

103. McCorquodale, D. and C. Burney. 1993. Biscayne Bay Sewage Pollution Indicators, final report - South Florida Water Management District. #C-3242.

It is clear that total and fecal coliforms are poor indicators of sewage pollution in marine waters. Coliphage appear more appropriate because of their improved survival and inability to reproduce outside their host. The Miami River plume study indicates that there is no detectable die off or reproduction of coliphage when the river water mixes with seawater. The coliphage counts simply decline with increasing salinity due to dilution. Saltwater resistant pathogens would be expected to behave in the same way. Likewise, the results of the Virginia Key sewage outfall drogue study lead to the same conclusion. As the drogue passed through the discharge plume eddies, higher coliphage counts were detected from samples from within the plume (lower salinity) than the higher salinity samples immediately outside. Although coliforms were undetectable in the plume, the increased resistance of phage to chlorination allowed its detection. Coliphage meet the general requirements of an improved indicator of fecal pollution in marine waters. The interpretation of coliphage titers relative to current coliform based regulations is still uncertain. McCorquodale (1987) suggested that the relatively constant mathematical relationship of fecal and total coliforms to coliphage found in fresh waters might be used to estimate the coliform counts for marine waters which would be present if there was no die off. It is clear that more field data is necessary to confirm the validity of this interpretation. Even if the variability in coliform to coliphage ratios prove to be unacceptable, sufficient field data from polluted and unpolluted areas could still be used to establish coliphage based regulatory criteria.

104. Morinigo, M., D. Wheeler, C. Berry, C. Jones, M. Munoz, R. Corax and J. Borrego. 1992. Evaluation of different bacteriophage groups as faecal indicators in contaminated natural waters in southern England. Water Res. **26**:267-271.

F specific RNA bacteriophages showed no direct relationship with the levels of fecal pollution. This group was never detected in samples with a low level of enteroviruses. In contrast, coliphages were constantly detected in the same samples. The concentrations of coliphages detected in the samples correlated with concentrations of enteroviruses at both high and low concentrations. Coliphage would be considered as an optimal indicator of the microbiological quality of the natural waters.

105. Paul, J., J. Rose, S. Jiang, C. Kellogg and L. Dickson. 1993. Distribution of viral abundance in the reef environment of Key Largo Florida. Appl. Environ. Microbiol. **59**:718-724.

Study indicates that viruses are abundant in the Key Largo area, particularly in Florida Bay, and that processes governing their distribution in the water column (i.e, salinity and freshwater input) are independent of those governing their distribution in sediments. Water column viral abundance did not correlate with bacterial direct counts.

106. Paul, J., J. Rose, J. Brown, E. Shinn, S. Miller and S. Farrah. 1995. Viral tracer studies indicate contamination of marine waters by sewage disposal practices in Key Largo, Florida. Appl. Environ. Microbiol. **61**:2230-2234.

The fate and transport of sewage in the subsurface environment and the potential contamination of marine surface waters was investigated by employing coliphage as tracers in a domestic septic system and wells. Estimated rates of migration of viral tracers ranged from 0.57 to 24.2 m/h, over 500 fold greater than flow rates measured previously by subsurface flow meters.

107. Puente, I. 1991. Sources of coliphage to the marine environment. Thesis. Nova Southeastern University.

Coliphage were not detected in dolphin or sea lion feces, but were detected in seagull and pelican. They were present in only 3 of 7 human stool samples initially, however were present after several days. It was thought that lysogenic bacteria in human feces release coliphage through spontaneous induction outside the human intestine.

108. Torroella, J.J. 1991. Bacteriophages of *Bacteroides* as indicators of pathogenic human viruses in coastal seawaters. Development and testing of sampling and analytical techniques for monitoring of marine pollutants (Activity A). Final reports on selected microbiological projects., Map. Tech. Rep. Ser. **54**:1-10.

Bacteriophages of *Bacteroides* outnumbered human viruses, such as enterovirus and rotaviruses in coastal waters. They were also recovered in samples of polluted environments in which human viruses were not. In sediments when the same methods of recovery was used for phages and animal viruses, counts of *B. fragilis* phages correlated with numbers of enteroviruses and rotaviruses.

Summary - Bacteriophage/Coliphage and Virus

Many authors have recommended coliphage as superior indicators of fecal pollution in marine and fresh waters (86, 90, 92, 101, 105). Coliphage survival characteristics and inability to reproduce outside their host make them good fecal indicators (87, 103). The relatively constant mathematical relationship of fecal and total coliform to coliphage in fresh waters might be used to estimate the coliform counts in marine waters (103). Ratios of human enteric viruses and coliforms are subject to wastewater treatment and nonhuman fecal pollution (95). No coliphage were detected in feces from dolphin or sea lion, but were isolated from seagull and pelican (107). Coliphage in human feces often is not detectable until after several days and may be used to indicate age of the fecal contamination (107).

Bacteriophage from *Bacteroides sp.* have been used to evaluate fecal pollution (84, 89, 91, 97). Lower concentrations of *Bacteroides sp.* phage than coliphage were detected

in marine waters which is an important shortcoming as an indicator (85). *Bacteroides fragilis* phage were detected in 13% of human stool samples, but not in any animal feces. This may indicate that they may be used to distinguish human from nonhuman fecal pollution (93). *Bacteroides* phage correlate well with human viruses in sediments of coastal waters (108).

V. PILI PROTEINS

109. Ellender, R., F. Howell and C. Shows. 1984. Characterization of fecal coliform isolates by electrophoretic analysis of pili. Mississippi-Alabama Sea Grant Consortium: Ocean Springs, Mississippi.

Another attempt to distinguish human fecal pollution from other sources was examined. Pili (microscopic hairlike surface structures) proteins of *E. coli* isolated from different species of animals were characterized according to a hemagglutination (HA) sequence and by electrophoresis. A total of 1280 isolates were analyzed by HA. *E. coli* strains exhibited a finite number of HA combinations, but isolates from different animals and environmental samples commonly produced the same HA pattern. Techniques for isolation and purification of pili were conducted on 112 strains. The time required for bacterial growth and pili extraction were lengthy: only four isolates could be examined per week. Data indicated that isolates differed according to the number and the molecular weight of gel bands. Although differences were observed, the system was not sensitive enough to determine the source of the bacterium.

VI. PROTOZOA

110. Chauret, C., N. Armstrong, J. Fisher, R. Sharma, S. Springthorpe and S. Satter. 1995. Correlating *Cryptosporidium* and *Giardia* with microbial indicators. J. AWWA **87**:76-84.

None of the microorganisms surveyed (total and fecal coliforms, fecal streptococci, *Aeromonas sp.*, *Pseudomonas aeruginosa*, *Clostridium perfringens* and coliphage) would be a reliable indicator of the presence of the protozoan.

VII. CHEMICAL INDICATORS

A. DETERGENTS/OPTICAL BRIGHTNERS

111. Albaiges, J., J. M. Bayona and M. Valls. 1989. Use of trialkylamines as an indicator of urban sewage in sludges, coastal waters, and sediments. Nature **337**:722-724.

Coastal areas receive a variety of organic inputs from land both natural and synthetic. The identification of specific organic markers in different marine areas can trace the pathways of transport and regions of concentration. Trialkylamines (TAMs), originating from sewage discharges, were detected in coastal waters in the Mediterranean Sea and North Sea. The authors have identified TAMs as trace impurities in quaternary ammonium salts used as fabric softeners in household laundry detergents, for which reason they are found in urban sewage, primarily associated with the particulate phase. They correlate in the sedimentary record with other domestic surfactant markers, such as the long chain alkylbenzenes (LABs).

112. Alhajjar, B., G. Chesters and J. Harkin. 1990. Indicators of chemical pollution from septic systems. Ground Water. **28**:559-568.

Chloride was a conservative tracer and the most suitable indicator of contaminant plumes; electrical conductance was semiconservative; and fluorescence was unacceptable.

113. Fernandez, P., M. Valls, J. Bayona and J. Albaiges. 1991. Occurrence of cationic surfactants and related products in urban coastal environments. Environ. Sci. Technol. **25**:547-550.

Surfactants were detected in varying amounts in polluted coastal waters.

114. Poiger, T., J. Field, T. Field and W. Giger. 1993. Determination of detergent derived fluorescent whitening agents in sewage sludges by liquid chromatography. Anal. Methods Instrum. **1**:104-113.

Fluorescent whitening agents (aromatic sulfonates) were quantitatively determined in sewage sludges by extraction with methanol and followed by reverse phase HPLC. Total concentrations of detergent measured in sludges ranged from 85-170 mg/kg dry weight.

B. IMMUNOGLOBIN ALPHA (IgA)

115. Barrilleaux, A. M. 1992. A study of the feasibility of using secretory IgA as a species specific indicator of fecal pollution in environmental waters. Thesis. University of Southern Mississippi.

116. Griffis, J. N. 1992. An evaluation of human secretory immunoglobulin alpha as a specific indicator of human fecal pollution. Thesis. University of Southern Missippi.

Two studies were published as master's thesis that explored the use of human IgA which is specifically associated with enteric bacteria. IgA appears to be the major immumoglobin isotype found in the external secretions of humans. Secretory IgA is secreted into the gut and other mucosa as the primary specific immune defense against

infection of these surfaces. They are excreted in the feces and may serve as a human specific indicator of human pollution. These feasibility studies examined the degradation rate of human secretory immunoglobulin alpha (sIgA) and the effects of a number of physical, chemical and biological factors on those rates. Sensitive immunoassay methods capable of detecting and quantitating sIgA were developed.

The authors indicate that sIgA is not significantly effected by freezing, salinity, temperature or pH. Chlorine was the only parameter evaluated that resulted in the nondetection of the molecule. It was reported that sIgA persists in detectable levels in natural water for one to three days. A major advantage of IgA is its species specificity. A disadvantage of this system is the inactivation by chlorine which would make it questionable for treated wastes. Detection levels were not low without utilizing an amplified bioluminescent assay. The problems associated with stability of the components of the indicator system make this technique expensive and difficult to produce consistent results. As with other new techniques the only studies found are from a single university.

C. ENZYMES

117. Brill, J., G. W. Chang and R. Lum. 1989. Proportion of beta-D-glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.

The significant proportion of GUR negative *E. coli* in fecal samples from health subjects means that although GURU may be a convenient tool for detecting some *E. coli*, it cannot be used for taxonomic purposes.

118. Clark, D.L., B. B. Milner and M. H. Stewart. 1991. Comparative study of commercial 4-methylumbelliferyl-b-D-glucuronide preparations with the standard methods membrane filtration fecal coliform test for the detection of *Escherichia coli* in water samples. Appl. Environ. Microbiol. **57**:1528-1534.

Commercial test kits have shown a number of false positive results as compared to the MF procedure.

119. Fiksdal, L., M. Pommepuy, M. Caprais and I. Midttun. 1994. Monitoring of fecal pollution in coastal waters by use of rapid enzymatic techniques. Appl. Environ. Microbiol. **60**:1581-1584.

Enzyme assay for 4-methylumbelliferyl-beta-D-galactopyranosidase and 4-methylumebelliferyl-beta-D-glucuronidase activities were used for rapid detection of fecal pollution in water. Method was not very sensitive and could only estimate fecal coliform levels on the order of 100 to 1000 per 100 ml.

D. GENETIC FINGERPRINTING

To avoid the problem of nonspecific culturing of indicator organisms, genetically based procedures for detection of organisms by the recovery of DNA have been developed. Amplification of target nucleotide sequences specifically associated with indicator bacteria by using the polymerase chain reaction (PCR) and detection of the amplified DNA with gene probes have been studied.

120. Bej, A., R. Steffan, J. DiCesare, L. Haff and R. Atlas. 1990. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. **56**:307-314.

The use of PCR and gene probes permits both the specificity and sensitivity necessary for monitoring coliforms as indicators of human fecal contamination of waters. The method involves collection of cells, PCR amplification of regions of the lacZ and lamB genes and detection of the amplified target DNA by gene probes. Using lacZ as the target gene sensitivity of detection is 1-5 viable target microorganisms per ml sample and the test procedure takes a few hours. Results with PCR gene probe tests were found to be comparable to those of conventional plating procedures. If simplified the DNA extraction method and the development of an appropriate nonisotropic gene probe detection technique, PCR can permit a rapid and reliable means of assessing the bacteriological safety of waters.

121. Hernandez, J., J. Alonso, A. Fayos, I. Amoros and R. Owen. 1995. Development of a PCR assay combined with a short enrichment culture of detection of *Campylobacter jejuni* in estuarine surface waters. Fems. Microbiol. Lett. **127**:201-206.

It was found that DNA recovered from *Campylobacter jejuni* lysed by the CTAB method was more suitable for use as a PCR template than DNA released by the boiling method. The region targeted for PCR amplification was 1.73-kb portion of the flagellum A gene of *C. jejuni*. The detection limit was lower than 30 cells per 100 ml in artificially contaminated waters. PCR assay and conventional culturing method had the same sensitivity, but results of the PCT technique were available within 48 hours and so shortened the time necessary for detection by 48 hours.

122. Kreader, C. 1995. Design and evaluation of *Bacteroides* DNA probe for the specific detection of human fecal pollution. Appl. Environ. Microbiol. **61**:1171-1179.

PCR probes were designed for several bacteroides species thought to be abundant in humans, but not in nonhumans. The results indicate that these probes can distinguish human from nonhuman feces in many cases. Simple procedures must be developed to overcome interferences before PCR detection can be used to monitor fecal pollution.

Preliminary results indicate that *Bacteroides* DNA, detectable by PCR-hybridization assay, rapidly disappears after a day or two when whole human feces are dispersed into natural waters. Further study is needed.

123. Puig, M., J. Joffe, F. Lucena, A. Allard, G. Wadell and R. Girones. 1994. Appl. Environ. Micro. **60**:2963-2970.

A procedure was developed for the rapid detection of enteroviruses and adenoviruses in environmental samples. Twenty-five samples of sewage and polluted river water were analyzed and showed a much higher number of positive isolates by nested PCR than by tissue culture analysis. Further studies are needed to determine the extent and diversity of adenovirus contamination in sewage and polluted water over longer periods.

124. Roll, B. M. 1995. Development of polymerase chain reaction techniques for the detection of waterborne pathogens in environmental waters. Dissertation. University of Hawaii.

A set of PCR primers were developed to detect *Bacteroides spp.*, the most common inhabitant of the human intestinal track. These primers were specific for four *Bacteroides spp.* and did not cross react with 27 non bacteroides isolates. These primers were able to detect *Bacteroides spp.* in a variety of environmental waters. Survival experiments using *E. coli* and *Salmonella typhimurium* were conducted in ocean water and demonstrated a loss of *E. coli* and *S. typhimurium* gene sequence after exposure for 14 and 16 days, respectively. In summary this study supports the use of PCR as a water quality monitoring tool.

125. Samadpour, M. and N. Chechowtz. 1995. Little Soos Creek Microbial Source Tracking - A Survey. King County Dept. Public Works. Seattle, Washington.

Microbial Source Tracking (MST) was a survey conducted to determine the contribution to contamination of the Little Soos Creek by animal and human sources. Polluted water and fecal samples were collected and processed to isolate *E. coli* populations in each sample. Genetic fingerprinting (using ribosomal RNA typing) was performed on each *E. coli* isolate. The DNA types referred to as ribotypes were used to match specific strains of *E. coli* from a contaminated site to its source. A regional database for each site must be developed, as the database becomes more comprehensive and refined its effectiveness in characterizing the nature of the contamination at a site is improved.

E. coli were isolated from the samples by membrane filtration and positively identified by biochemical analysis. Bacterial cells were suspended in Tris-EDTA buffer and lysed with sodium dodecyl sulfate and proteinase K. This was followed by a phenol extraction to remove non-DNA cellular material. The DNA was further purified by chloroform extraction. DNA was precipitated and spooled onto a glass pipette and washed with absolute methanol and resuspended in distilled water. Restriction endonuclease digestions

of each DNA prep were done using the appropriate restriction enzymes. The fragments of DNA produced by the enzyme digestion were resolved by agarose gel electrophoresis and transferred by blotting onto a Nitran filter.

The blotted DNA was hydrolyzed with a radioactive RNA probe. The probe was labeled with dCTP. Hybridization of the probe to the blotted DNA was done under stringent conditions. After hybridization the blots were exposed to X-ray film. The X-ray image of the DNA banding produced is termed an audiogram and the pattern produced is a ribotype. In this study 33% of ribotypes obtained from *E. coli* isolated from water were matched to source types other than water and 71% of water isolates belonged to the strains represented by these match ribotypes. MST identified the sources of approximately three-fourths of the fecal contamination. In Little Soos Creek the primary sources of contamination were determined to be cows, dogs, and horses. Unmatched ribotypes may represent unsampled source types such as cats or wild animals.

Summary - Genetic Fingerprinting

The use of PCR and gene probes to identify and quanitate specific organisms have been reported for several organisms. Results of PCR gene probe tests were found to be comparable to those of conventional plating techniques (120). This method may prove to be a rapid and reliable means of determining sources of fecal pollution. PCR techniques identified the sources of approximately three-fourths of the fecal contamination in one study (125). PCR probes designed for *Bacteroides* can distinguish human from nonhuman feces in many cases (122). Techniques must be simplified to reduce interferences. A recent study was able to detect *Bacteroides sp.* in a variety of environmental samples (124). Enteroviruses were detected by PCR in much higher numbers than by tissue culture analysis (123).

E. TRACERS/DISCHARGE

126. Kloepper-Sams, P. J. and J. Owens. 1993. Environmental biomarkers as indicators of chemical exposure. J. Hazard. Mater. 35:283-294.

Biomarkers are anatomical, physiological and biochemical responses of an organism which indicates exposure to a stress. They can provide valuable insight into initial responses of an organism to exposure and may predict higher level affects. The biomarkers studied are not human specific and have limited use for human health considerations.

127. Owczarczyk, A, M. Strzelecki, S. Szpilowski and R.Wierzchnicki. 1994. Application of tracer method for investigation of dilution and decay of petrochemical effluent discharged into Big River. Water Air Soil Pollut. **78**:199-213.

Nine tracer experiments were performed using the isotope 82Br as a radioactive tracer. The 82Br was detected using the submersible scintillation probe NaI/Tl connected to radiometric set RZP-10. The tracer method appears to be convenient to investigate the effluent transport and dilution processes in natural water. This makes possible the evaluation of decomposition rates of certain pollutants. The data obtained constitute the basis of assessment of actual state of pollution and for prediction procedures of expected states and hazards.

F. UROBILIN

128. Miyabara, Y., N. Sugaya, J. Suzuki and S. Suzuki. 1994. Estimation of urobilin as a fecal pollution indicator in the aquatic environment. Bull. Environ. Contam. Toxicol. **53**:77-84.

129. Miyabara, Y., J. Suzuki and S. Suzuki. 1994. Classification of urban rivers on the basis of water pollution indicators in river sediment. Bull. Environ. Contam. Toxicol. **52**:1-8.

130. Miyabara, Y., Y. Sakata, J. Suzuki and S. Suzuki. 1994. Estimation of faecal pollution based on the amounts of urobilins in urban rivers. Environ. Pollut. **84**:117-122.

131. Miyabara, Y., K. Miyata, J. Suzuki and S. Suzuki. 1994 Evaluation of faecal pollution of river sediment by detection of urobilin. Environ. Pollut. **84**:111-115.

132. Miyabara, Y., K. Sakamoto, J. Suzuki and S. Suzuki. 1993. Evaluation of contribution of drainage from sewage treatment plants to water pollution based on the amounts of urobilin in rivers. Jap. J._Toxicol. Environ. Health **39**:401-408.

133. Miyabara, Y., J. Suzuki and S. Suzuki. 1994. Estimation of i-urobilin movement in an aquatic environment. Jap. J. Toxicol. Environ. Health **40**:13-19.

Urobilins are formed from conjugated bilirubin by hydrolysis and reduction by intestinal microflora and only originate from mammalian faeces and urine. Urobilin is produced in the spleen and secreted from the duodenum through the gall bladder. These urobilins have clinically been used to indicate hepatic function based on a quantified colorimetric method. The authors have developed a highly sensitive method to detect these compounds utilizing HPLC with fluorometry based on the Jaffe Schlesinger reaction. They have attempted to estimate human fecal pollution , based on the amount of urobilin present in surface water and sediment samples. Their studies indicate: 1) Urobilin degradation rates are similar to that of total and fecal coliform. 2) Urobilin appears more stable in sediments than indicator bacteria. 3) The methods for measuring urobilins are simple.

All published articles on urobilin as an indicator of fecal pollution located in this survey originate from the same university in Japan. Other authors may be investigating this area but have not published. It is also unclear how specific to human origin are these results. The methods described require expensive instrumentation and trained technicians. The pre-instrument extraction is a source of error and requires a high level of training to achieve low detection limits. The method is simple to a well equipped and trained chemist. Urobilins appear to be a potentially productive indicator, however, additional research by other investigators is needed.

G. LIPID BIOMARKERS/COPROSTANOL

Coprostanol, a fecal sterol present in the feces of humans and other animals, has been investigated as a fecal indicator. It is found in relatively large amounts in human feces and its degradation rate is slow enough to rate as a possible indicator. The feces of humans and higher animals appear to be the only source of this sterol.

Bile acids are a class of sterols that are necessary in the digestive processes of vertebrates. They are produced in the liver and function as emulsifiers that solublize lipids. A small amount is excreted in the feces and have been examined as a fecal indicator.

134. Aminot, A., P. LeCorre, Y. Marty and G. Thoumelin. 1990. Laboratory investigation of the degradation of organic matter in estuarine and coastal waters: sterols variations. Oceanol. Acta. **13**:53-60.

Sterols formed during the first day of incubation are subsequently degraded, decomposition occurring at a relatively rapid pace and being complete after twenty to twenty-five days. Several processes control the degradation of the organic matter including the degradation by microbial exoenzymes of complex organic compounds. These may originate from higher plants or planktonic organisms or may be of anthropogenic origin.

135. Bautista, J.M., J. C. Del Rio, F. J. Gonzalez-Vila and F. Martin. 1994. Identification of anthropogenic compounds in peat filters used for waste water depuration. Fresenius Environ. Bull. **3**:540-545.

The lipid fraction extracted from samples of peat filters used for depuration of municipal wastewater has been analyzed by GC and GC/MS. The main compounds identified were the trialkylamines (TAMs) and long chain alkylnitriles (LANs), that are markers of the cationic surfactants. Coprostanol, a marker for the presence of fecal matter, was also a distinctive component detected.

136. Bayona, J., M. P. Fernandez and J.O. Grimalt. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environ. Sci. Technol. **24**:357-363.

137. **Bolognani, F., P. Brigidi, C. Cerre, D. Matteuzzi and M. Rossi.** 1993. Cloning of the gene for cholesterol oxidase in *Bacillus spp.*, *Lactobacillus reuteri* and its expression in *Escherichia coli*. Lett. Appl. Microbiol. **17**:61-64.

In the intestinal tract of humans, cholesterol is metabolized by bacterial microflora to coprostanol (65%) and coprostanone (10%) and to a lesser extent to cholestanone and epicoprostanol.

138. **Brown, D. W., D. G. Burrows, S. L. Chan, M. M. Krahn, W. D. MacLeod and C. A. Wigren.** 1989. Rapid, automated methods to analyze for organic contaminants in environmental samples. Oceans '89: The Global Ocean. Volume 2: Ocean Pollution 397-401.

Coprostanol was isolated by chromatographic cleanup on normal phase HPLC columns and was quantified by GC with flame ionization detection (FID).

139. **Crewe, N.F., J. D. Leonard and R. Pocklington**. 1987. Coprostanol as an indicator of fecal contamination in seawater and marine sediment. Oceanol Acta **10**:83-90.

140. **Dolci, F., V. U. Fossato, M. R. Sherwin and E. S. Vanvleet.** 1993. Coprostanol in lagoonal sediments and mussels of Venice, Italy. Mar. Pollut. Bull. **26**:501-507.

Coprostanol concentrations found in mussels were lower and less variable than the sediments, indicating that sedimentary coprostanol concentrations appear to be a useful indicator of potential ecological problems in the lagoon and canals of Venice due to the direct input of untreated waste.

141. **Eganhouse, R.P., B. R. Gould, D. P. Olaguer and C. S. Phinney.** 1988. Use of molecular markers for the detection of municipal sewage sludge at sea. Mar. Environ. Res. **25**:1-22.

The concentration of two classes of waste specific molecular markers (the linear alkylbenzenes and the fecal sterols, coprostanol and epicoprostanol) were determined in six municipal sewage sludges. Experiments were performed to establish the limits of detection and quantification of these compounds in small volume bulk water samples using high resolution gas chromatography and gas chromatography/mass spectrometry. The results indicate that molecular markers can be used to monitor the short term fate of sewage sludge in the ocean.

142. **Ellis, J.T., J. S. Latimer, L. A. LeBlanc and J. G. Quinn.** 1992. The geochemistry of coprostanol in waters and surface sediments from Narragansett Bay. Estuar. Coast Shelf Sci. **34**:439-458.

Coprostanol concentrations in the waters (0.02-0.22 mu g/l) and surface sediments (0.22-33.0 mu u/l) from the bay were as high or higher than any values reported in the literature, indicating that the bay is severely impacted by sewage. Coprostanol was a good indicator of sewage treatment plant efficiency.

143. Green, G., R. Leeming, P. D. Nichols and J. H. Skerratt. 1992. Hydrocarbon and coprostanol levels in seawater, sea-ice, algae and sediments near Davis Station in eastern Antarctica: A regional survey and preliminary results for a field fuel spill experiment. Mar. Pollut. Bull. **25**:293-302.

Coprostanol has been detected in known polluted waters and not in unpolluted waters. A survey of hydrocarbons and coprostanol in a coastal marine environment in Antarctica showed a wide distribution predominantly of marine origin.

144. Han, B.C. and W. L. Jeng. 1994. Sedimentary coprostanol in Kaohsiung Harbour and the Tan-Shui Estuary, Taiwan. Mar. Pollut. Bull. **28**:494-499.

A significant log-log correlation is found between total coprostanol concentration and oil hydrocarbon concentration ($r=0.803$). Sediment coprostanol ranges in concentration from 1.00 to 230 mu g/g with an average of 63.5 mu g/g. Significant correlations have been found for coprostanol and cholesterol; and for cholestanol and cholesterol; these relationships mediated predominantly by sediment microorganisms in reducing environment.

145. Joo, Y.H., K.Y. Jung, M. Kawano, and R. Tatsukawa. 1994. Determination of fecal pollution for well and surface waters from livestock raising areas in Korea using coprostanol as an indicator. Toxicol. Environ. Chem. **45**:57-67.

Coprostanol content in well water samples ranged from less than one to 20 ng/l. The results indicate the level of coprostanol in well water depends on the number and species of livestock, soil texture and well depth. Coprostanol found in surface water was higher compared with recreational water level (<500 ng/l).

146. Kaplan, I.R., E. Ruth and M. Venkatesan. 1986. Coprostanols in Antarctic marine sediments: A biomarker for marine mammals and not human pollution. Mar. Pollut. Bull. **17**:554-557.

147. Venkatesan, M. and I. Kaplan. 1990. Sedimentary coprostanol as an index of sewage addition in Santa Monica basin southern California USA. Environ. Sci. Technol. **24**:208-214.

The flux and composition of solvent extractable lipid fractions were measured in particulate matter. The progressive seaward decline of coprostanol relative to total sterols from the outfalls represents dilution of sewage by biogenic sterols. The ratio of

coprostanol to dinosterols appears to be a better indicator of the degree of sewage addition.

148. Kirchmer, C. J. 1989. 5 beta-cholestan-3 beta-ol an indicator of fecal pollution. Dissertation. University of Florida.

149. Kuebler, B., A. Pittet and R. Stettler. 1990. Use of coprostanol as a specific allochthonous fecal indicator in surface sediment of the Lake of Neuchatel. Aquat. Sci. **52**:130-143.

Coprostanol has proven to be a good specific allochthonous fecal indicator in sedimentary surface samples of a lake. Its concentration is slightly affected in surface sediment by the microbial reduction of cholesterol to cholestanol and coprostanol.

150. Marty, Y. and M. Quemeneur. 1994. Fatty acids and sterols in domestic wastewaters. Water Res. **28**:1217-1226.

Three 5 beta-stanols: coprostanol, 24-methylcoprostanol and 24-ethylcoprostanol, accounted for 50-60% of the total sterols, the coprostanol/cholesterol and the 24-ethylcoprostanol/beta-sitosterol ratios were greater than 1. These characteristics were still recognizable in treated sewage.

151. Mirsadeghi, H. and M. I. Venkatesan. 1992. Coprostanol as sewage tracer in McMurdo Sound, Antarctica. Mar. Pollut. Bull. **25**:328-333.

Sediment samples close to the point source contain as much as 3 mg/g of coprostanol, whereas samples farther from the source contain only trace levels to 40 ng/g. It appears that sewage particles are very quickly incorporated into the sediment layers close to discharge point.

152. Murphy, T. 1992. Feasibility of using chemicals as indicators of fecal pollution: bile acids. Thesis. Univ. of Cincinnati.

Conclusions included:
1. Bile acids were identified in primary effluents. The enormous volumes of water that must be extracted hamper the practicality of this method.
2. Bile acids were not found in secondary effluent, river water or tapwater controls. The bile acids may be absent or below detection limits.
3. The extraction method reported is qualitative for environmental samples.
4. Continued research is needed to improve the extraction technique for environmental samples.

153. Nichols, P., R. Leeming, M. Rayner and V. Latham. 1995. CSIRO Oceanography, GPO Box 1538, Hobart, Tasmania 7001 (Australia). unpublished manuscript.

Abstract:
The sterol composition, including the fecal biomarker coprostanol, from a variety of sample types was determined by capillary GC and GC-MS. The coprostanol concentration in field samples readily provided an estimate of human fecal pollution. The technique was successfully used for stormwater, the sea-surface microlayer, beach sands and grease, and in regional studies of coastal sediments. Sterol profiles can be used to distinguish human and non-human sources of sewage pollution and algal blooms. Development of appropriate component ratios, both, within the sterol fraction and between compound classes, may provide a useful mechanism to further exploit sterol data. For sewage-containing samples, it may be possible to extend the data comparison and calculation of key ratios to include bacteriological parameters. Collectively, the use of sterol compositional data complement other physical, chemical and biological measurements obtained in environmental studies.

154. Pierce, R. H. 1987. A Survey of Coprostanol Concentrations in Biscayne Bay Sediments, prepared for Dade County Department of Environmental Resource Management. Mote Marine Laboratory, Sarasota, Florida, May 26.

This study was designed to assess the sanitary conditions of Biscayne Bay, especially concerning sewage input from liveaboard boats. The fecal sterol coprostanol, 5B-cholestan-3B-ol, was extracted with cyclohexane using the reflux-moisture trap procedure to remove moisture, followed by complete reflux extraction with dichloromethane. Extracts were combined and evaporated to lust dry on a rotary-evaporator, and the residue dissolved in cyclohexane for column chromatographic clean up, followed by gas chromatographic analysis.

A Varian Model 6000 GC with a flame ionization detector coupled with a Varian CDS-401 data system was used. They used a 30m x 0.32 capillary fused silica J&W, DB-5 column. The temperature program was from 180 C to 280 C at 5/min with a 20 minute hold at 280C; He carrier gas with N2 make-up at the detector.

Samples were taken at four sites, two tributaries (Biscayne Canal - septic tanks and Little River - sewer) and two marinas (King's Bay - no liveaboards and Dinner Key - liveaboards). Both tributaries exhibited the presence of the sterol with the greatest concentrations in the upper reaches declining when entering Biscayne Bay. The marinas had detectable levels with a greater concentration at Dinner Key. Levels decreased rapidly with distance from the source. A control station in the bay had no detectable sterol (< 10 ppb).
The conclusions from this study were:
1. All sample areas, except control, exhibited varying degrees of impact by sewage derived material.
2. Areas known to harbor liveaboards exhibited a higher degree of contamination than areas that do not have liveaboards.
3. Additional information useful for interpretation of results are:
    a. tidal and current data
    b. land and water use activities

c. other sources of sewage input

A parallel study was conducted by Donald S. McCorquodale, Jr. (Spectrum Laboratories, Inc.) for DERM that measured total and fecal coliform, fecal streptococci and coliphage at the sampling events. Bacterial and viral indicators were detectable long after the coprostanol was undetectable. The coliphage was the most persistent indicator.

155. Venkatesan, M. and I. Kaplan. 1990. Sedimentary coprostanol as an index of sewage addition in Santa Monica basin southern California USA. Environ. Sci. Technol. **24**:208-214.

Sewage derived coprostanol is trapped in sediments in polluted areas. Ventkatesan and Santiago in 1989 reported that coprostanol from the feces of sea mammals was detected in Antarctic sediment cores estimated to have been 3600 years old. These findings also indicate a persistence of coprostanol in the environment, which might make the fecal sterol useful for tracing historical occurrences of sewage pollution but useless for assessing pathogenic contamination.

Summary - Lipid Biomarkers/Coprostanol

Fecal sterols have been studied as indicators of fecal pollution by many investigators (136, 139, 143, 144, 153). Coprostanol has been detected in wastewater filters (135), sewage (142), livestock waste (145), marine mammal excrement (146) and residue from extinct sea mammals (155). In humans coprostanol is produced by bacteria metabolizing cholesterol in the intestinal tract (137) and degraded by several processes once excreted (134). Coprostanols in sediments has been reported to be a useful indicator of fecal pollution (140), but are affected by the microbial reduction (149). Concentrations near a point source are considerably higher than those farther from the source (151). Coprostanol can be used to monitor the short term fate of sewage sludge in the ocean (141). Marinas with live-aboard boats had detectable levels at higher concentrations than marinas with no live-aboards (154). Under some specific conditions coprostanol may persist for long periods (155).

H. CAFFEINE

USGS scientists have reported that caffeine was detected throughout the 1900 mile length of the Mississippi River, with the highest concentrations near metropolitan areas. Elevated concentrations were found in the Illinois River probably from the large population of Chicago. The results are from three surveys that collected about 450 samples of the Mississippi River in 1991 and 1992. No published papers were found in this search that document these studies.

VIII. LITERATURE CITED

Abdel, G.A., L. El-Attar, F. El-Sharkawi and S. Molazem. 1989. Some environmental factors affecting survival of fecal pathogens and indicator organisms in seawater. Water Sci. Technol. **21**:115-120.

Ahtiainen, J., M. Niemi and H. Jousimies-Somer. 1991. Staphylococci in polluted waters and in waters of uninhabited areas. Water Sci. Technol. **24**:103-108.

Albaiges, J., J. M. Bayona and M. Valls. 1989. Use of trialkylamines as an indicator of urban sewage in sludges, coastal waters, and sediments. Nature **337**:722-724.

Alhajjar, B., G. Chesters and J. Harkin. 1990. Indicators of chemical pollution from septic systems. Ground Water. **28**:559-568.

Alhajjar, B., S. Stramer, D. Cliver and J. Harkin. 1988. Transport modeling of biological tracers from septic systems. Water Res. **22**:907-915.

Alonso, J.L., L. Amoros, M. Botella and A. Rambach. 1992. *Salmonella* detection in marine waters using a short standard method. Water Res. **26**:973-978.

American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed., Washington D.C.

Aminot, A., P. LeCorre, Y. Marty and G. Thoumelin. 1990. Laboratory investigation of the degradation of organic matter in estuarine and coastal waters: sterols variations. Oceanol. Acta **13**:53-60.

Araujo, R.M., F. Lucena and R. Pares. 1990. The effect of terrestrial effluents on the incidence of *Aeromonas spp*. in coastal waters. J. Appl. Bacteriol. **69**:439-444.

Araujo, R.M., R. M. Arribas, F. Lucena and R. Pares. 1989. Relation between *Aeromonas* and fecal coliform in fresh waters. J. Appl. Bacteriol. **67**:213-217.

Armon, R. and Y. Kott. 1995. Distribution comparison between coliphages and phages of anaerobic bacteria (*Bacteroides fragilis*) in water sources, and their reliability as fecal pollution indicators in drinking water. Water Sci. Technol. **31**:215-222.

Avila, M.J., M. A. Morinigo, R. Cornax, P. Romero and J. Borrego. 1989. Comparative study of coliform-enumeration media from seawater samples. J. Microbiol. Methods. **9**:175-193.

Balebona, M.C., D. Castro, R. Cornax and M. A. Morinigo. 1991. Significance of several bacteriophage groups as indicators of sewage pollution in marine waters. Water Res. **25**:673-678.

Barbe, D.E. and J. Francis. 1995. An analysis of seasonal fecal coliform levels in the Tchefuncte River. Water Res. Bull. **31**:141-146.

Barnhart, H. M. and O. C. Pancorbo. 1992. Microbial pathogens and indicators in estuarine environments and shellfish: critical need for better indicators of human specific fecal pollution. J. Environ. Health **54**:57-63.

Barrilleaux, A. M. 1992. A study of the feasibility of using secretory IgA as a species specific indicator of fecal pollution in environmental waters. Thesis. University of Southern Mississippi.

Bartlett, M.S., J. H. Dorsey and J. A. Lemay. 1989. Microbiological monitoring of recreational waters in Santa Monica Bay, California, and the effects of storm drain effluents on three bacterial indicators. Oceans '89: The Global Ocean **2**:684-689.

Barzily, A. and Y. Kott. 1991. Survival of pathogenic bacteria in an adverse environment. Health Related Water Microbiology, Water Sci. Technol. **24**:395-400.

Bautista, J.M., J. C. Del Rio, F. J. Gonzalez-Vila and F. Martin. 1994. Identification of anthropogenic compounds in peat filters used for waste water depuration. Fresenius Environ. Bull. **3**:540-545.

Bayona, J. M., P. Fernandez and J. O. Grimalt. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environ. Sci. Technol. **24**:357-63.

Bej, A., R. Steffan, J. DiCesare, L. Haff and R. Atlas. 1990. Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. **56**:307-314.

Bester, D., C. M. E. de Wet, M. Grundlingh, and B. Louw. 1991. Evaluation of fecal enterococci isolation media to indicate fecal pollution in chlorinated water. Water Sci. Technol. **24**:777-801.

Bifulco, J., J. Shirey and G. Bissonnette. 1989. Detection of *Acinetobacter spp.* in rural drinking water supplies. Appl. Environ. Microbiol. **55**:2214-2219.

Bolognani, F., P. Brigidi, C. Cerre, D. Matteuzzi and M. Rossi. 1993. Cloning of the gene for cholesterol oxidase in *Bacillus spp.*, *Lactobacillus reuteri* and its expression in *Escherichia coli*. Lett. Appl. Microbiol. **17**:61-64.

Bonnefont, J.L., B. Guiennet and Y. Martin. 1990. Experimental studies on the survival of fecal bacteria from urban sewage in seawater. Water Res. **24**:267-273.

Bonnefont, J.L., P. Lelong and Y. Martin. 1984. Experimental study of fecal coliform survival of urban sewage in seawater. Second International Colloquium on Marine Bacteriology. **3**:567-572. Brest, France: 1-5 October.

Boone, D. 1996. Survival of Fecal Bacteria in Surface Waters. USF, unpublished manuscript.

Borrego, J. J., R. Cornax, M. A. Morinigo and P. Romero. 1990. Survival of pathogenic microorganisms in seawater. Curr. Microbiol. **20**:293-298.

Borrego, J., R. Cornax, A. de Vincente, M. Morinigo and P. Romero. 1987. Coliphages as an indicator of fecal pollution in water. Its relationship with indicator and pathogenic microorganisms. Water Res. **21**:1473-1480.

Borrego, J., R. Cornax, E. Martinez-Manzanares, M. Morinigo and P. Romero. 1990. Coliphages as an indicator of fecal pollution in water. Their survival and productive infectivity in natural aquatic environments. Water Res. **24**:111-116.

Borrego, J.J., D. Castro, R. Cornax, M. Morinigo and E. Martinez-Manzanares. 1990. Viability of *Salmonella spp* and indicator microorganisms in seawater using membrane diffusion chambers. Antonie van Leeuwenhoek **57**:109-117.

Borrego, J.J., R. Cornax, E. Martinez-Manzanares, M. Morinigo and M. A. Munoz. 1992. Presence of indicators and *Salmonella* in natural waters affected by outfall wastewater discharges. Marine Disposal Systems, Water Sci. Technol. **25**:1-8.

Bosch, A., F. Lucena, R. Girones and J. Jofre. 1988. Occurrence of enteroviruses in marine sediment along the coast of Barcelona Spain. Can. J. Microbiol. **34**:921-924.

Brickler, S.K., J. D. Doyle, R. Kramer, R. Kuehl and B. Tunnicliff. 1992. Instability of fecal coliform populations in waters and bottom sediments at recreational beaches in Arizona. Water Res. **26**:979-988.

Brill, J., G. W. Chang and R. Lum. 1989. Proportion of beta-D-glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.

Broward County. 1994. Chapter 27, Pollution Control, of the Code of Broward County, Florida. Municipal Code Corporation. Tallahassee, Florida.

Brown, D. W., D. G. Burrows, S. L. Chan, M. M. Krahn, W. D. MacLeod and C. A. Wigren. 1989. Rapid, automated methods to analyze for organic contaminants in environmental samples. Oceans '89: The Global Ocean. Volume 2: Ocean Pollution 397-401.

Buckhouse, J.C., J. R. Miner, J. A. Moore and B. M. Sherer. 1992. Indicator bacterial survival in stream sediments. J. Environ. Qual. **21**:591-595.

Carballo-Cuervo, S., M. C. de la Rosa-Jorge, R. M. Fernandez-Alvarez and J. Rodriguez-de Lecea. 1991. The influence of agricultural run-off on bacterial populations in a river. J. Appl. Bacteriol. **70**:437-442.

Charrel, M., J. F. Collin and D. Zmirou. 1988. Comparison of bacterial indicators and sampling programs for drinking water systems. Appl. Environ. Microbiol. **54**:2073-2077.

Chauret, C., N. Armstrong, J. Fisher, R. Sharma, S. Springthorpe and S. Satter. 1995. Correlating *Cryptosporidium* and *Giardia* with microbial indicators. J. AWWA **87**:76-84.

Cheung, W., K. Chang and R. Hung. 1991. Variations in microbial indicator densities in beach waters and health related assessment of bathing water quality. Epidemiol. Infect. **106**:329-344.

Chowdhury, M.A.R., S. Miyoshi, S. Shinoda and H. Yamanaka. 1990. Ecology of mesophilic *Aeromonas spp.* in aquatic environments of a temperate region and relationship with some biotic and abiotic environmental parameters. Zentralbl. Hyg. Umweltmed **190**:344-356.

Chung, H. 1993. F-specific coliphages and their serogroups, and *Bacteroides fragilis* phages as indicators of estuarine water and shellfish quality (fecal contamination). Dissertation. University of North Carolina at Chapel Hill.

Clark, D.L., B. B. Milner and M. H. Stewart. 1991. Comparative study of commercial 4-methylumbelliferyl-b-D-glucuronide preparations with the standard methods membrane filtration fecal coliform test for the detection of *Escherichia coli* in water samples. Appl. Environ. Microbiol. **57**:1528-1534.

Cliver, D. O. and J. Snowdonl. 1989. Coliphages as indicators of human enteric viruses in groundwater. Crit. Rev. Environ. Control **19**:231-249.

Codina, J.C., A. de Vicente and P. Romero. 1991. Relationship between *Pseudomonas aeruginosa* and bacterial indicators in polluted natural waters. Water Sci. Technol. **24**:121-124.

Cooney, J.J. and G. W. Pettibone. 1986. Effect of organotins on fecal pollution indicator organisms. Appl. Environ. Microbiol. **52**:562-566.

Cornax, R., M. Morinigo, I. Paez, M. Munoz and J. Borrego. 1990. Application of direct plaque assay for detection and enumeration of bacteriophages of *Bacteroides fragilis* from contaminated water samples. Appl. Environ. Microbiol. **56**:3170-3173.

Cox, F., G. Plews and R. Seabloom. 1990. Effect of sewage discharge from pleasure craft on marine water and shellfish quality. Canadian Waste Management Conference 101-118. 3-5 Oct.

Cox, M.E., B. A. Dixon and D. A. Wadford. 1992. Techniques for the enhanced recovery of *Bacteroides vulgatus* as an indicator of fecal contamination in shellfish. J. Shellfish Res. **11**:557.

Crewe, N.F., J. D. Leonard and R. Pocklington. 1987. Coprostanol as an indicator of fecal contamination in seawater and marine sediment. Oceanol Acta **10**.1:83-90.

Criswell, C. F. 1994. The effect of the West Indian Manatee (*Trichechus manatus*) on fecal coliform levels in the Indian River Lagoon, Brevard County, Florida. Thesis. Florida Institute of Technology.

Davies, C., J. Long, M. Donald and N. Ashbolt. 1995. Survival of fecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. **61**:1888-1896.

Davis, E., D. Manville, J. Mathewson and G. Meriwether. 1992. Investigation of bacterial populations producing elevated MPN values and false positive fecal coliform counts and applicable disinfectants in an industrial wastewater. Water Sci. Technol. **26**:9-11.

de Araujo, M.A., V. F. Guimaraes, L. Mendonca-Hagler and A. N. Hagler. 1990. *Staphylococcus aureus* and fecal streptococci in fresh and marine surface waters of Rio de Janeiro, Brazil. Rev. Microbio. **21**:141-147.

De Villiers, J.C., W. O. Grabow and P. Jagals. 1994. Evaluation of indicators for assessment of human and animal fecal pollution of surface run-off. Water Sci. Technol. **31**: 235-242.

Dean, K. S. Hayes, K. Morgan, and L. Newland. 1990. Septic tank and agricultural non-point source pollution within a rural watershed. Toxicol. Environ. Chem. **26**:137-155.

Derrien, A. and E. Dupray. 1995. Influence of the previous stay of *Escherichia coli* and *Salmonella spp.* in waste waters on their survival in seawater. Water Res. **29**.4:1005-1011.

Ding, M. and F. Huang. 1992. A preliminary analysis of the distribution of fecal coliform bacteria in the near shore waters of the south of Fildes Peninsula, Antarctica. Antarctica Research/Nanji Yanjiu **4**:84-87.

Dolci, F., V. U. Fossato, M. R. Sherwin and E. S.Vanvleet. 1993. Coprostanol in lagoonal sediments and mussels of Venice, Italy. Mar. Pollut. Bull. **26**:501-507.

Dosso, M., D. Guiral and A. Kouassi. 1990. Seasonal variations of microbial contamination of an estuarine tropical lagoon urban area. The case of the city of Abidjan (Ivory Coast). Rev. Hydrobiol. Trop. **23**:181-194.

Dutka, B.J., E. M. Janzen and G. A. Palmateer. 1991. Coliphage and bacteriophage as indicators of recreational water quality. Water Res. **25**:355-357.

Eganhouse, R.P., B. R. Gould, D. P. Olaguer and C. S. Phinney. 1988. Use of molecular markers for the detection of municipal sewage sludge at sea. Mar. Environ. Res. **25**:1-22.

Ellender, R., F. Howell and C. Shows. 1984. Characterization of fecal coliform isolates by electrophoretic analysis of pili. Mississippi-Alabama Sea Grant Consortium: Ocean Springs, Mississippi.

Ellis, J.T., J. S. Latimer, L. A. LeBlanc and J. G. Quinn. 1992. The geochemistry of coprostanol in waters and surface sediments from Narragansett Bay. Estuar. Coast Shelf Sci. **34**:439-458.

Evison, L.M. 1988. Comparative studies on the survival of indicator organisms and pathogens in fresh and sea water. Water and Wastewater Microbiology, Water Sci. Technol. **20**:309-315.

Fernandez, P., M. Valls, J. Bayona and J. Albaiges. 1991. Occurrence of cationic surfactants and related products in urban coastal environments. Environ. Sci. Technol. **25**:547-550.

Fiksdal, L., M. Pommepuy, M. Caprais and I. Midttun. 1994. Monitoring of fecal pollution in coastal waters by use of rapid enzymatic techniques. Appl. Environ. Microbiol. **60**:1581-1584.

Florida (State of). 1995. Administrative Code (FAC), Surface Water Quality Standards, Chapter 62-302.

Fujioka, R., A. Ueno and O. Narikawa. 1990. Unreliability of KF agar to recover fecal streptococcus from tropical waters. J. Water Pollut. Control Fed. **62**:27-33.

Gerba, C. and S. M. Goyal. 1988. Enteric virus: risk assessment of ocean disposal of sewage sludge. IAWPRC Conference on Water and Wastewater Microbiology. Newport Beach, CA. 8-11 Feb.

Grabow, W., T. Neubrech, C. Holtzman and J. Jofre. 1994. *Bacteroides fragilis* and *E. coli* bacteriophages: excretion by humans and animals. The 17th Biennial Conference of the International Association on Water Quality. Budapest. 24-30 July.

Green, G., R. Leeming, P. D. Nichols and J. H. Skerratt. 1992. Hydrocarbon and coprostanol levels in seawater, sea-ice, algae and sediments near Davis Station in eastern Antarctica: A regional survey and preliminary results for a field fuel spill experiment. Mar. Pollut. Bull. **25**:293-302.

Green, J. and B. O'Keefe. 1989. Coliphages as indicators of fecal pollution at three recreational beaches on the Firth of Forth. Wat. Res. **23:**1027-1030.

Griffis, J. N. 1992. An evaluation of human secretory immunoglobulin alpha as a specific indicator of human fecal pollution. Thesis. University of Southern Mississippi.

Han, B.C. and W. L. Jeng. 1994. Sedimentary coprostanol in Kaohsiung Harbour and the Tan-Shui Estuary, Taiwan. Mar. Pollut. Bull. **28**:494-499.

Havelaar, A., M. Van Olphen and Y. Drost. 1993. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. Appl. Environ. Microbiol. **59**:2956-2962.

Hernandez, J., J. Alonso, A. Fayos, I. Amoros and R. Owen. 1995. Development of a PCR assay combined with a short enrichment culture of detection of *Campylobacter jejuni* in estuarine surface waters. Fems. Microbiol. Lett. **127**:201-206.

Hoeller, C. 1988. Long-term study of occurrence, distribution, and reduction of *Campylobacter sp.* in the sewage system and wastewater treatment plant of a big town. Water and Wastewater Technology, Water Sci. Tech. **20**:529-531.

Howell, J., M. Coyne and P. Cornelius. 1995. Fecal bacteria in agricultural waters of the bluegrass region of Kentucky. J. Environ. Qual. **24:**411-419.

Howington, J.P., G. McFeters and J. Smith. 1994. Survival, physiological response, and recovery of enteric bacteria exposed to a polar marine environment. Appl. Environ. Microbiol. **60**: 2977-2984.

Ijzerman, M. M. 1993. Development and Evaluation of a colorimetric coliphage assay detection system (coliphage). Dissertation. Virginia Polytechnic Institute and State University.

Jenkins, A., D. Kay, J. Wilkinson and M. Wyer. 1995. Modeling fecal coliform dynamics in streams and rivers. Water Res. **29**:847-855.

Jiang, S., C. Kellogg, J. Paul, J. Rose and E. Shinn. 1995. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Appl. Environ. Microbiol. **61**: 2235-2241.

Joncas, M., S. Michaud, J. Carmichael and M. Lavoie. 1985. Detection of false positives among total and fecal coliform counts by factorial analysis of correspondence. Appl. Environ. Microbiol. **49**:229-231.

Joo, Y.H., K.Y. Jung, M. Kawano and R. Tatsukawa. 1994. Determination of fecal pollution for well and surface waters from livestock raising areas in Korea using coprostanol as an indicator. Toxicol. Environ. Chem. **45**:57-67.

Juanico, M., D. Ronen and S. Gedaliah. 1990. The use of non-conservative parameters to trace wastewater effluents in water bodies. Water Res. **24**:1245-1250.

Kaplan, I.R., E. Ruth and M. Venkatesan. 1986. Coprostanols in Antarctic marine sediments: A biomarker for marine mammals and not human pollution. Mar. Pollut. Bull. **17**:554-557.

Kator, H. and M. Rhodes. 1988. Survival of *Escherichia coli* and *Salmonella spp.* in estuarine environments. Appl. Environ. Microbiol. **54**:2902-2907.

Kim, S.J., D. H. Lee, E. J. Lee, S. H. Lee and S. J. Park. 1995. Spectrofluorometric assay for rapid detection of total and fecal coliforms from surface water. Appl. Environ. Microbiol. **61**:2027-2029.

Kirchmer, C. J. 1989. 5 beta-cholestan-3 beta-ol an indicator of fecal pollution. Dissertation. University of Florida.

Kloepper-Sams, P. J. and J. Owens. 1993. Environmental biomarkers as indicators of chemical exposure. J. Hazard. Mater. **35**:283-294.

Kone, K. 1994. Detection and enumeration of bacteriophages of *Bacteroides fragilis* HSP40 in food and sewage samples by novel procedures. Dissertation. Kansas State University.

Korhonen, L. and P. Martikainen. 1991. Comparison of the survival of *Campylobacter jejuni* and *Campylobacter coli* in culturable form in surface water. Can. J. Microbiol. **37**:530-533.

Korhonen, L.K., T. U. Kosunen and P. J. Martikainen. 1990. Occurrence of thermophilic campylobacters in rural and urban surface waters in central Finland. Water Res. **24**:91-96.

Kreader, C. 1995. Design and evaluation of bacteroides DNA probe for the specific detection of human fecal pollution. Appl. Environ. Microbiol. **61**:1171-1179.

Krikelis, V. 1991. Research on enteric viruses in aquatic environments. Development and testing of sampling and analytical techniques for monitoring of marine pollutants (Activity A). Final reports on selected microbiological projects., Map. Tech. Rep. Ser. **54:**23-29.

Krstulovic, N. and M. Solic. 1994. Presence and survival of *Staphylococcus aureus* in the coastal area of Split (Adriatic Sea). Mar. Pollut. Bull. **28**:696-700.

Kuebler, B., A. Pittet and R. Stettler. 1990. Use of coprostanol as a specific allochthonous fecal indicator in surface sediment of the Lake of Neuchatel. Aquat. Sci. **52**:130-143.

Kuhn, I., G. Allestam, T. Stenstrom and R. Mollby. 1991. Biochemical fingerprinting of water coliform bacteria, a new method for measuring phenotypic diversity and for comparing different bacterial populations. Appl. Environ. Microbiol. **57**:3171-3177.

Lechevallier, M., C. Cawthon and R. Lee. 1988. Factors promoting survival of bacteria in chlorinated water supplies. Appl. Environ. Microbiol. **54**:649-654.

Legendre, P. and M. Troussellier. 1989. Dynamics of fecal coliform and culturable heterotrop densities in an eutrophic ecosystem: Stability of models and evolution of these bacterial groups. Microb. Ecol. **17**:227-235.

Lewis, G.D. 1995. F-specific bacteriophage as an indicator of human viruses in natural waters and sewage effluents in northern New Zealand. Water Science and Technology: J. Internat. Assoc. Water Poll. Res. **31**:5-6.

Loh, C., H. Zain, Y. Ngeow, C. Wang and Y. Ho. 1990. Assessment of standard methods for the detection of faecal contamination in the monitoring of streams in an equatorial rain forest. Malayan Nat. J. **44**:77-83.

Loh, C.L., W. J. Robertson, S. A. Sattar and V. S. Springthorpe. 1993. In situ survival of indicator bacteria, MS-2 phage and human pathogenic viruses in river water. Health-Related Water Microbiology, Water Sci. Technol. **27**:413-420.

Lucena, F., J. Lasobras, D. McIntosh, M. Forcadell and J. Jofre. 1994. Effect of distance from the polluting focus on relative concentrations of *Bacteroides fragilis* phages and coliphages in mussels. Appl. Environ. Microbiol. **60**:2272-2277.

Marino, R. P. 1990. Survival of fecal coliforms and fecal streptococci in storm drain sediment. Dissertation. University of Michigan.

Marty, Y. and M. Quemeneur. 1994. Fatty acids and sterols in domestic wastewaters. Water Res. **28**:1217-1226.

Mates, A. and M. Schaffer. 1992. Quantitative determination of *Escherichia coli* from coliforms and fecal coliforms in sea water. Microbios. **286**:27-32.

McCorquodale, D. and C. Burney. 1993. Biscayne Bay sewage pollution indicators, final report - South Florida Water Management District. #C-3242.

McCorquodale, D. S. 1987. Coliphage as an indicator of fecal pollution in marine waters: assay, validation, and application. Dissertation. Nova University.

McFeters, G.A. and A. Singh. 1991. Effects of aquatic environmental stress on enteric bacterial pathogens. Pathogens in the Environment. J. Appl. Bact. **70** supplement:115s-120s.

Mirsadeghi, H. and M. I. Venkatesan. 1992. Coprostanol as sewage tracer in McMurdo Sound, Antarctica. Mar. Poll. Bull. **25**:328-333.

Miyabara, Y., J. Suzuki and S. Suzuki. 1994. Classification of urban rivers on the basis of water pollution indicators in river sediment. Bull. Environ. Contam. Toxicol. **52**:1-8.

Miyabara, Y., J. Suzuki and S. Suzuki. 1994. Estimation of i-urobilin movement in an aquatic environment. Jap. J. Toxicol. Environ. Health **40**:13-19.

Miyabara, Y., K. Miyata, J. Suzuki and S. Suzuki. 1994 Evaluation of faecal pollution of river sediment by detection of urobilin. Environ. Pollut. **84**:111-115.

Miyabara, Y., K. Sakamoto, J. Suzuki and S. Suzuki. 1993. Evaluation of contribution of drainage from sewage treatment plants to water pollution based on the amounts of urobilin in rivers. Jap. J. Toxicol. Environ. Health **39**:401-408.

Miyabara, Y., N. Sugaya, J. Suzuki and S. Suzuki. 1994. Estimation of urobilin as a fecal pollution indicator in the aquatic environment. Bull. Environ. Contam. Toxicol. **53**:77-84.

Miyabara, Y., Y. Sakata, J. Suzuki and S. Suzuki. 1994. Estimation of faecal pollution based on the amounts of urobilins in urban rivers. Environ. Pollut. **84**:117-122.

Monfort, P. and B. Baleux. 1991. Distribution and survival of motile aeromonas spp. in brackish water receiving sewage treatment effluent. Appl. Environ. Microbiol. **57**:2459-2467.

Morinigo, M., D. Wheeler, C. Berry, C. Jones, M. Munoz, R. Corax and J. Borrego. 1992. Evaluation of different bacteriophage groups as faecal indicators in contaminated natural waters in southern England. Water Res. **26**:267-271.

Muniz, I., L. Jimenez, G. Toranzos and T. Hazen. 1989. Survival and activity of *Streptococcus faecalis* and *Escherichia coli* in tropical freshwater. Microb. Ecol. **18**:125-134.

Murphy, T. 1992. Feasibility of using chemicals as indicators of fecal pollution: bile acids. Thesis. Univ. of Cincinnati.

Natarajan, R., T. Ramamurthy and S. Ramesh. 1989. Incidence of *Salmonella* in coastal environment of Porto-Novo (South East India). Eighth International Ocean Disposal Symposium. Dubrovnik, Yugoslavia.

Nichols, P., R. Leeming, M. Rayner and V. Latham. 1995. CSIRO Oceanography, GPO Box 1538, Hobart, Tasmania 7001 (Australia). unpublished manuscript.

Niemi, J.S. and R. M. Niemi. 1990. Monitoring of fecal indicators in rivers on the basis of random sampling and percentiles. Water Air Soil Pollut. **50**:331-342.

Niemi, J.S. and R. Niemi. 1991. Bacterial pollution of waters in pristine and agricultural lands. J. Environ. Qual. **20**:620-627.

Okuofu, C. 1989. Comparative study of *Bifidobacteria*, faecal coliform and faecal streptococci as indicators of water pollution. Water Res. **1**:197-199.

Opara, A.A. 1993. Assessing anaerobic enteric bacteria as indicators of fecal pollution in tropical waters. Trop. Freshwater Bio. **3**:309-318.

Owczarczyk, A, M. Strzelecki, S. Szpilowski and R.Wierzchnicki. 1994. Application of tracer method for investigation of dilution and decay of petrochemical effluent discharged into Big River. Water Air Soil Pollut. **78**:199-213.

Palmer, M. 1988. Bacterial loadings from resuspended sediments in recreational beaches. Can. J. Civ. Engin. **15**:450-455.

Paul, J., J. Rose, J. Brown, E. Shinn, S. Miller and S. Farrah. 1995. Viral tracer studies indicate contamination of marine waters by sewage disposal practices in Key Largo, Florida. Appl. Environ. Microbiol. **61**:2230-2234.

Paul, J., J. Rose, S. Jiang, C. Kellogg and L. Dickson. 1993. Distribution of viral abundance in the reef environment of Key Largo Florida. Appl. Environ. Microbiol. **59**:718-724.

Pierce, R. H. 1987. A Survey of Coprostanol Concentrations in Biscayne Bay Sediments, prepared for Dade County Department of Environmental Resource Management. Mote Marine Laboratory, Sarasota, Florida, May 26.

Poggi, R. 1991. Sanitary impacts of microbiological contamination. National Colloquium The Sea and Urban Waste Disposal **11**:115-132.

Poiger, T., J. Field, T. Field and W. Giger. 1993. Determination of detergent derived fluorescent whitening agents in sewage sludges by liquid chromatography. Anal. Methods Instrum. **1**:104-113.

Pommepuy, M., Cormier, M., A. Derrien, E. Dupray, J. F. Guilland and F. Le Guyander. 1992. Enteric bacteria survival factors. Water Management in Coastal Areas, Water Sci. Technol. **25**:93-103.

Pourcher, A., L. Devriese, J. Hernandez and J. Delattre. 1991. Enumeration by a miniaturized method of *E. coli*, *S. bovis* and enterococci as indicators of the origin of fecal pollution of waters. J. Appl. Bacteriol. **70**:525-530.

Puente, I. 1991. Sources of coliphage to the marine environment. Thesis. Nova University.

Puig, M., J. Joffe, F. Lucena, A. Allard, G. Wadell and R. Girones. 1994. Appl. Environ. Microbiol. **60**:2963-2970.

Rector, D. D. 1977. Densities of indicator microorganisms in surface microlayers of recreational fresh waters. Thesis. Georgia State University.

Rhodes, M. and H. Kator. 1988. Survival of *Escherichia coli* and *Salmonella spp*. in estuarine environments. Appl. Environ. Microbiol. **54**:2902-2907.

Ripp, S. and R. Miller. 1995. Effects of suspended particulates on the frequency of transduction among *Pseudomonas aeruginosa* in a freshwater environment. Appl. Environ. Microbiol. **61**:1214-1219.

Rivera, S., T. Hazen and G. Toranzos. 1988. Isolation of fecal coliforms from pristine sites in a tropical rain forest. Appl. Environ. Microbiol. **54**:513-517.

Roll, B. M. 1995. Development of polymerase chain reaction techniques for the detection of waterborne pathogens in environmental waters. Dissertation. University of Hawaii.

Samadpour, M. and N. Chechowtz. 1995. Little Soos Creek Microbial Source Tracking - A Survey. King County Dept. Public Works. Seattle, Washington.

Seyfried, P., R. Tobin, N. Brown and P. Ness. 1985. A prospective study of swimming related illness. II Morbidity and the microbiological quality of water. Am. J. Publ. Health **75**:1071-1075.

Sjogren, R. E. 1994. Prolonged survival of an environmental *Escherichia coli* in laboratory soil microcosms. Water Air Soil Pollut. **75**:389-403.

Tartera, C., F. Lucena and J. Jofre. 1989. Human origin of *Bacteroides fragilis* bacteriophages present in the environment. Appl. Environ. Microbiol. **55**:2696-2701.

Torroella, J.J. 1991. Bacteriophages of *Bacteroides* as indicators of pathogenic human viruses in coastal seawaters. Development and testing of sampling and analytical techniques for monitoring of marine pollutants (Activity A). Final reports on selected microbiological projects., Map. Tech. Rep. Ser. **54**:1-10.

Venkatesan, M. and I. Kaplan. 1990. Sedimentary coprostanol as an index of sewage addition in Santa Monica basin southern California USA. Environ. Sci. Technol. **24**:208-214.

Wright, R.C. 1989. The survival patterns of selected fecal bacteria in tropical fresh waters. Epidemiol. Infect. **103**:603-611.

Yoshpe-Purer, Y. 1989. Evaluation of media for monitoring fecal streptococci in seawater. Appl. Environ. Microbiol. **55**:2041-2045.

Zaccone, R., E. Crisafi and G. Caruso. 1995. Evaluation of fecal pollution in coastal Italian waters by immunofluorescence. Aquat. Microb. Ecol. **9**:79-85.

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