Bacterial Indicators of Recreational Water Quality

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The currently recommended bacterial indicator for measuring the quality of recreational waters in the United States is the fecal coliform group. This indicator was first proposed as a monitoring tool in 1968[1] and since that time it has been adopted by more than 95% of the states and trust territories of the United States. A very small percentage of the states still use total coliforms as the indicator of choice and a few of the states use both total coliforms and fecal coliforms. Prior to 1968, total coliforms were generally accepted as the indicator for monitoring outdoor bathing waters. Although the total coliform group had been used by individual states for many years, it gained wide acceptance only after the American Public Health Association adopted a bathing water classification scheme in 1943. The classification scheme, which had been proposed by Scott[2] in 1934, was based on four classes of water. The upper limits for the four classes were 50, 500, 1000 and greater than 1000 coliforms per 100 ml. Scott developed the classification after an extensive survey of the Connecticut shoreline indicated that 92.8% of the samples contained less than 1000 coliforms per 100 ml. This classification scheme agreed well with a sanitary survey classification which showed that only 6.9% of the shoreline was designated as poor. He concluded that water having coliform densities of less than 1000 per 100 ml was probably acceptable for bathing.

The use of the 1000 coliforms per 100 ml density as the value for separating high quality from poor quality bathing waters provides a good example of how microbial standards have been chosen and how, one they are set, there is a great reluctance to change to another indicator or standard. The classification of bathing waters suggested by Scott, for instance, was based on the principle of attainment. Since over 92% of Connecticut's shoreline waters contained less than 1000 coliforms per 100 ml very little in the way of intervention measures would be required and therefore the standard could be attained with little or no difficulty. Quite independently, the State of California arbitrarily adopted a standard of 10 coliforms per ml in 1943[3]. This standard was not based on epidemiological evidence or on extensive surveys, but rather on the perception that it related well with the drinking water standard of that time, there was no epidemiological evidence of health effects within the standard, the 10 coliforms per ml could easily be attained and any less stringent standard might result in waters that would be asthetically unacceptable. A third means for determining bathing water quality was the analytical method adopted by Streeter[4] in 1951. He used the coliform to *Salmonella* ratio developed by Kehr and Butterfield[5], the number of bathers exposed, the approximate volume of water ingested daily per bather and the average coliform density per ml of bathing water to develop a bather risk factor. Streeter speculated that in water containing 1000 coliforms or less per 100 ml there would be no great health hazard for individual bathers, at least from *Salmonella typhosa*. It is interesting to note that in spite of the different means used for obtaining a standard measure for water quality, either arbitrarily as was done in California, or on a practical basis as Scott had done or even through the use of an analytical technique, as Streeter had done, the final results were approximately the same, 1000 coliforms per 100 ml.

Total coliforms also were an integral element in the establishment of fecal coliforms as a monitoring tool for surface waters. The committee that was deliberating the potential use of a new indicator for evaluating water quality in 1968[6] proposed fecal coliforms, a subset of the total coliforms, because it was felt that this indicator group was more fecal specific and less subject to seasonal variation than the non-thermotolerant members of the total coliform group. However, water quality measured with fecal coliforms had never been linked epidemiologically to swimming-associated illness and therefore the committee had to look back to the 1949 United States Public Health Service (USPHS) studies which used total coliforms to monitor the water[7]. In 1949 the USPHS determined that an excess of swimming-associated gastroenteritis occurred in freshwater swimmers when the total coliform density was about 2300 per 100 ml. The 2300 per 100 ml value was related to fecal coliforms by estimating the ratio of fecal coliforms to total coliforms in the mid-1960s on the same portion of the Ohio River where the initial

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study was conducted. The proportion of fecal to total coliforms was about 18%, and therefore it was assumed that a health effect would be detected in recreational waters when the fecal coliform density was 400 per 100 ml. The committee further reasoned that a detectable health effect was undesirable and that by setting the standard at 200 fecal coliforms per 100 ml a built-in safety factor would be present. Thus, the currently used standard is the equivalent of about 1100 total coliforms per 100 ml. Although it is probably coincidental, it is remarkable that the suggested fecal coliform standard was the equivalent of the one being abandoned, namely 1000 total coliforms per 100 ml.

About the same time that fecal coliforms were being proposed as a replacement for coliforms the first descriptions of what an ideal indicator should be appeared in the literature. Table I is a compilation of those characteristics proposed by Bonde[8] in 1966 and of the many others that followed in the ensuing years[9,10,11,12]. The various authors were in close agreement on the first four characteristics, which address correlation to pathogens, "aftergrowth", resistance to disinfection and enumeration methods, but they did not agree on the remaining six characteristics. All characteristics are important, but two among them are more important than the others and have been circled. The first is that of Scarpino[9] who indicated that the density of the indicator should have a direct relationship to the degree of fecal contamination. The basis for his suggestion was that fecal contamination is related to the potential for the presence of enteric pathogens, and therefore, if there was a direct relationship between the indicators and fecal contamination, the degree of risk could be estimated.

The second is the suggestion of Cabelli[11] that an indicator density should correlate with health hazards from given types of pollution. Cabelli's proposed characteristic is much broader than Scarpino's, but it has the same objective, to have a microbial indicator that will provide an estimate of the risk level from a pollution source. If this characteristic is present, especially if it was determined with a highly specific method, most of the other characteristics will also be present. Much of the research described below will address this important characteristic of microbial indicators.

EPIDEMIOLOGICAL-MICROBIOLOGICAL STUDIES

In 1972 the U.S. Environmental Protection Agency (USEPA) began a series of studies which examined the relationship between swimming-associated illness and water quality[13]. A number of potential bacterial indicators of water quality were examined in order to determine which exhibited the best relationship to the rate of swimming-associated illness. Each indicator was intensively monitored during the periods of peak swimming activity, usually at multiple sites at each beach. A study unit was made up of six to eight weekends during the swimming season. The overall water quality measure for a study unit was obtained by taking the geometric mean of the indicator densities for all of the weekend days. A corresponding illness rate was calculated by subtracting the illness rate for non-swimmers from the illness rate in swimmers. Gastroenteritis was defined as having multiple gastrointestinal symptoms.

Correlation analysis was performed on the swimming-associated gastroenteritis rates and the corresponding geometric mean densities of each indicator from multiple beaches and study units. The correlation coefficient was used as the measure of the "strength" of the relationship between each indicator of water quality and swimming-associated gastroenteritis. Table II shows the correlation coefficients for eleven indicator systems against highly credible and total gastrointestinal symptoms obtained from the

TABLE II
Strength of Indicator-Illness Relationships from Marine Studies

Indicator	Correlation Coefficient HCGI	Correlation Coefficient Total GI	Number of Study Units
Enterococci	.75	.84	8
E. coli	.52	.56	8
Klebsiella	.32	.35	8
Enterobact.-Citrobact.	.26	.23	8
Total Coliforms	.19	.12	8
C. perfringens	.19	.38	5
P. aeruginosa	.19	.25	8
Fecal Coliforms	-.01	.01	8
A. hydrophila	-.09	-.08	7
V. parahemolyticus	-.20	.19	5
Staplylococci	-.23	.71	5

Data from Reference 13

TABLE I
Ideal Characteristics of Bacterial Indicators of Fecal Contamination

Ideal Characteristics	Bonde 1966	Scarpino 1971	Dutka 1973	Cabelli 1977	Barrow 1981
1. Be present where pathogens are	X	X	X	X	
2. Unable to grow in aquatic environments	X	X	X		X
3. More resistant to disinfection than pathogens	X		X	X	X
4. Easy to isolate and enumerate	X	X	X	X	X
5. Applicable to all types of water		X			
6. Not be subject to antibiosis	X				
7. Absent from sources other than sewage or be exclusively associated with sewage				X	X
8. Occur in greater numbers than pathogens	X				
9. Density of indicator should have direct relationship to degree of fecal contamination		X			
10. Indicator density should correlate with health hazard from a given type of pollution				X	

studies conducted in New York City from 1973 to 1975[13]. Enterococci showed the strongest relationship to the highly credible symptoms of gastroenteritis. *E. coli* was a very weak second and fecal coliforms showed no association with symptomatic illness. *Aeromonas hydrophila, Vibrio parahemolyticus* and *Staphylococcus aereus,* three bacteria not commonly associated with human or animal feces also showed no relationship to swimming-associated gastrointestinal illness.

The marine bathing beach studies were continued at Lake Pontchartrain near New Orleans, Louisiana and at Boston, Massachusetts from 1976 to 1978. During this period the water quality monitoring effort was directed mainly at measuring enterococci, *E. coli* and fecal coliforms. These three indicators maintained their positions relative to the rankings observed in the first three years of the study. Enterococci showed the strongest association with illness due to swimming, confirming the earlier findings of the New York City beach study.

In 1979 another series of studies was begun at freshwater bathing beaches at Tulsa, Oklahoma and Erie, Pennsylvania. These studies were completed in 1982[14]. The conduct of the freshwater studies was the same as that used in the marine studies. However, only three indicators were examined. Enterococci and *E. coli* were used because they showed the best relationship to swimming-associated gastroenteritis in the marine studies and fecal coliforms were examined because this is the currently accepted bacterial indicator of water quality. The results are shown in Table III. The correlation coefficient for *E. coli* was slightly greater than that for enterococci, although this difference was not statistically significant. This finding was quite different from the results of the marine studies. The correlation coefficient for fecal coliforms, however, was very similar to that found in the marine studies, indicating again there was no association

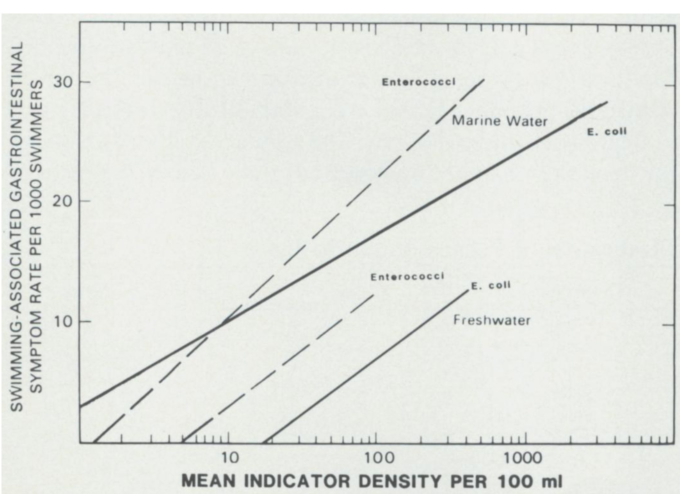

Figure 1. Relationship of Gastrointestinal Symptom Rates to *E. coli* and Enterococcus Densities at Marine and Freshwater Bathing Beaches (13, 14).

TABLE III
Strength of Indicator-Illness Relationships from Freshwater Studies

Indicator	Correlation Coefficient HCGI	Correlation Coefficient Total GI	Number of Study Units
Enterococci	.774	.673	9
E. coli	.804	.528	9
Fecal Coliforms	-.081	.249	7

Data from Reference 14

between fecal coliforms and swimming-associated gastroenteritis. Figure 1, which is a compilation of the marine and freshwater regression lines for swimming-associated gastroenteritis on *E. coli* or enterococci illustrates more clearly the similarity of the enterococci slopes and the difference in the *E. coli* slopes in fresh and marine waters. This figure also shows that, with the exception of *E. coli* in seawater, the illness rates all approach zero before the indicator densities reach one. This can be interpreted as meaning that the pathogen disappears before the indicator does, an important factor in choosing an indicator.

SELECTION OF A BACTERIAL WATER QUALITY INDICATOR

The results of the marine and freshwater studies indicate that one bacterial indicator meets most of the ideal characteristics discussed above. Table IV is a list of the characteristics possessed by enterococci, *E. coli* and fecal coliforms as they relate to those ideal characteristics. Enterococci appear to fulfill most of the suggested characteristics. Most important is the fact that the density of this indicator correlated well with gastrointestinal disease associated with swimming. The regression line of swimming-associated gastrointestinal illness rates on enterococci density indicates that the illness rate approaches zero before the indicator density reaches one, as pointed out above, which suggests that this indicator possesses two of the other desired characteristics. At least in the freshwater studies, where the effluents were chlorinated before being discharged, it must be assumed that the etiologic agent was not more resistant to disinfection than the indicator. Similarly, the enterococci must occur in greater numbers than the pathogen, or they die off more slowly, since the effects of the pathogen disappear before the indicator does. Furthermore, enterococci do not grow in aquatic environments[15] and they are relatively easy to enumerate. They are also applicable to both marine and fresh waters, the two environments where they would be used most often. *E. coli* has fewer of the desirable characteristics than enterococci and is deficient in the following areas. It is not applicable to marine waters and is not more resistant to disinfection than some of the enteric pathogens. *E. coli* also is quite sensitive to antibiosis, at least in marine waters[16]. However, this is a moot point since, as noted above, this indicator is not adequate for use in seawater.

Fecal coliforms lack many of the ideal characteristics.

Some members of the group are able to grow in the environment, even after chlorination. The fecal coliform group is similar to *E. coli* with respect to chlorination in that some enteric pathogens are more resistant. One member of the fecal coliform group, *Klebsiella*, has known extra-enteral sources. This genus occurs in pulp and paper mill effluents[17], textile processing plant wastes[18], cotton mills[19] and other industrial sources in the absence of fecal contamination[20]. Lastly, as pointed out above, fecal coliform densities do not correlate with health hazards from given sources.

It would appear that if a bacterial indicator were to be chosen on the basis of scientific dependability, enterococci would be the prime indicator of choice. *E. coli* also could be an appropriate indicator if fresh waters only were under consideration. Similarly, on the basis of currently available information, fecal coliforms would be a poor choice for monitoring recreational water quality.

DISCUSSION

The almost total lack of a relationship between fecal coliforms and swimming-associated gastroenteritis deserves some further discussion because of the current status of this indicator. Fecal coliforms are a heterogeneous group which comprises several genera. *Escherichia coli* and *Klebsiella* species are usually found in the highest densities in surface water samples. *Klebsiella* species also are sometimes found in high densities in domestic sewage effluents. Table IV shows that the median percentage of thermotolerant *Klebsiella* in fecal coliform populations in the secondary effluents of seven sewage treatment plants was 30%, with a range of 13 to 42%. Bathing beach water samples from one of the freshwater beaches reflected a similar proportion of thermotolerant *Klebsiella* in the fecal coliform population (data not shown). The median percentage of *Klebsiella* was about 36%, with a range of 17 to 74%. The potential for *Klebsiella* growth in polluted surface waters has been documented[21] and this ability may be a contributing factor for the lack of a relationship between health risk in swimmers and water quality as measured with fecal coliforms. A second factor that could affect the above relationship is the fecal coliform test itself. It is well known that the cellular injury or stress suffered by fecal coliforms in aquatic environments can be overcome through the use of resuscitation techniques, which, with water samples from some aquatic environments, increases the recoveries between 100 and 200% relative to the recoveries without resuscitation. Thus, the fecal coliforms could be grossly underestimated if they are sampled from certain stressful environments and measured with the standard methods technique. On the other hand, if certain segments of the fecal coliform population grow in the water environment, then the density of fecal coliforms may be grossly overestimated. Both of these factors could have been at work in the USEPA studies which examined swimming-associated illness and water quality. Thus, if low indicator densities are inflated because of fecal coliform growth or if high densities are deflated because of environmental stress and the use of the standard methods fecal coliform technique, the paired data points would have a tendency to move to the center of the scatter diagram. The end result of these potential shifts if that the data may be distorted to the point where a valid relationship would not exist. Although this scenario is speculative, some of its elements are compatible with the known characteristics of fecal coliforms and it could easily account for the effects observed in the marine and freshwater health effects water quality studies conducted by the USEPA.

Some other results observed while analyzing the water quality data are pertinent to the indicator systems used and therefore should be discussed more fully. The first is the failure of *E. coli* to show a strong relationship to swimming-associated gastrointestinal illness in the marine water studies as it did in the freshwater studies and the second is the significantly greater illness rate in marine swimmers versus freshwater swimmers. The overall mean swimming-associated gastroenteritis rates for marine and freshwater swimmers are shown in Table VI. The illness rates from the two groups were tested with the Wilcoxon rank sum test which indicated that it was highly improbable that the two groups came from the same population ($p < 0.05$). The overall geometric mean densities of *E. coli* and enterococci for these two types of water also are shown in the table. Although the illness rates were statistically different, the indicator densities were not. The similarity of mean indicator densitites was not unexpected, since one of the restric-

TABLE IV

Occurrence of Ideal Characteristics in Three Indicators of Fecal Contamination

Ideal Characteristics	Enterococci	E. coli	Fecal Coliforms
1. Be present when pathogens are	Yes	Yes	Yes
2. Unable to grow in aquatic environments	Yes	Yes	No
3. More resistant to disinfection than pathogens	Yes	No	No
4. Easy to isolate and enumerate	Yes	Yes	Yes
5. Applicable to all types of water	Yes	No	No
6. Not be subject to antibiosis	?	?	?
7. Absent from sources other than sewage or be exclusively associated with sewage	No	No	No
8. Occur in greater numbers than pathogens	Yes	Yes	Yes
9. Density of indicator should have direct relationship to degree of fecal contamination	Yes	Yes	Yes
10. Indicator density should correlate with health hazard from a given type of pollution	Yes	Yes	No

TABLE V
Ratio of Thermotolerant *Klebsiella* to Fecal Coliforms in Sewage Treatment Plant Secondary Effluents

Plant No.	Distribution of Thermotolerant *Klebsiella* in Fecal Coliform Populations	
	Klebsiella[1]/Fecal Coliform[1]	Percent Klebsiella
1	1.1/8.3	13
2	0.2/1.7	13.5
3	0.8/4.0	20
4	0.4/1.3	30
5	1/2.9	34
6	1.3/3.8	34
7	80/190	42

[1]Density per ml x 10^{-4}

tions of the study site selection process was that each site had to meet local and state water quality standards. All of the study locations used the 200 fecal coliform per 100 ml standard, and furthermore, most of the sites were in compliance with these regulations during the course of the studies. This restriction could be an important contributing factor to the difference in gastrointestinal illness rates observed between marine and freshwater swimmers. The most reasonable hypothesis is that at beach sites there are more pathogens in marine waters than in fresh waters and the reason for the higher densities of pathogens is the differential indicator die-away in the two types of water environments. There is some evidence to support this hypothesis.

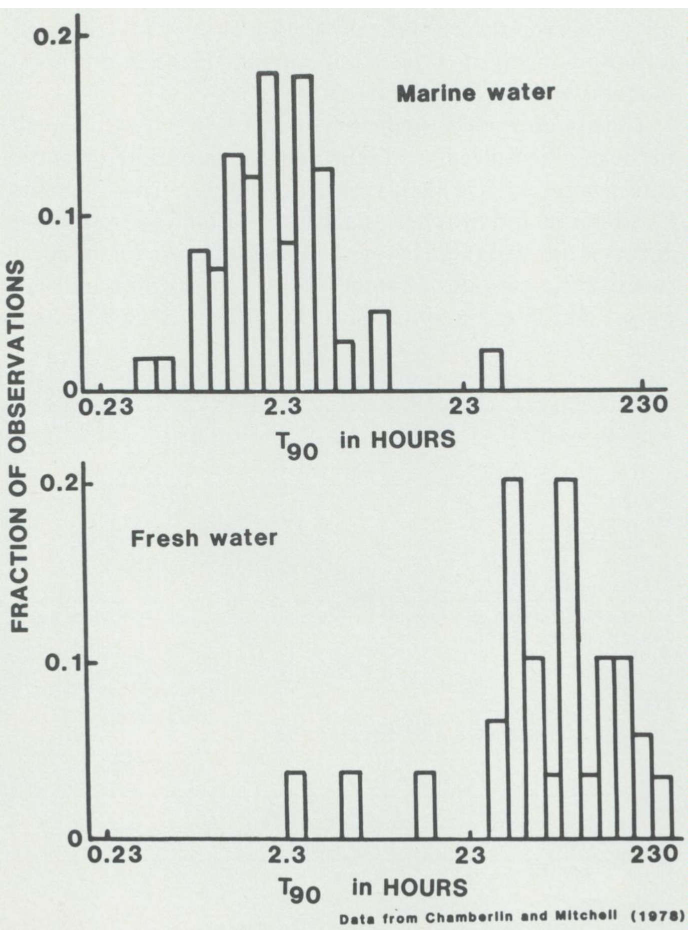

Figure 2. Distributions of Coliform Die-off Rates Under Natural Conditions in Marine and Freshwater Environments (22).

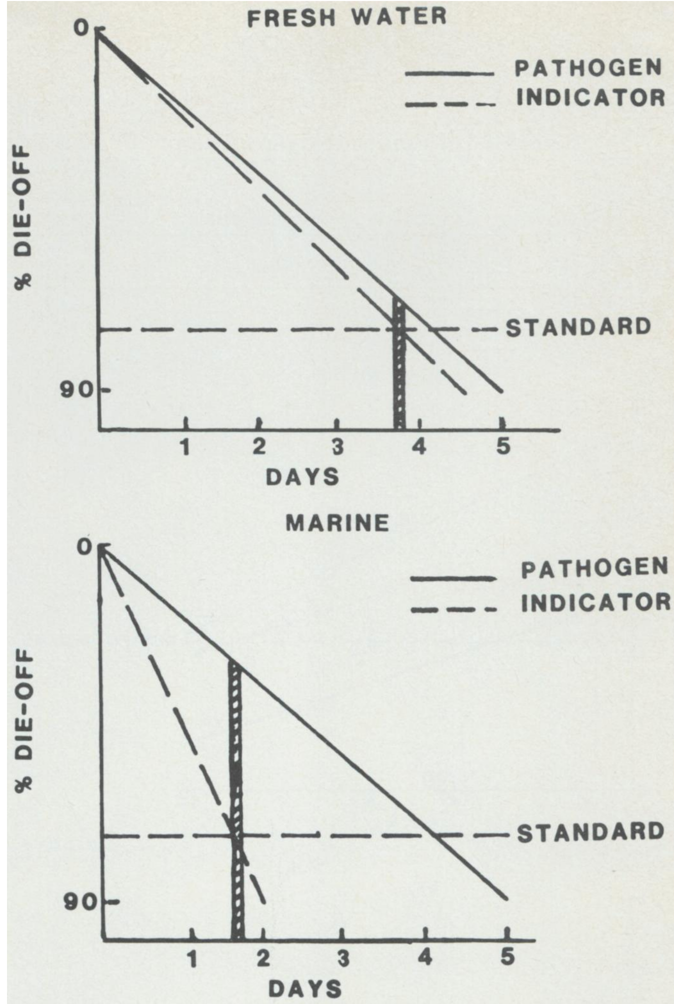

Figure 3. Model for Indicator, Pathogen Die-off in Marine and Fresh Waters.

Figure VI is taken from some of the analyses of environmental data done by Chamberlin and Mitchell[22] in 1978. They analyzed 87 seawater studies and 28 freshwater studies on coliform indicator die-off in natural environments. Their analysis indicated that in the seawater studies the median T_{90} value, that is, the time it takes for 90% of the bacterial population under study to die-off, was 2.2 hours, whereas in the freshwater studies, the mean T_{90} values was 57.6 hours. This great discrepancy in die-off rates could well account for the difference in illness rates observed in fresh and seawater swimmers. Figure 3 is a schematic representation of possible die-off effects in marine and freshwater environments. These hypothetical relationships predict that if indicators discharged into either environment have similar sources, then the indicators in seawater would reach compliance levels long before those discharged into freshwater. If the pathogen had a tendency to die-off at the same rate in seawater and freshwater, then the difference in illness rates would have a rational explanation. Thus, when the indicator is within the standard in marine waters, there would be a great excess of pathogen, whereas in freshwater by the time the indicator reached the standard, the pathogen would also be at low densities. The vertical cross-hatched bar indicates

TABLE VI
Summary of Epidemiological-Microbiological Data from Marine and Freshwater Bathing Beach Studies

Type of Water	Number of Trials	Swimming-Associated Gastroenteritis Rate[1]	Indicator Density[2]	
			E. coli	Enterococci
Marine	16	15.2	56	25
Fresh	9	5.7	72	20

[1]Arithmetic mean
[2]Geometric mean

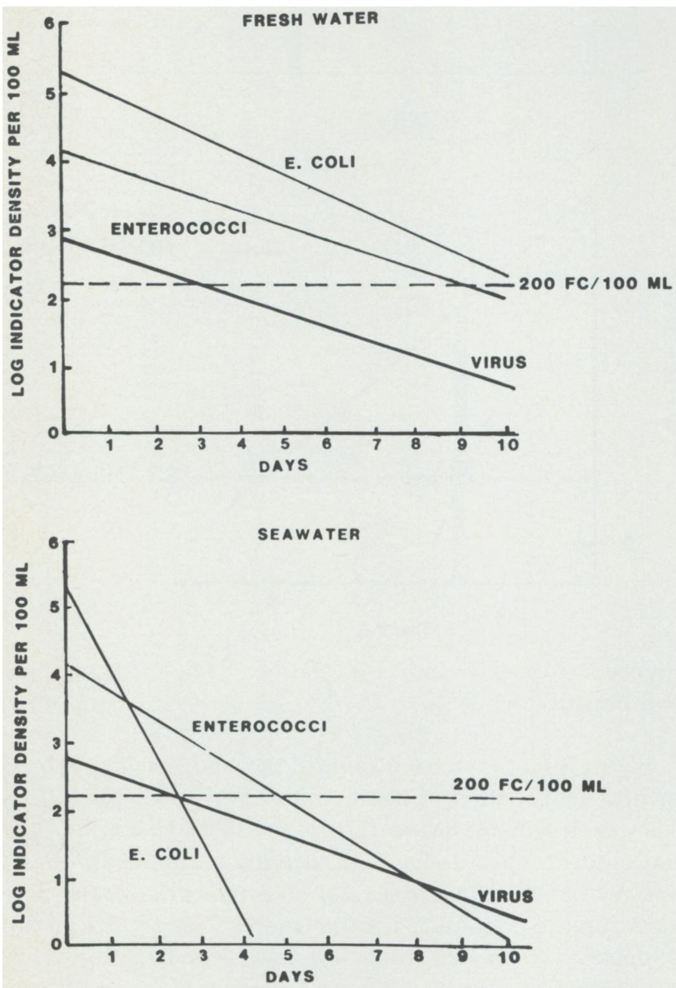

Figure 4. Die-off Characteristics of Enterococci, *E. coli* and Viruses in Marine and Fresh Waters.

the relative densities of pathogen which might occur when the indicator density is at the limit of the standard. The question is, do enteric viruses, the suspected pathogen[23], die-off at the same rate in seawater and freshwater? A search of the literature revealed only one paper which addresses the question directly. Cioglia and Loddo[24] have shown that strains of Polio, $ECHO_6$ and Coxsackie virus had similar die-off rates in river and seawater held at 25° C. The results of a portion of their experiments are shown in Table VII. Although the data are limited, it is obvious that the die-off rates in river and seawater are about the same. There is no evidence that swimming-associated gastroenteritis is caused by any of these three enteric viruses, and therefore one can only speculate that the unidentified pathogen which causes swimming-associated gastroenteritis may have similar decay characteristics. If the decay rate of the etiologic agent is similar in marine and fresh waters, then the proposed model is valid.

The next question is whether *E. coli* and enterococci will fit the model. Since *E. coli* represents a major portion of the coliform group, it is likely to react as the coliforms did in the Chamberlin and Mitchell analysis of marine and freshwater decay. However, there is very little information on the comparative die-off rates of enterococci in freshwater and seawater. There are a number of laboratory studies which

TABLE VII
Survival of Enteroviruses in Seawater and River Water

Virus Strain	Die-Off Rates	
	Seawater	River Water
Polio I	8[1]	15
Polio II	8	8
Polio III	8	8
$ECHO_6$	15	8
Coxsackie B_3	2	2

[1]Maximum number of days required to reduce the virus population by three logs
Data from Reference 24

TABLE VIII
Decay Rate Estimates for *E. coli* and Enterococci in Seawater and Freshwater

Reference	Die-Off Rates			
	Freshwater		Seawater	
	E. coli	Enterococci	E. coli	Enterococci
Bitton *et al.* (29)	6.3[1]	34.7		
McFeters & Stewart (30)	2.7	4.2		
Keswick *et al.* (27)	3.1	4.5		
Hanes & Fragala (25)	4.6	3.0	0.8	2.4
Omura *et al.* (31)			0.7	2.6
Median	3.9	4.4	0.8	2.5

[1]Time required for 90% of the population to die-off in days.

compared *E. coli* and enterococci die-off rates in freshwater or in seawater, but only one[25] compared both indicators in fresh and marine waters. Table VIII is a collection of data from five studies that examined the decay rates of these two indicators. The data are given as the time required for 90% of the population to die-off in days. The summary data given in the last row of the table were obtained by calculating the median value for each column. Enterococci in freshwater had the slowest die-off rate and *E. coli* in seawater died-off most rapidly. Figure 4 is a graphic presentation of the median decay rates for *E. coli* and enterococci given in Table VIII. These figures illustrate more clearly what is probably happening in the marine and freshwater environments. The initial densities shown for both indicators were the mean levels from secondary effluents of seven sewage treatment plants reported by Miescier[26]. The *E. coli* decay rates, as predicted, are similar to the coliform decay rates in seawater and freshwater. Enterococci decay rates are similar in both marine and fresh waters, but there is a more rapid decay in seawater. If these die-off rates are considered in concert with an estimated viral decay rate, then a pattern emerges that is very much like the proposed model. In both graphs the horizontal line represents the 200 fecal coliform per 100 ml standard. The average die-off rate for enteric viruses was calculated from data given by Keswick[27] and the initial density of viruses shown in the figure is the estimate given by Gerba[28]. The model appears to answer two of the questions raised earlier. The answer to the first question — "Why is *E. coli* a poor indicator of quality for marine waters?" — is obvious from the data of Chamberlin and Mitchell and the data in Table VIII. The variation and rapid die-off of coliforms and *E. coli* in marine waters is consistent with a low correlation coefficient and the disappearance of the indicator while the illness rate is still observable. These results from other studies support the conclusion reached from the epidemiological data, that *E. coli* is a poor indicator in marine waters. The answer to the second question — "Why is the gastroenteritis rate significantly higher in seawater swimmers?" — also can be found in the die-off characteristics of the indicators. The rapid die-off of *E. coli* and coliforms in the probable absence of pathogen decay in marine waters could account for the observed differences. The restriction that the bathing water had to meet a 200 fecal coliform per 100 ml standard assured that the illness rate difference would be large because of the extreme sensitivity of coliforms to marine environments. However, the difference in illness rates between freshwater and marine swimmers probably would have been observed even if enterococci had been used for the site selection process since this indicator also shows appreciable differences in die-off rates between these two types of water.

CONCLUSIONS

The selection of an indicator to measure the quality of recreational bathing water has been considered with respect to a number of suggested characteristics for an ideal bacterial indicator. Although none of the indicators examined could fulfill all of the suggested characteristics, enterococci were able to meet more of them than *E. coli* or fecal coliforms. Even more important, enterococci density had a stong direct relationship to swimming-associated illness in both seawater and freshwater. *E. coli* density showed a strong relationship to health risk in freshwater, but not in seawater whereas fecal coliform density was not related to swimming-associated gastroenteritis in either aquatic environment.

The differential die-off rates of bacterial indicators in marine and fresh waters, coupled with the apparent similarities in the decay rates of pathogens in these two water environments, preclude the use of a single criterion or standard for both marine and fresh waters.

Finally, the epidemiologic method has an inherent advantage over previous methods used for justifying bacterial indicators of water quality. Indicators were selected in the past on the basis of some relationship to pathogens in the same contaminated aquatic environment. This method of selection is not possible today because many of the known pathogens, especially viral pathogens, cannot be cultured in the laboratory. The epidemiologic method, on the other hand, does not require establishing pathogen to indicator relationships since only illness incidence is measured. This technique may be useful for developing bacterial indicator systems for other types of regulated waters.

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