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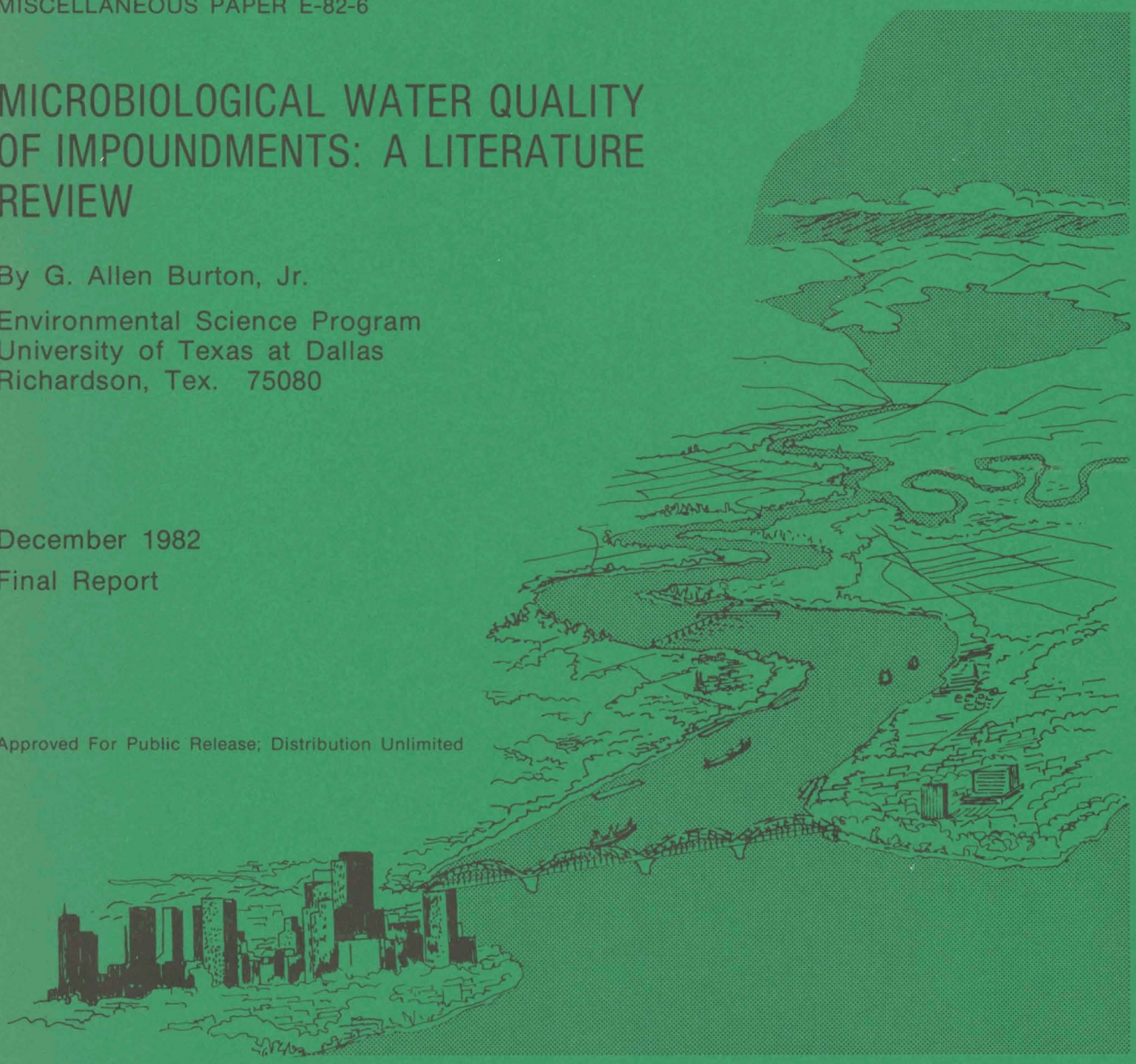
MICROBIOLOGICAL WATER QUALITY OF IMPOUNDMENTS: A LITERATURE REVIEW

By G. Allen Burton, Jr.

Environmental Science Program
University of Texas at Dallas
Richardson, Tex. 75080



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Assessing the microbiological water quality of impoundments and the potential for waterborne disease outbreaks is a difficult task when using traditional sampling programs. Problems associated with using fecal coliform bacteria as indicators of human pathogen presence complicates assessments of future water quality in preimpoundment areas.

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Reliable determination of future and present microbiological water quality requires knowledge of how the chemical, physical, and biological characteristics of the watershed and impoundment interrelate to influence microbial indicator and pathogen densities. Accurate estimates of microbial indicator and pathogen densities, obtainable by using the enumeration methods and their modifications suggested in this report, will allow monitoring of the proper indicator organisms and estimation of potential sites of pathogen occurrence, density, and survival. Sampling programs must be geared toward critical time periods and areas; i.e., summer months, storm flows, feeder streams, agricultural and urban runoff, and swimming areas, including water and sediments. Frequency of sampling should be dictated by variability of water conditions, confidence level of data, and extent of human contact.

Choice of proper indicator organisms and enumeration methods and appropriate sampling strategies will allow sound preimpoundment assessment and reservoir management to greatly reduce the risk of waterborne disease outbreaks.

PREFACE

This report was prepared by G. Allen Burton, Jr., Environmental Science Program, University of Texas at Dallas, Richardson, Texas, for the U. S. Army Engineer Waterways Experiment Station (WES) under Purchase Order No. DACW-39-82-M-2075 dated 30 March 1982. This study forms part of the Environmental and Water Quality Operational Studies (EWQOS) Program, Work Unit IIF, Reservoir Site Preparation. The EWQOS Program is sponsored by the Office, Chief of Engineers, and is assigned to the WES under the purview of the Environmental Laboratory (EL).

The study was conducted under the direct WES supervision of Dr. Douglas Gunnison, and the general supervision of Mr. Donald R. Robey, Chief, Ecosystem Research and Simulation Division, EL, and Dr. John Harrison, Chief, EL. Dr. Jerome L. Mahloch was the EWQOS Program Manager.

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MICROBIOLOGICAL WATER QUALITY OF IMPOUNDMENTS: A LITERATURE REVIEW

PART I: INTRODUCTION

1. The United States has a large number of impoundments which provide flood control and serve as water supplies and recreational sites for millions of individuals. Each of these impoundments possesses unique and complex hydrologic and water quality characteristics. Impoundments are subject to contamination from multiple sources including: industrial and municipal discharges; agricultural, rural, and urban runoff; septic tanks; recreational user discharges; and natural processes. These sources introduce chemicals, fertilizers, and fecal wastes which add microorganisms and alter the physicochemistry of impoundments, thereby altering the natural microbial makeup of aquatic systems.

2. Many microorganisms found in runoff, discharges, and impoundments are pathogenic to humans, fish, and wildlife. During the period from 1971 to 1978, 224 waterborne disease outbreaks were reported in the United States, resulting in two deaths and 48,193 illnesses. Of the illnesses reported, 11,435 occurred in 1978 (Craun 1980 and Haley et al. 1980). In most of the outbreaks the waters were contaminated with chemicals or pathogenic microorganisms, with drinking water being epidemiologically implicated as the source of illness. Table 1 lists the etiologic agents identified in outbreaks from 1946 through 1978. All reported waterborne illnesses are not linked to ingestion of contaminated water, and many cases are not reported at all; thus the true number of illnesses due to waterborne pathogens is probably underestimated. Most reported outbreaks occurred during the summer months (Craun 1978).

3. A summary of waterborne infectious diseases which may occur in North American impoundments is given in Table 2. The list includes agents which produce disease from water contact activities (e.g., swimming, boating, fishing) as well as drinking water; it serves as a general guide to potential waterborne disease transmission in reservoirs.

4. The constant potential for outbreaks of waterborne disease from

TABLE 1

Waterborne Illness in the United States

(from Haley et al. 1980, Craun 1978, Center for Disease Control 1977, 1980, Pipes 1978, Craun and McCabe 1973)

	1946-1970		1971-1975		1976		1977		1978*	
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
Gastroenteritis**	178	45,255	63	17,752	25	1,469	19	-†	16	2,014
Salmonella typhi	53	610	4	222	0	0	0	0	0	0
Other *Salmonella*	13	16,730	2	37	1	750	2	-	2	89
Infectious Hepatitis-A	53	1,833	14	368	0	1	0	0	0	0
Shigella	33	7,400	14	2,803	2	2,175	1	-	4	178
Entamoeba hystolytica	5	75	-	-	-	-	-	-	-	-
Enterotoxigenic *Escherichia coli*	4	188	1	1,000	0	0	0	0	1	-
Giardia lamblia	3	176	13	5,136	3	639	4	-	4	5,171
Leptospira	1	9	-	-	-	-	-	-	-	-
Tularemia	2	6	-	-	-	-	-	-	-	-
Naegleria fowleri	-	-	-	-	-	-	-	-	3	3
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	4††	256
Schistosome	-	-	-	-	-	-	-	-	1	30
Poliovirus	1	16	-	-	-	-	-	-	-	-
Campylobacter fetus	0	0	0	0	0	0	0	0	1	3,000
Parvovirus-like agents	-	-	-	-	-	-	-	-	3	937
Chemical poisoning	12	60	12	511	3	35	6	-	2	46

* In 1978, 55% of the outbreaks were of unknown etiology, 11% were caused by *Giardia*, 10% by chemicals, 9% by *Shigella*, 8% by virus, and 5% by *Salmonella*.

** Agent unknown.

†Figures unavailable.

††Two outbreaks similar to *P. aeruginosa*, but not identified.

TABLE 2

Agents of Waterborne Disease
(from Pipes 1978)

Disease	Agent
Bacterial	
Shigellosis	*Shigella* spp.
Diarrhea	Enterotoxigenic *E. coli*
	Campylobacter fetus spp. *jejuni*
Salmonelliosis	*Salmonella* spp.
Yersiniosis	*Yersinia enterocolitica*
Leptospirosis	*Leptospira* spp.
Typhoid fever	*S. typhi*
Tularemia	*Francisella tularensis*
Melioidosis	*Pseudomonas pseudomallei*
Otitis externa	*P. aeruginosa*
Pustular dermatitis	*P. aeruginosa*
Folliculitis (dermatitis)	*P. foliculitis*
Wound infections	*Aeromonas hydrophila*
Legionelliosis*	*Legionella pneumophila*
Viral	
Gastroenteritis	Parvovirus-like agents (e.g. Norwalk)
	Enteroviruses (e.g. Coxsackie A and B, Polio, Echo)
	Unknown
Hepatitis	Hepatitis A
Poliomyletis	Poliovirus
Parasitic	
Amebic dysentery	*Entamoeba hystolytica*
Giardiasis	*Giardia lamblia*
Primary Amebic Meningoencephalitis	*Naegleria fowleri* & *Acanthamoeba*
Ascariosis	*Ascaris lumbricoides*
Trichuriosis	*Trichuris trichura*
Balantidial dysentery	*Balantidium coli*
Coccidiosis	*Isopora* spp.
Swimmer's itch	Schistosomes

*no reported cases of waterborne infection.

water supplies makes routine monitoring for contamination necessary. However, attempting to assess the occurrence of microbial pathogens in aquatic systems is very difficult due to a multitude of factors. Because low levels of pathogens may be present, it is necessary to monitor water supplies using "indicator" organisms. Indicator organisms are easier to identify than pathogens because they occur in higher numbers. The basic assumption of this approach is that the presence of indicator organisms is associated with the presence of pathogens. Criteria (McFeters et al. 1978) for an ideal indicator are as follows:

a. An indicator should be applicable to all types of waters subject to investigation.

b. Microorganisms used as indicators should be present in greater numbers than the pathogen in all cases where the latter is found.

c. Numbers of any indicator microorganism should not increase significantly in the absence of a health hazard.

d. Indicator microorganisms should be more resistant to the physiological stress within aquatic environments, hence exhibit greater survival, than pathogens.

e. Indicator reaction or test data should be unique and characteristic of that microorganism or determination.

f. Indicator methodology should be of minimal complexity, rapid, and inexpensive.

g. Indicator microorganisms should be harmless to man under usual conditions.

h. The indicator or test should be proportional to the health hazard that is present.

5. The traditional indicator group for microbial pathogens has been the coliform bacteria. As a result of the incorporation of coliform standards into Federal criteria and state water quality standards, continued use of coliform tests is required, despite the numerous shortcomings of these tests as indicators of microbial pathogens.

6. Not only are coliform bacteria inadequate as indicators, there are problems monitoring their presence in aquatic systems. Isolated "grab" samples from impoundments frequently result in data on coliform numbers which are essentially meaningless. In recent years many of the problems and shortcomings associated with using coliform bacteria as indicators for microbial pathogens in aquatic systems have been identified and alternatives suggested. These problems, alternate indicators, and appropriate sampling methods for impoundments will be discussed in the following sections.

PART II: FECAL COLIFORMS VERSUS OTHER PATHOGEN INDICATORS

Fecal Coliforms: Advantages and Disadvantages

7. As a result of the widespread presence of "total coliform" bacteria in nature, the use of the coliform group as an indicator is generally discouraged, except in finished drinking water Presence of coliform bacteria in drinking water has greater meaning since it indicates inadequate treatment. Fecal coliforms are those bacteria which are gram negative, asporogenous rods and produce gas from lactose at an incubation temperature of 44.5^{o}C (Dufour 1978). The presence of fecal coliforms in water suggests either animal or human wastes have contaminated the system and their associated pathogens may also be present (McKee and Wolf 1963, Moore 1959). Estimates of the percentages of microbial flora in human feces are listed in Table 3 and the percentages of warmblooded animals excreting enteric pathogens in Table 4.

8. Development of indicator standards, prediction of risk of waterborne disease, and assessment of pathogen levels requires knowledge of an indicator-pathogen relationship. For a given concentration of indicator organisms, there should be a related concentration of pathogens under a known set of conditions. This hypothesis is based on the assumption that there are relatively constant levels of pathogens present in sewage which usually seems to be the case in large municipal sewage systems; but as the number of individuals who contribute to fecal wastes becomes smaller, the indicator-to-pathogen ratio variance increases. So a waste discharge into a lake from a healthy recreational user may be completely free of pathogens, or it may contain a high density of virulent pathogens if the user is infected (Cabelli 1979). Nearly 40 years ago it was suggested that a ratio of 3 to 120 of Salmonella typhi per million coliforms existed for a typhoid rate of 0.01/1000 to 30/1000 population per year (Kehr & Butterfield 1943). If one assumes one person per 100,000 individuals excretes Salmonella typhi, there would be roughly one pathogen per litre of sewage (Pipes 1978). Correlation between fecal coliform (FC) densities and the presence of Salmonella in recreational waters has been reported (Geldreich 1970, Bonde 1977, Smith et al. 1973, Smith and Twedt 1971, Dutka 1973). Geldreich (1970) reported isolating Salmonella in approximately 30% of samples with less than 200 FC/100 mL but in more than 98% when the density was greater

TABLE 3

Estimated Percentages of Microbial Flora in Human Feces

(from Geldreich et al. 1978 and Cabelli 1979)

Agent	Occurrence (% individuals)	Log_{10} Density/gram*
Streptococcus faecalis	26	4-5
Enterococcus	74-76	5-6
Fecal Streptococcus	100	5-6
Mycobacterium	43	0-2
Total coliforms	87-100	7-9
E. coli	87-100	7-9
Intermediate coliform types	0-72	<1-6
Enterobacter/Klebsiella	0-98	<1-9
K. pneumoniae	26-30	6-8
Fecal coliforms	96-100	7-9
Pseudomonas aeruginosa	3-15	3-5
Aeromonas hydrophila	0.2-0.7	-**
Clostridium perfringens	13-35	6-7
C. tetani	1-35	-
Coliphage	-	<1
Poliovirus	0-70	0-7 (PFU)†
Coxsackie virus	0-88	-
Echovirus	0-43	0-8 (PFU)
Adenovirus	0-77	-
Candida albicans	-	<0-4
Giardia lamblia	3-15	-
Entamoeba coli	3-32	-
Endolimax nana	9-16	-
Dientamoeba fragilis	0.2-6	-
Iodamoeba butschlii	1.4-5	-
Trichomonas hominis	0.3-4	-
Chilomastix mesnili	0.4-6	-
Enteromonas hominis	0.1-3	-
Retortomonas intestinalis	0.1-1.3	-

*The number of organisms, in logarithms, per gram of feces.
**Data unavailable.
† Plaque-Forming Units.

TABLE 4

Percentages of Warmblooded North American Animals Excreting Pathogens

(from Geldreich et al. 1978)

Pathogen	Animal	% Excreters in N. America
Salmonella	Human	1.0
	Cattle	13.0
	Sheep	3.7
Shigella	Human	.46
Enterotoxigenic *E. coli*	Human	1.2-15.5
	Pig	9
Enterovirus	Human	0-88
Giardia lamblia	Human	1.5-20
Leptospira	Human	<1-3
	Cattle	2.3
	Mice	33.0
	Dogs	26.6
Entamoeba histolytica	Human	10

than 2000 FC/100 mL. Bonde (1977) reported Salmonella usually occurred at concentrations of E. coli greater than 1000/100 mL. Many times, however, when the actual density of Salmonella is examined, no correlation exists (Cabelli 1976). Several studies have discounted the coliform-pathogen relationship (Smith et al. 1973, Smith and Twedt 1971, Dutka 1973, Gallagher and Spino 1968). Enteric pathogens have been found when low levels of indicator bacteria were present (Dutka 1973, Muller 1964, Colberg et al. 1974, Kraus 1977). Moreover, no studies have shown FC to indicate the presence of fecal pathogens such as Yersinia enterocolitica and Campylobacter fetus spp. jejuni. Y. enterocolitica and other species have been implicated in waterborne disease from waters which have been found to be relatively free from FC (Schieman 1978, Ghirelli and Marker 1977). Small mammals, snails, poultry, and migratory waterfowl have been shown to be reservoirs of Yersinia and Campylobacter and are partially responsible for contaminating water (Botzler et al. 1976, Hacking and Sileo 1974, Kapperud 1975, Luechtefeld et al. 1980, Center for Disease Control 1979a).

9. Fecal coliforms do not serve as indicators of bacterial pathogens which are ubiquitous in aquatic environments. Pseudomonas aeruginosa, Aeromonas hydrophila, and Klebsiella spp. are naturally occurring aquatic bacteria which have been implicated in disease. These organisms usually do not pose a health threat unless present in high numbers, yet they are frequently present in conjunction with fecal pollution (Cabelli 1980b).

Protozoa and Viruses

10. Fecal coliform indicator validity is especially tenuous in predicting health hazards resulting from presence of pathogenic protozoa and viruses. Recent studies have demonstrated the existence of enteric viruses in waters containing low levels of fecal coliforms (Gerba 1980). No normal viral flora exists in humans, and any relationship between indicators and virus density is hampered by the following: (a) only a small percentage of the population may harbor and excrete enteric viruses, (b) intestinal infection and excretion of viruses is transient, (c) most cases are subclinical, (d) excretion of viruses is subject to seasonal variation, (e) no methods are available for the in vitro cultivation and quantitation of many viral pathogens, and (f) techniques for isolation and quantitation of the hundreds of viruses are primitive with poor recovery rates (Pipes 1978). Virus-to-coliform ratios have been established in one study (U.S.

Environmental Protection Agency 1975) as follows:

feces 1:65,000
polluted water 1:50,000
sewage 1:92,000

Bonde (1977) reported higher ratios of E. coli:virus when E. coli densities were greater than 1000 organisms per 100 mL. Large variations in the numbers of coliphage in sewage and in the coliphage:coliform bacteria ratio have been reported (Pretorius 1962). These ratios obviously change with environments and time; furthermore, improved isolation techniques for viruses in recent years have raised doubt about previously reported ratios (Mechalas et al. 1972). Several studies have demonstrated that no consistent virus: coliform ratio exists (Gerba 1980, Goyal et al. 1977, 1979, Berg 1976a, Duma 1980); for example enteroviruses were detected 44% of the time in recreational waters considered acceptable as judged by FC criteria (Gerba 1980).

11. In the South, Naegleria and Acanthamoeba have been found to be ubiquitous in aquatic systems. Relatively high densities have been reported in warm water such as thermal discharges from power plants and hot springs (Duma 1980, Stevens et al. 1977, Wellings 1977, O'Dell 1979). These pathogens have not been associated with fecal pollution; therefore, use of the FC as an indicator of their presence is inaccurate.

12. Another protozoal pathogen, Giardia lamblia, has been reported with increasing frequency as an etiologic agent in waterborne disease (Jakubowski and Hoff 1979). It is found in waters which are relatively free of FC (Craun 1979); moreover, its ability to encyst allows extended survival, thus preventing the use of FC as an indicator (Craun and McCabe 1973, Craun 1976, Rendtorff and Holt 1954, Rendtorff 1954).

Presence of Sediments

13. The relationship of FC densities in the water column to pathogen densities in the sediments is also unclear. Sediment has been shown to harbor much higher numbers of bacteria, viruses, and protozoa, both pathogenic and nonpathogenic (Goyal et al. 1977, Grimes 1975 & 1980, Hendricks 1971, LaBelle et al. 1980, Rittenberg et al. 1958, Van Donsel & Geldreich 1971, Gerba & Goyal 1978, Smith et al. 1978, U.S. Public Health Service 1965, Goyal et al. 1978, Metcalf & Stiles 1968, Slanetz et al. 1965, DeFlora et al. 1975, Matson et al. 1978, LaBelle & Gerba 1979, Farmer 1980, Gerba et al. 1977, Winslow 1976). When large numbers of enteric organisms are present in the sediment,

a relationship exists between their numbers and the degree of contamination of the overlying water (Allen et al. 1953). Nearshore sediments at reservoir swimming areas have shown FC concentrations as high as 48,000/100 cc (Winslow 1976). Van Donsel and Geldreich (1971) reported that a minimum of 150 FC/100 mL in the water was required for Salmonella to be present in the sediment. In their studies, they sampled various streams and lakes and found 100-to 1000-fold more FC in the sediments than in overlying waters. They recovered Salmonella spp. from 46% of their sediment samples, whereas only 8% of the overlying waters contained this pathogen (Van Donsel and Geldreich 1971). Similar ratios of FC in sediment and water were found in the Mississippi River (Grimes 1980). Goyal et al. (1977) reported 47% of the sediment samples compared to 3% of the overlying water samples to be positive for Salmonella spp. Hendricks (1971) recovered 90% more Salmonella spp. from sediments, than from overlying waters. As a consequence of the great variability of sediments, it usually is not possible to establish a correlation between FC levels in the sediment and overlying waters (Winslow 1976).

14. There is no doubt that the present indicator system works to some degree. Fecal coliforms do indicate fecal pollution, and the present acceptable levels are so low that infection by fecal bacterial pathogens is unlikely. However, there are still insufficient data to support a direct, consistent relationship between indicator bacteria, pathogen concentration, and infection. It may be concluded that as indicator density increases there is a deterioration of water quality, but not necessarily an increase in health hazards (Pipes 1978).

Die-Off Rates

15. Because of the tenuous relationship between indicator organisms and pathogens, it may be feasible to develop an indicator index based on survival as a function of exposure time in water. Such an index could relate a numerical survival ratio for a pathogen to an indicator survival value once pathogen survival data for different aquatic environments were known. If infective dose levels were known for each pathogen, then quantitative risk could be assigned to recreational activities. At the present, the data for such a system are inadequate (Mechalas et al. 1972, Andre et al. 1967, Geldreich et al. 1968, Klock 1971, Gordon 1972, Moore 1971, Rudolphs et al. 1950, Gyllenberg et al. 1960).

16. Numerous studies have measured the survival rates of fecal coliform and pathogenic bacteria in water (Carter et al. 1967). Enteric organisms are physiologically adapted to the nutrient-rich environment of warm-blooded animal intestines. When placed in dilute, nutrient-poor, aquatic systems they become stressed and die (Bissonnette 1975). Factors affecting their rates of survival are many; with sunlight, nutrient availability, pH, and temperature, presence of protozoa, phage, and toxins, and other physicochemical factors predominating (Faust et al. 1975, Gameson and Saxon 1967, Van Donsel et al. 1967, Mitchell 1967). Since such a multitude of environmental variables influences survival, die-off varies as much between aquatic systems as do the variables. In general enteric bacteria die in fresh water within three days (Rudolphs 1950). Attempts at determining whether certain types of bacteria such as E. coli survive as long as pathogens such as Salmonella have produced conflicting results. The majority of studies have shown E. coli to be a good indicator because it survives as long or longer than Salmonella spp. (Geldreich 1970, Geldreich et al. 1968, Rudolphs et al. 1950, Mitchell and Starzyk 1975, Orlob 1956), while others have reported the opposite (Gudding and Krogstad 1975, McFeters et al. 1974, Vasconcelos and Swartz 1976). The inconsistency of these findings can be attributed to varied strain characteristics and different methodologies and, more importantly, to unknown environmental variables. One study (McFeters et al. 1974) comparing die-offs of fecal indicator bacteria and enteric pathogens reported that 50% reductions in initial population required an average of 17 hours for coliforms and from 2.4 to 19.2 hours for Salmonella spp. These survival rates are somewhat lower than die-off in marine waters (Chamberlin and Mitchell 1978). Chamberlin and Mitchell (1978) compared numerous freshwater and seawater investigations of coliform survival and calculated average die-off rates, assuming that first order decay followed the relationship known as Chick's law; i.e., $dB/dt = -KB$, where B is the bacterial density at time t, K is the die-off rate, and d is the difference from beginning to end. In fresh water, rates of decrease averaged 0.015 to 0.02/hr, while a rate of 1/hr was found in seawater. Their studies indicated sunlight was the most important factor contributing to die-off. Thornton et al. (1970) measured coliform densities associated with turbidity when storm flows were tracked into a reservoir. The authors attributed die-off to be most closely associated with water temperature. The

model developed predicted relatively well disappearance of coliforms associated with storm flow.

17. Die-off of other pathogens varies considerably from the FC survival. Survival of FC may not be representative of other pathogens because FC and Salmonella have the most rapid die-off of all microorganisms of health significance tested (McFeters and Stuart 1972). Y. enterocolitica, a pathogenic bacterium, survives for long periods in cold waters which are low in nutrients (Shillinger and McFeters 1978). High densities of heterotrophic and coliform bacteria are inhibitory to Yersinia spp. survival, as they are also to survival of Shigella, Leptospira, and enteric viruses (Schieman 1978, Highsmith et al. 1977, Geldreich 1972). Aeromonas hydrophila, P. aeruginosa, L. pneumophila, and N. fowleri are not only pathogens, but are also naturally occurring aquatic organisms with indefinite survival. Giardia, parasitic ova, and enteric viruses survive adverse conditions such as water treatment much better than the coliform indicators (Craun and McCabe 1973, Craun 1976, Rendtorff and Holt 1954, Rendtorff 1954, Berg 1973, Malina 1976, Lin et al. 1971, Mack et al. 1972, Petrilli et al. 1974, Nestor and Coston 1976, Berg 1973). This characteristic of greater persistence and the fact that, in contrast to bacterial pathogens, very low numbers of organisms may cause infection when ingested makes the occurrence of these organisms critical (Pipes 1978, Mechalas et al. 1972, Rendtorff 1979, Center for Disease Control 1979b, Melnick 1976). Viruses may persist for several weeks to months in cold-water environments (Hill et al. 1971, Katzenelson 1978). One study showed survival of viruses was closely related to temperature: fewer than 5 days at 37°C, 2.5 to 9 days at 22-25°C, and 40 to 90 days at 3-5°C (Bitton 1978). These survival periods are much longer than the periods for coliform indicators. Kott (1981) reported that enteroviruses survive twice as long as FC in secondary wastewater; however, E. coli B die-off was similar to that of the viruses. Viruses, like bacteria, vary in their ability to survive in waters, therefore adding another complicating factor in determining water quality (Colwell and Foster 1980, Metcalf 1971).

18. Sediments greatly extend the survival of most microbial organisms of health significance, as indicated by the much higher organism numbers reported (Winslow 1976, Horak 1974). Studies of survival in sediments are few (Van Donsel and Geldreich 1971, Gerba and McLeod 1976, LaBelle and Gerba 1982, LaLiberte and Grimes 1982, Schaub et al. 1974, Roper and Marshall

1978). Van Donsel and Geldreich (1971) reported a 90% die-off in seven days of both FC and Salmonella spp. in various sediments. Studies by the authors comparing survival of S. newport, E. coli, P. aeruginosa, and K. pneumoniae in five different sediments showed E. coli and Salmonella to have comparable die-off rates of 2 to 5 logarithms in 2 weeks, whereas P. aeruginosa and K. pneumoniae decreased only 1 to 2 orders of magnitude. At initial concentrations of 10^8 colony-forming units per milliliter (CFU/mL) such as are found in sewage, these pathogens could survive in the sediments for months. This increased survival partially accounts for the high numbers of indicators and pathogens in sediments.

Other Indicator Organisms

19. Several other bacteria have been proposed as indicator organisms for microbial pathogens. These include E. coli, fecal streptococci, enterococci, total bacteria, C. perfringens, K. pneumoniae, Aeromonas, and Pseudomonas (McFeters et al. 1978, Cabelli 1979).

20. E. coli has been used in many European countries as the primary fecal indicator organism. It has the advantage of being specific for warmblooded animals and is not found in nature as are some fecal coliforms such as Klebsiella, Enterobacter, and Citrobacter. For this reason it serves as a better indicator of recent fecal pollution. In a 3-year epidemiological study, the occurrence of E. coli was shown to correlate better to incidences of waterborne illness than FC, fecal streptococci, total coliforms, Aeromonas, P. aeruginosa, Klebsiella, and Enterobacter-Arthrobacter (Geldreich et al. 1978, Ktsanes et al. 1981, Cabelli et al. 1976).

21. There was originally no scientific justification for using fecal coliforms rather than E. coli as indicators other than that facile (easily accomplished) methods for enumerating E. coli were not available. However, now accurate, facile methods do exist which are specific for E. coli (Cabelli 1979, Dufour et al. 1981).

22. Another widely used indicator has been the fecal streptococci (FS). This includes S. faecalis, S. faecium, S. bovis, S. equinus, and S. avium (Bordner and Winter 1978). However, some species of Streptococcus are capable of reproducing outside of warmblooded animals and are widespread in nature (Mundt 1962a, 1962b), which violates one essential requirement of indicators. When FS information is combined with FC data to provide a

factor known as the FC/FS ratio, sources of contaminants are more clearly identified (Galvani 1974, Geldreich and Kenner 1969). FC/FS ratios of 4 or greater indicate human feces, whereas ratios of less than 0.7 are indicative of animal wastes (Geldreich and Kenner 1969, Geldreich et al. 1980). However, due to differing die-off rates, dilution, and the occurrence of background coliforms levels, these ratios are often useless in determining animal or human origin unless derived from the immediate source within a few hours of pollution (Cabelli 1980b, Geldreich and Kenner 1969). Some studies have shown FC to survive longer than FS, but most report longer survival of FS (Pipes 1978, McFeters et al. 1974, Geldreich et al. 1980). This would cause the FC/FS ratio to decrease with time, thus attributing the pollution to animals. Some lower animals have an intestinal flora with high FC/FS ratios similar to humans, which adds to the problems of using such a ratio (Wheater et al. 1979, Thomas and Levin 1978).

23. Enterococci are a subgroup of the FS and are more specific for human wastes (Bordner and Winter 1978). Enterococci have been observed to survive longer than fecal streptococci (McFeters et al. 1974, Geldreich et al. 1980). They have been reported to be an adequate indicator for organisms that have a longer survival, such as viruses. Studies by Cabelli (1979, 1980b) and Cabelli et al. (1974) of lake beaches have shown numbers of enterococci to correlate highly with waterborne illness and be better indicators than E. coli, FC, or others. Their use as an indicator does not account for possible input of pathogens from animals.

24. Clostridium perfringens has been proposed by some as an indicator organism (Bonde 1963). The occurrence has correlated well with other indicators. Due to its ability to form spores under stressful conditions, it is a good indicator when disinfectants (water treatment) are present and when water samples cannot be analyzed quickly. Its presence in water or sediments does not necessarily indicate recent pollution, due to this same spore-forming characteristic; however, information concerning the occurrence of past pollution is often desireable. On the other hand, for water quality maintenance and enforcement information, it is essential to know if pollution was recent. Two additional factors which limit the usefulness of this organism as an indicator are its requirement of anaerobic conditions for growth and its widespread occurrence in nature (McFeters et al. 1978, Bonde 1963).

25. In recent years, the use of K. pneumoniae as an indicator of sanitary

significance has frequently been challenged. Although it is associated with E. coli in fecal material and is an opportunistic pathogen, it has been isolated in many natural sources and is capable of proliferating in water (McFeters et al 1978, Menon and Bedford 1973, Seidler et al. 1975, Dufour and Cabelli 1976). Its presence as a constituent of the FC population contributes to the shortcomings of FC as an indicator (Cabelli 1980b). It is, however, a good indicator of organic pollution, being found in high numbers in textile, paper, and pulp mills, beet processing, and other wastes (Bordner and Winter 1978).

26. Two other bacteria have recently been proposed as indicators, Aeromonas and P. aeruginosa (McFeters et al. 1978). There has, however, been much controversy concerning their significance as indicator organisms (Colwell and Foster 1980). Both organisms are widespread in nature and are capable of reproducing in water, thus maintaining relatively stable populations (Carson et al. 1973, Fliermans et al. 1977, Nemedi and Layni 1971). A. hydrophila is often a good indicator of nutrient loading (including sewage), thus its densities are associated with water quality and serve as an index of the trophic state of a water body (Cabelli 1980b, Cabelli et al. 1974, Rippey and Cabelli 1980). Other studies have shown good correlations with conductivity, redox potentials, and temperature but question use of this organism as a trophic-level indicator (Colwell and Foster 1980). It has a seasonal distribution, being present in high numbers only during warmer months unless thermal discharges are present (Colwell and Foster 1980, Straskrabova 1974). As with many bacteria, densities in the sediment are elevated and remain relatively stable throughout the year. P. aeruginosa is also frequently present in high numbers in sewage (Wheater et al. 1979, Miescier 1977, Dutka 1979). Cabelli et al. (1976) suggest P. aeruginosa-to-FC ratios greater than 20 indicate the source is not of fecal origin.

27. The use of the yeast, Candida albicans, as an indicator has been gaining acceptance in recent years (Dutka 1979, Buck 1977). It is a known pathogen, and its occurrence is apparently directly related to man's activities. It can be isolated from the mouth, throat, skin, and feces of normal individuals; but it is not a good fecal indicator because only about 18% of the population has C. albicans present in their feces (Cabelli et al. 1976). Survival in fresh waters is relatively longer than most enteric bacteria, often lasting several weeks (Cabelli et al. 1976). Its presence

in uncontaminated water is rare. Beaches with high FC counts produced samples containing C. albicans counts of 20-25/litre, while low FC areas had counts of 0-2/litre (Buck 1977).

28. Two absolute indicators of fecal pollution which have potential for use are numbers of bifidobacteria and the fecal sterol, coprostanol. Bifidobacteria are anaerobic organisms found only at high densities in feces of humans and higher animals and have survival characteristics similar to E. coli. Their use as water quality indicators has been suggested by a few authors, but further verification of enumeration procedures and natural sources is needed (Evison and James 1975, Levin 1977). Coprostanol is a fecal component that has several traits which make it an ideal chemical indicator of fecal pollution; however, it's identification requires complex laboratory methods which prevent its use at present (Colwell and Foster 1980, Smith and Gouron 1969, Dutka et al. 1974, Dutka and El-Shaarawi 1975, Murtaugh and Bunch 1967).

29. Perhaps the most important organisms of sanitary significance are the viruses, yet they are the most difficult to detect. No facile method has been developed for identifying virus indicators, and much disagreement and uncertainty exists regarding proper indicators for viruses of fecal origin (Carson et al. 1973). There is agreement, however, that a significant number of waterborne cases of gastroenteritis are probably caused by enteroviruses (Pipes 1978). Relatively involved isolation methods have implicated viruses directly and indirectly in waterborne outbreaks of illness (Pipes 1978). There has been much discussion on the use of coliphage as a virus indicator (Kott 1981, Colwell and Foster 1980, Leahy et al. 1980). It has been suggested by Kott et al. (1976) that the f2 coliphage was a good indicator for human enteroviruses and this was supported by studies on the frequency of these viruses in sewage treatment. There are several characteristics of coliphage which qualify its use as an indicator: (a) it is prevalent in sewage at ratios to enterovirusus of 10^3:1, (b) it can be enumerated within 24 hrs , (c) it is more resistant to chlorine than the enteroviruses tested, (d) it survives as long or longer than enteroviruses in water, and (e) reliable isolation methods exist which are less expensive than most virus isolation methods (Colwell and Foster 1980). However, coliphage does have the following shortcomings as a fecal indicator:

(a) it is not present at consistent densities in fecal material, (b) it is not associated exclusively with fecal wastes of warmblooded animals, (c) its survival rates compared to many viruses of sanitary significance are not known, and (d) its survival rates vary (Cabelli 1980b, Cabelli et al. 1976).

PART III: STANDARD AND RECOMMENDED METHODS FOR ENUMERATING INDICATORS

30. Numerous methods and materials exist which allow easy enumeration of most microbial indicators and pathogenic organisms. Care must be taken, however, in choosing which methods and materials to use because recovery rates vary substantially depending on the methods and materials and may prevent comparison of data.

Standard Methods

31. The majority of marketed methods and materials dealing with indicators concern FC. Presently, there are two standard methods which predominate: the membrane filtration (MF) and the most-probable-number (MPN) methods (Bordner and Winter 1978).

Problems

32. There are, however, many problems associated with these methods for FC, and these must be recognized. Among the major problems are: (a) occurrence of false positive and false negative results, (b) varying rates of recovery among different brands and batches of membrane filters, (c) effects of turbidity on enumeration by MF, (d) varying recovery rates between MF and MPN, and (e) inability to recover stressed FC.

32. False positives. Some bacteria fit the definition of FC when, in fact, they are not of fecal origin. Some which have been reported to give these false positive results are *Aeromonas*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia* species. This problem is the result of both the crude definition of the FC and the inability of the popular FC isolation media to inhibit nonfecal bacteria. False positives have been noted frequently by investigators attempting to isolate FC from sewage wastewaters, soils, and groundwaters (Bordner and Winter 1978, Neilson 1978, Leahy et al. 1980, Hussong et al. 1981).

33. False negatives. False negatives are normally a greater problem in analyses and may be attributed to numerous factors. Many waters will contain such a high number of bacteria capable of growth on FC isolation media that they inhibit the growth of coliforms (Mundt 1962b). This is especially true in very turbid samples. Bacteria tend to adsorb to suspended particulate matter as a result of charge interactions (Weiss 1951). This causes three problems. First, noncoliform organisms will be associated

with the suspended particulate matter in high numbers and through competition and antagonism will prevent growth of some of the coliforms (Geldreich et al. 1978, Herson 1980). Second, the turbid sample will tend to clog pores of membranes used in the MF procedure, preventing nutrients from the media from reaching the FC and allowing only small quantities of water to be filtered (Bordner and Winter 1978, Sladek et al. 1975). If samples are prefiltered to remove turbidity, significant numbers of FC may be lost (Geldreich et al. 1978). Finally, when counting plates from MF samples, one must assume each colony arose from one FC; however, in turbid samples several FC may adsorb to a particle and grow into one colony, resulting in an underestimation of the true FC density (Bordner and Winter 1978). Use of MPN instead of MF when turbidity is greater than 5 nephlometer turbidity units (NTU) is recommended (Geldreich et al. 1978, LeChevallier et al. 1981) but will not totally alleviate the problems. Since heavy growth of heterotrophic bacteria frequently accompanies turbidity, growth of FC may be suppressed (Center for Disease Control 1980, Geldreich et al. 1978, Herson 1980). The numerous problems associated with estimating FC densities in turbid samples with high noncoliform densities have been described in numerous studies (Bordner and Winter 1978, Herson 1980, LeChevallier 1981).

35. Another significant factor contributing to false negatives is the inability of standard methods to recover stressed or injured FC cells (Bissonnette et al. 1975, Bordner 1977). Since these organisms are physiologically adapted to the warm, nutrient-rich guts of animals, dilute aquatic environments cause FC to be stressed, injured, and eventually killed. Injured cells are of sanitary significance because, if they are ingested, the favorable environment will allow resuscitation and possible pathogenesis. Since standard FC isolation media contain ingredients which are inhibitory to many bacteria and incubation is at 44.5^{o}C, stressed FC cells are frequently unable to grow. Incubation at this high temperature may grossly underestimate levels of _E. coli_ (Dutka et al. 1979); many studies have shown high percentages of FC to be injured and not recoverable by standard methods (Bissonnette et al. 1975, Bordner et al. 1977, Stuart et al. 1977). Preliminary incubation at lower temperatures or in nutrient-rich media allows injured cells to recover and multiply with subsequent incubation in the routine manner. Unstressed freshly isolated FC grow very well at 44.5^{o}C.

36. Effects of different filters and selective media. Another complicating problem is the variability associated with different lots and brands of membrane filters and selective media. A review of these problems was presented in the Environmental Protection Agency's Symposium on the Recovery of Indicator Organisms Employing Membrane Filters (See Bordner et al. 1977). There is a disagreement on whether some of these problems are significant, but the following points should be noted:

a. Dyes used in media to distinguish indicator organisms vary in their content from lot to lot. Some background bacteria which may be present can cause the dyes to fade.

b. Membrane filters vary in their ability to trap and allow subsequent growth of indicator organisms. Many studies have observed that on polycarbonate filters recovery of water quality indicator bacteria is less efficient than on other major brands.

c. Turbid samples clog membrane pores, preventing filtration of adequate quantities of water and preventing growth media from soaking through to achieve contact with the bacteria. Particles on the filter serve as sites of attachment for numerous bacteria which prevent estimation of true densities; these particles also allow background bacteria to overgrow indicator colonies with less intense color development.

d. Use of standard selective media, such as M-FC which contains inhibitory agents, in combination with a high temperature of incubation (44.5°C) will prevent growth of some sublethally stressed indicators. Prior incubation at lower temperatures and/or with nonselective nutrient-rich media produces higher rates of recovery, thus a better picture of the true bacterial density in the water system (Bordner et al. 1977).

Advantages of using the standard methods

37. Numerous studies (Evans et al. 1981, Tobin et al. 1981, Strathman 1979) have compared the two basic techniques used to quantify bacterial/pathogen densities in water, the MPN and MF procedures. Both methods have advantages and shortcomings.

38. Most-probable-number method. The MPN method is more precise when greater numbers of replicate tubes are used. However, the number of tubes in this multistep procedure can quickly become resource-limiting if many water samples are tested. The probability tables used in the tabulation of the bacterial densities are designed to include a positive bias, thus possibly overestimate the true density. The MPN method produces imprecise measurements which in a given test may range from 30 to 289 percent of the absolute value

(Pipes 1978). There are several advantages of the MPN test. It is relatively facile and has been used extensively for many years, which allows comparison with historical data. This method is recommended over the MF procedure when turbidity greater than an NTU of 5 exists (Geldreich et al. 1978, LeChevallier et al. 1981, Evans et al. 1981). A greater recovery rate of bacterial indicators is possible in turbid samples because the lack of prefiltration and clogging of membranes allows all indicators present in the test aliquot to be in direct contact with the isolation media. This is also thought to provide a less stressful environment than the MF (Hufham 1974).

39. Membrane filtration method. Many of the shortcomings of the MF procedure have been previously pointed out (Bordner et al. 1977). However, it does have some characteristics which make it very popular. It is quicker and less resource-intensive than the MPN method. Larger quantities of water may be sampled, provided suspended particulate matter is low. This permits better estimates of indicator/pathogen densities. Bacterial counts obtained from filters are more accurate than MPN estimates, provided that: (a) proper dilution is used, (b) uniform distribution in the sample and on the filter is obtained, (c) the sample does not contain nonrecoverable stressed organisms, and (d) there are no inhibitory substances and microflora present.

New methods of identification

40. Many new methods have been developed in recent years to quantify fecal indicators (Dufour 1981, Bordner and Winter 1978, Mundt 1962b, Stuart et al. 1977). Those dealing with FC which have the greatest potential for being adopted on a widespread basis are simple modifications of the MF and MPN procedures. These recent developments are significant because in many cases they provide adequate solutions to the procedural problems which have been identified. These modifications can be divided into four areas: (a) new selective media, (b) preincubation at lower temperatures, (c) preincubation in nonselective media, (d) subsequent analyses on false-negative samples.

41. Any of the modifications, when used properly, should alleviate some traditional problems encountered with the standard methods. It is desirable to use a method which is simple and consistant on a regular basis so that comparisons between present and historical data can continually be made. The goal of any modification should be to obtain a more accurate

picture of water quality; therefore, successful modifications should be implemented as soon as possible.

Recommended Methods

42. The following methods are modified versions of the standard MF and MPN procedures. These modifications are slightly more involved but allow the investigator to better assess the potential for waterborne disease:

a. MF and MPN tests for fecal coliforms will give increased recoveries of stressed organisms if preincubated at 35°C for 4 hours. When testing chlorinated waters or effluents, preincubated test results should be initially compared to one-step results for possible toxic effects of preincubation. When turbidity and background growth are problems, use of the MPN method with preincubation is recommended. In the presumptive test of the MPN method, when tubes become turbid due to bacterial growth but do not produce gas, transfer a loopful of bacterial suspension to M-FC plates without rosolic acid and streak. Pick some bacterial growth from the M-FC plates after 24 hours at 44.5°C and reinoculate presumptive media. If gas is subsequently produced, transfer a loopful of bacteria from the presumptive tubes to EC confirmatory tubes. This method follows that of Evans et al. (1981), except that it is modified to identify FC rather than total coliforms by streaking M-FC plates, inoculating EC media, and incubating the tubes at 44.5°C.

b. Streamlined MF methods exist for enumerating *E. coli*, enterococci, *P. aeruginosa*, and *A. hydrophila*. They involve filtering known quantities of water and placing the membranes on organism-specific selective media for a few hours with subsequent transfer of the membrane to a medium which confirms the organisms of interest by color reactions. These are explained in detail in the Dufour et al. (1981), Rippey and Cabelli (1979), and Brodsky and Ciebin (1978) references.

43. Methods for collecting water samples are well known (Bordner and Winter 1978). Thorough rinsing of the sampler with sampling-site water is usually adequate for these tests. Cross-contamination is only a problem when sampling of contaminated waters is followed by subsequent sampling of relatively "clean" waters (Kittrell 1969).

44. When sampling sediments in swimming areas, the sample can often be collected by hand by scooping the upper few centimeters into a sterile wide-mouth bottle or plastic bag. Deeper samples may be obtained using a Van Donsel-Geldreich sampler or an Eckman or Ponar dredge (Bordner and Winter 1978). The sediment sample should be kept near 4°C and analyzed within a few hours. Thoroughly mix the sample to ensure homogeneity. Remove one gram or one milliliter of mixed sediment and suspend in 99 mL of sterile phosphate buffer. From this, tenfold dilutions may be made by mixing the sediment-buffer suspension, removing one milliliter, and transferring it to a tube of sterile 9-mL

buffer solution. Three or four dilutions may then be analyzed by the appropriate MF or MPN test to obtain accurate organism counts. Dilution and mixing of sediment samples for analyses is explained in many references, along with other laboratory techniques and quality assurance methods (Bordner and Winter 1978, American Public Health Association 1981).

45. As previously discussed, all present indicators of potential waterborne disease have their own characteristic shortcomings. In order to adequately assess water quality, a combination of several indicators should be used. Choosing which indicators to monitor will require knowledge of several factors: the indicator's growth characteristics; the pathogens it represents; when and where the indicator is likely to occur; its sources; and the physical, biological and chemical characteristics of the water body and its watershed. Simple methods exist for quantification of many indicators and pathogens and should be used when conditions exist for their potential occurrence. These are summarized in Table 5.

TABLE 5

Suggested Indicator and Pathogen Enumeration Methods, by Reference

Agent	Method (Reference)
E. coli	Dufour et al. 1981
Enterococci	Cabelli 1979
Fecal coliform	This reference
Fecal streptococci	Bordner and Winter 1978
Aeromonas hydrophila	Rippey and Cabelli 1979
Pseudomonas aeruginosa	Brodsky and Ciebin 1978
Salmonella spp.	American Public Health Association 1981 or Spino 1966
Klebsiella pneumoniae	Bagley and Seidler 1978
Clostridium perfringens	Cabelli 1979
Candida albicans	Buck 1977
Yersinia enterocolitica	Highsmith et al. 1977
Shigella spp.	American Public Health Association 1981
Giardia lamblia	Jakubowski and Ericksen 1979
Coliphage	Goyal et al. 1980
Naegleria spp.	Wellings et al. 1979
Legionella pneumophila	Orrison et al. 1981
Bifidobacteria	Evison and James 1975
Coprostanol	Dutka et al. 1974
Leptospira	American Public Health Association 1981

PART IV: DETERMINATION AND DETECTION OF CONTAMINATION

Exposure

46. The actual risk of contracting a waterborne illness from contaminated waters is uncertain. About all that can be said with certainty is that the risk is very low (Pipes 1978, Cabelli 1979, Moore 1975). The current microbiological criteria and standards for waters are derived from weak epidemiological studies (Cabelli 1980b). No studies have been conducted which can substantiate the current standards. Only recently were epidemiological studies completed which did show significant relationships between rates of waterborne illness and levels of E. coli and enterococci in lake swimming areas (Cabelli 1979, 1980a, Cabelli et al. 1979). An attack rate for gastroenteritis of about 1% was associated with E. coli or enterococci densities of approximately 10/100 mL. This indicates that very low densities of pathogens can cause infection, contradicting other studies which have reported infective dose requirements to be as high as 10^{11} cells (Bonde 1981, Hornick et al. 1970). However infective doses range over several orders of magnitude for enteric bacteria. This is not surprising, since virulence in an organism can change easily and be lost quickly outside an ideal environment, particularly in a stressful aquatic environment. Some studies have suggested that one virion may cause infection; however, these studies did not simulate normal waterborne pathogen ingestion (Mechalas et al. 1972). A standard of 1 virus per 10 gallons of recreational water has been proposed, but this standard lacks epidemiologic foundation (Melnick 1976). Due to the greater resistance and survival of viruses relative to FC, the probability of a viral infection increases more rapidly than does the risk from Salmonella (Mechalas et al. 1972).

47. Eye, ear, nose, and throat ailments represent more than half of all the illnesses recorded among swimmers, gastrointestinal disturbances up to 20 percent, and skin irritation the remainder (Stevenson 1953, Hendry and Toth 1982). The following list gives the more significant waterborne diseases with a brief description of when they may occur.

a. The incidence and cause of gastroenteritis is largely unknown. Many cases in recent years have been attributed to viruses. The source of the contaminant is probably human; however, FC levels quite often are low. The incidence of gastroenteritis peaks in the

summer as do most other waterborne outbreaks (Craun et al. 1978).

b. Infections of the skin and eyes may be caused by Aeromonas hydrophila, P. aeruginosa, and schistosome species. As mentioned previously, these organisms are not always present in conjunction with high FC levels. Aeromonas may be present at high densities in warm waters which are relatively nutrient-rich (Craun 1978, Rippey and Cabelli 1979). Temperature appears to be the most important factor in mesotrophic waters (Colwell and Foster 1980, Straskrabova 1974). Particularly high densities have been noted in thermal discharge waters (Fliermans et al. 1977). P. aeruginosa has been associated with high nutrient levels; however this organism is also found in oligotrophic waters (Carson et al. 1973, Nemedi and Layni 1971). Various species of snail-transmitted bird schistosomes produce dermatitis, including human "swimmer's itch," in recreational waters. Infections due to A. hydrophila and P. aeruginosa have been increasing in occurrence in recent years, and detection of these organisms should be part of any monitoring program in recreational waters (Cabelli et al. 1976).

c. Diarrhea associated with waterborne infections may be caused by several organisms including: Salmonella, Shigella, Yersinia, Campylobacter, viruses, and Giardia. Viruses probably predominate as etiologic agents, with several types being implicated. As noted, their detection is relatively complicated and their incidence of occurrence uncertain. Yersinia, Campylobacter and Giardia are the dominating causes of enteric infections in some areas (Center for Disease Control 1979a, Pai et al. 1979, Sands et al. 1981). Yersinia enterocolitica is particularly common in Europe and Canada, with increasing isolations across the United States (Bottone 1977). Small animals, rodents, and pigs have been shown to be possible reservoirs of Yersinia (Kapperud 1975, Toma and Diedrick 1975, Kaneko and Hashimoto 1981). Infections have occurred from waters containing low FC levels. The extended time required for the more successful Yersinia enumeration methods precludes most laboratories from including it in routine isolations, so the incidence of this organism may be underestimated. Likewise, Campylobacter fetus is on the increase, with cattle, pigs, poultry and migratory waterfowl known as possible reservoirs of this organism (Luechtefeld et al. 1980, Center for Disease Control 1979a). The true incidence is probably also underestimated because of the failure to include it in routine methods. Giardia has received widespread attention in recent years as an etiologic agent: infection rates as high as 16% have been observed in some states (Center for Disease Control 1976b). Most backpackers are aware that the "clean, pure" mountain water is often contaminated with Giardia due to infestation from beavers and other animals. Giardia's ability to encyst enables it to survive long periods and disinfection treatments (Craun and McCabe 1973, Craun 1976, Rendtorff and Holt 1954, Rendtorff 1954, Berg 1973a, Malina 1976, Lin et al. 1971, Mack et al. 1972, Petrilli et al. 1974, Nestor and Coston 1976, Berg 1973b). Prolonged diarrhea typical of Giardia is often mistaken

as being caused by Campylobacter or rotavirus.

d. Two protozoa, Naegleria and Acanthamoeba, which may produce death upon infection have been recently found to be widespread in natural waters (Wellings et al. 1977, 1979, O'Dell 1977). Fortunately, the incidence of infection is extremely low. Cases of Primary Amoebic Meningoencephalitis (PAME) due to Naegleria have been higher in recent years, but this may have been due to warmer weather which increased both water temperatures and swimming (Duma 1980). The organism thrives in warmer waters, overwintering in the sediments (Duma 1980, Wellings et al. 1977, Stevens et al. 1977). Isolation methods are presently too complex for Naegleria and Acanthamoeba to be included in routine monitoring programs (Duma 1980, Wellings et al. 1979, O'Dell 1977).

e. Concern over Legionella pneumophila has increased in recent years with the realization that it is widespread and a cause of numerous illnesses and deaths (Fraser and McDade 1979, Fliermans et al. 1981). It has been shown to be ubiquitous, occurring in waters of many types (Fliermans et al. 1979, 1981). Since it has not been implicated in waterborne disease outbreaks and requires involved isolation techniques, routine monitoring is impractical.

Sampling Problems

48. Problems associated with attempting to assess water quality, in particular the microbiological quality, of an impoundment reservoir or lake are many and complicated. Stream monitoring is somewhat easier because at times flows are stable and areas are well mixed. However, the hydrology of an impoundment is very complex, with varying components such as currents, mixing, and retention time.

49. In order to get a clearer picture of the microbiological status of impoundments, thus the potential for waterborne disease, a strategic sampling program must be established. Data collected by this program, when combined with knowledge of watershed, water quality, and hydrologic characteristics of the impoundment, will allow for well-informed management of recreational areas and decreased transmission of waterborne disease.

50. The hydrology of an impoundment is perhaps the most critical factor in the determination of microbiological quality. Generally, impoundment of streams results in improved bacteriological quality because more self-purification occurs through increased hydraulic retention time of the waters (Geldreich and Kenner 1969). Since the microbes of concern are planktonic, they move with the currents and settle with sediments. When human and

animal wastes are discharged, they remain near shore at high densities unless mixed by wind, water turbulence, stream inflows, or destratification (Geldreich and Kenner 1969, Thornton et al. 1980, McFeters and Stuart 1972, Schillinger and McFeters 1978). Stratification dynamics play a key role in bacterial distribution (Asthana and Burdick 1972, Lighthart 1975, Menon et al. 1971, Drury and Gearheart 1975). In nearshore shallow areas, vertical distribution is fairly uniform due to mixing and a uniform temperature (Geldreich and Kenner 1969). But during the summer, the remaining reservoir usually undergoes thermal stratification into three layers, which results in current restriction and inhibits nutrient transfer and bacterial distribution (Asthana and Burdick 1972, Menon et al. 1971, Drury and Gearheart 1975, Wetzel 1975). Bacteria in the water column will predominate in the layers above the thermocline where biological productivity predominates (Geldreich and Kenner 1969, Drury and Gearheart 1975, Wetzel 1975, Weiss and Oglesby 1960, Collins 1963). Sedimentation of clay, silt, and organic matter results in higher levels of bacteria and viruses at the sediment-water interface. This is a result of the tendency of the organisms to attach to particles with densities approximating 3,000 to 15,000 organisms per mL (Tsernoglou and Anthony 1971). As noted earlier, the sediments provide protection, nutrients, and extended survival to high concentrations of microorganisms. In fall and winter, cold water flows into the reservoir causing destratification (overturn) which results in relatively complete mixing, therefore a more uniform dispersion of bacteria, until warmer weather returns. When this mixing occurs, nutrients and microbes trapped in the hypolimnion circulate and a temporary deterioration in water quality may occur, including increased bacterial levels (Geldreich et al. 1980).

51. During stratification most mixing is horizontal, with slight vertical currents existing. Naturally the currents are greater in the old river channel and in open waters and less in coves and isolated portions of the impoundments (Wetzel 1975). Knowledge of these currents aids in determining the ability of areas to dilute contamination.

52. Water quality in reservoirs directly interacts with hydrologic and watershed characteristics to affect the microbiological status of the system. During summer stratification, runoff from the watershed and feeder

streams tends to remain in the upper layers of the reservoir. These inflows may contain organic matter, nutrients, animal and human wastes, and other contaminants, so increased densities of microorganisms may occur above the thermocline (Geldreich et al. 1980, Wetzel 1975, Weiss and Oglesby 1960, Collins 1963).

53. Survival of indicator pathogen organisms has been observed to decrease near the surface due to the detrimental effects of ultraviolet light. Geldreich et al. (1980) showed greatest survival was at 0.9 m below the surface. Less penetration of ultraviolet light and cooler temperatures at this level probably account for this occurrence (Geldreich et al. 1980, McCambridge and McMeekin 1981). Extracellular products which are excreted by algae also promote growth and survival of bacteria. Therefore, algal blooms may allow bacterial densities to reach high levels in the absence of significant fecal pollution (McFeters et al. 1978).

54. Low dissolved oxygen levels in the hypolimnion have not been shown to affect survival of enteric organisms (Geldreich et al. 1980). Anoxic conditions will allow increased transfer from the sediment of inorganic species which cause numerous water quality problems at overturn periods. These problems include hydrogen sulfide release, high concentrations of reduced iron and manganese, fish kills, and increased bacterial levels (Wetzel 1975).

55. Storm events have been shown to play a major role in the water quality of impoundments (Nix et al. 1975). Increased flows from feeder streams carry the majority of the annual supply of nutrients during storm events (Nix et al. 1975). Also associated with increased flow and turbidity are high levels of indicator organisms. Indicator densities in swimming areas have been shown to increase dramatically to unsafe levels following rainfall as a result of high concentrations in runoff (Horak 1974, Hendry and Toth 1982). Sources of these fecal wastes and pathogens can be farm animals, wildlife, pets in urban and recreational areas, inadequate waste treatment systems, and septic tanks. Increased densities of enteric organisms in feeder streams and storm runoff have been observed in many studies (Thornton et al. 1980, Geldreich et al. 1980, Hendry and Toth 1982). Their dramatic increase during a storm is a function of many factors such as watershed area, land use, and duration and intensity of the rainfall.

The FC generally exhibit the "first-flush" phenomenon (Davis et al. 1977); i.e., like many nutrients and chemical species they are "flushed" through the stream during the initial increased storm flow. In large reservoirs, high FC densities in the streams are diluted out once the flow reaches the impoundment, with sedimentation, dispersion, and die-off predominating. (Thornton et al. 1980, Geldreich et al. 1980, Coutant and Shapple 1966, Churchill 1958, Powell and Berthouex 1967). Storm flows enter reservoirs in turbid plumes, proceeding through reservoirs as overflows, interflows, or underflows, depending on relative densities of the river water and the reservoir water. Good correlations for decreasing bacterial densities versus time have been shown from models which utilize water temperature, level of turbidity, the speed at which a turbid storm plume proceeds through the reservoir, and the distance it covers (Thornton et al. 1980).

56. Sediments are perhaps the most important yet most underutilized source of information on microbiological quality of impoundments. As previously pointed out, densities of indicator organisms and pathogens in sediments are often several orders of magnitude higher than in overlying waters and remain relatively stable over time, unlike microbial water densities. Microbial sediment densities are dependent on a multitude of variables; nearby sources of contamination are undoubtedly the most important. Clays and silts of smaller grain sizes usually have more organic matter associated with them as well as increased surface area and physical protection,which allow microorganisms much longer survival in clays and silts than in sandy bottoms (Gerba and McLeod 1976, Weiss 1951). Hydrology and recreational use determine the health significance of contaminated sediments. In areas where sediments are resuspended due to runoff, boats, swimming, wading, and water turbulence, high densities of organisms can be recirculated into the water column (Grimes 1975, 1980, Matson et al. 1978, LaLiberte & Grimes 1982). Sediments in areas where there are currents, or which are away from shore or free from the influence of contaminated runoff or discharges, seldom support high densities of organisms in overlying waters.

Detection of Contamination

57. Well-informed management strategies with respect to microbio-

logical water quality are possible with a well-planned monitoring system. Water densities of enteric organisms will fluctuate with the seasons. As pointed out earlier, stratification, agriculture, recreational uses, and rainfall patterns change with the seasons and should determine sampling frequencies and stations.

58. Sampling must be geared toward potential problem areas, from both contamination and human contact aspects. Taking one "grab" sample from an area of concern usually is insufficient and provides relatively meaningless results (Pipes 1978) because the distribution of enteric organisms through the water varies significantly, both horizontally and vertically (Pipes 1978, Thornton et al. 1980, Wetzel 1975, Palmer et al. 1976). Variations in densities as high as three orders of magnitude over a few centimeters have been demonstrated (Thornton et al. 1980). When sample collection is not being conducted in shallow areas, vertical samples should be collected down to the thermocline. In shallow areas, emphasis should be placed on increased numbers of horizontal samples and sediment samples. In the initial phase of a monitoring system, potential contamination and use areas should be sampled through one year to identify sites of importance as they relate to seasonal changes.

59. Sample sites should include the mouths of feeder streams; point source discharges; areas subject to agricultural, urban, and recreational runoff; drinking water supply intakes; and swimming areas. Also during initial phases of monitoring, storm events should be monitored and tracked with storm-flow plume samples taken through the reservoir to determine the degree of contamination and the areas affected by feeder streams. Swimming areas must also be sampled during and immediately after periods of rain to assess runoff effects.

60. Once a thorough survey is complete and significant sources are pinpointed, a reduced sampling program is practical. Unless the reservoir is also a drinking water supply, concentrated microbiological sampling is only necessary during periods when there is primary contact by users, usually from late spring to early fall.

61. The confidence one can place on monitoring data depends on the degree of known variability associated with the results. Typical bacteriological data are erratic, fluctuating several orders of magnitude over

small areas, between replicates, and over short time periods (Pipes 1978, Horak 1974). Patchiness of organism distribution and fluctuating environmental variables in many areas means that confidence in data values will require replicate samples at high frequencies. Limited resources, however, often preclude this. Nonetheless, confidence intervals should be determined for the data. If the interval is unacceptably large, increased replicate numbers or sampling frequencies can be initiated to increase data confidence. For a 90% confidence coefficient, a confidence interval is calculated as:

$$\overline{X} \pm \frac{(1.645)\ \sigma}{\sqrt{n}}$$

where $\overline{X}$ is the mean of the data, σ is the standard deviation, and n is the number of samples (Ward and Nielsen 1978). To get equal precision at all stations, sample numbers at each station can be increased or decreased accordingly:

$$n_i = \frac{\sigma^2_i}{\sum_1^n \sigma^2_i} (N)$$

where n_i is the sample number to be taken at the station i of interest, σ^2 is the variance at station i, and N is the total number of samples to be allocated (Ward and Nielsen 1978).

62. After an adequate data base of microbiological levels has been collected for an impoundment, it may be possible to assess the extent of spatial and temporal fluctuations at fewer sampling stations and at less frequent sampling intervals. This would permit more replicates to be taken at each sampling, allowing increased data confidence.

PART V: CONCLUSIONS AND GENERAL RECOMMENDATIONS

63. Water quality conditions can be predicted for future impoundments by preimpoundment studies. Potential problems involving microbial pathogens may be detected by surveying the preimpoundment watershed area for manmade and natural factors which might promote problems in an impoundment.

64. Factors which should be assessed relating to man include surveys of population distribution; location of septic tanks; amount of urban runoff; municipal and industrial discharges; and agricultural runoff including fertilizers, pesticides, herbicides, and animal wastes. Since many of these factors and activities will affect streams in the preimpoundment area, streams should be monitored for FC, *E. coli*, and enterococci. If high densities of these indicators are encountered, further surveys for the incidence of pathogens should be conducted. If human wastes are the predominant source of contamination, monitoring of *Salmonella*, coliphage, *Campylobacter fetus*, and *Giardia* should be considered. Where agricultural wastes predominate, there will be a possibility of *Salmonella*, *Leptospira*, *Campylobacter fetus*, and parasitic ova occurring.

65. Interrelated with and in addition to manmade factors of contamination are the natural characteristics of the preimpoundment watershed. A survey of physical, chemical, and biological characteristics should include the following: soil types; drainage; amount and extent of vegetation; yearly rainfall; water demand of area; predicted retention time of impoundment; air temperature averages; and animal population including types, numbers, pathogen and parasite potential, and occurrence of parasite vectors. Stream flow will dramatically affect microbial densities and should be a factor in determining sample frequencies.

66. When impoundment studies of streams implicate wildlife as being a significant source of fecal contamination, microbial monitoring in streams should be suitably adjusted. Pathogens which possibly could occur are *Salmonella*, *Yersinia*, *Campylobacter fetus*, *Francisella tularensis*, *Giardia lamblia*, schistosomes and parasitic ova.

67. The physical and chemical characteristics of the preimpoundment area will determine if the presence of microbial pathogens will be a

significant problem. Watersheds in which erosion is a problem will likely lead to waters of high turbidity, thus high bacterial numbers. Soils of large clay and silt fractions will promote pathogen survival, but in early impoundment stages they could potentially cause numerous adverse water quality conditions such as high biological oxygen demand, excessive nutrient release, and hydrogen sulfide production. If the impoundment will be of significant depth, stratification may occur which decreases mixing of the water column, thus limiting natural purification. All of the significant natural and manmade factors which relate to the future water quality of the impoundment must be determined to accurately predict microbial problems.

68. A key factor in the potential for waterborne disease transmission from enteric organisms is the siting of swimming areas. Beaches which are established in areas where water currents are prevalent will allow fecal pollution to be flushed out of the swimming area and diluted to safe levels. Areas with active currents can be located by tracer dye studies. Coves and sheltered areas tend to lack adequate circulation, and enteric organisms may reach dangerous levels in the water and sediments. Boat ramps should be located well away from swimming areas because the water turbulence from boats can resuspend sediments and pathogens into the water column (Horak 1974). Swimming areas should have predominantly sandy bottoms. Enteric bacteria and viruses do not survive nearly so long in sand and, therefore, do not concentrate as they may do in sediments of silt and clay. Bacterial counts from sands can be several orders of magnitude less than equal volumes of fine-grained sediments.

69. Preliminary studies should reveal the level of contamination from feeder streams and point and nonpoint source discharges. Beaches should be placed an adequate distance from critical runoff areas and feeder streams so that even during storm events the storm plumes do not carry contaminated water into the beach areas. If studies reveal elevated indicator levels in swimming areas following periods of rain, the beaches should be temporarily closed to allow for die-off dilution of the organisms. Because of the known relationship between artificially warmed water (e.g. power plants' discharge plumes) and the frequency of certain pathogens (e.g. Naegleria and Aeromonas), swimming areas and boat ramps should not be permitted near thermal discharges. Water criteria for standards do not exist for indicator

bacteria in sediments as they do for bacteria in water. This shortcoming, however, does not negate the need for sediment monitoring, for the reasons previously mentioned. High levels of indicator bacteria in the sediment should be regarded as indicators that their resuspension into the water column could violate water quality standards or increase the possibility of waterborne disease outbreaks.

70. Knowing which organisms to monitor is a difficult problem to solve without thorough studies of each impoundment. Each water system has unique water quality and watershed characteristics which permit varying growth and survival rates for different microorganisms. Testing should include regular monitoring during high-use periods, employing simple, consistent, and reproducible enumeration methods for FC, *E. coli*, and enterococci. During the summer, *P. aeruginosa* and *A. hydrophila* should also be monitored as often as resources allow. Densities of these organisms are relatively constant, hence require less sampling. Sampling in swimming areas should always include sediment in addition to water.

71. Establishment of a monitoring system which is geared toward critical areas and critical periods, such as those of intense recreational use and storm events, can prevent unnecessary sampling. Using suggested sampling methods, frequencies, indicators, and isolation procedures will provide data which are meaningful and useful. This will permit sound reservoir management which will greatly reduce any risks of waterborne disease outbreaks.

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