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Tropical Source Water

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Over 2 billion people, or half of the world's population, have suffered from diseases due to drinking polluted waters (Barabas, 1986). More than 250 million new cases of waterborne disease are reported each year, resulting in more than 10 million deaths and nearly 75% of these waterborne disease cases occur in tropical areas. Indeed, nearly 50% of diarrheal disease deaths (4.6 million) occur in children under 5 years of age, living in the tropics (Snyder and Merson, 1982; Bockemühl, 1985). Many investigators and government administrators assume that the high morbidity and mortality rates simply indicate the level of contamination of water in tropical areas. However, determining biological contamination in tropical source water is much more difficult than most regulatory agencies perceive. Yet, the need to accurately determine the level of biological contamination is much greater in tropical areas than it is in temperate areas since these regions have a much greater number of waterborne diseases (Table 2.1 and 2.2). In turn, these diseases are exacerbated by the lack of adequate sewage treatment and a greater reliance on untreated (and possibly contaminated) waters as drinking water sources (Feachem, 1977; Esrey et al., 1985).

Source water quality in most tropical areas differs from that of temperate areas in three major ways: 1) physical and chemical; 2) biological; and 3) social and economic. We will examine each one of these categories, compare the standard microbial indicators in tropical source waters, and then look at techniques which may be more applicable to the tropics.

2.1 Physical and Chemical Characteristics of Tropical Waters

The physical and chemical differences between temperate and tropical source water are perhaps the most obvious, yet are quite often the most overlooked. Water temperatures in tropical areas are higher than those in temperate areas.

Table 2.1 Waterborne infectious bacteria

Organism	Disease	Infectious dose
Acinetobacter calcoaceticus	Nosocomial infections	?
Aeromonas hydrophila[a]	Enteritis, wounds	?
A. sobria[a]	Enteritis, wounds	?
A. caviae[a]	Enteritis, wounds	?
Campylobacter jejuni[a]	Enteritis	?
C. coli[a]	Enteritis	?
Chromobacterium violaceum[a]	Enteritis	?
Citrobacter spp.[a]	Nosocomial infections	?
Clostridum perfringens type C[a]	Enteritis	?
Enterobacter spp.[a]	Nosocomial	?
Escherichia coli serotypes[a]	Enteritis	$>10^6$ CFU[b]
Flavobacterium meningosepticum	Nosocomial, meningitis	?
Francisella tularensis	Tularemia	10 CFU
Fusobacterium necrophorum	Liver abscesses	10^6 CFU
Klebsiella pneumoniae[a]	Nocosomial, pneumonia	?
Leptospira icterohaemorrhagia[a]	Leptospirosis	?
Legionella pneumophila[a]	Legionellosis	>10 CFU
Morganella morganii[a]	Urethritis, nosocomial	?
Mycobacterium tuberculosis[a]	Tuberculosis	?
M. marinum	Granuloma	?
Plesiomonas shigelloides[a]	Enteritis	?
Pseudomonas pseudomallei	meliodosis	?
Salmonella enteritidis[a]	Enteritis	$>10^6$ CFU
S. montevideo B[a]	Salmonellosis	?
S. paratyphi A&B[a]	Paratyphoid fever	?
S. typhi[a]	Typhoid fever	10^5 CFU
S. typhimurium[a]	Salmonellosis	$>10^5$ CFU
Serratia marcescens[a]	Nosocomial	?
Shigella dysenteriae[a]	Dysentery	?
Staphylococcus aureus[a]	Wounds, food poisoning	?
Vibrio alginolyticus[a]	Wounds	?
V. cholerae[a]	Cholera dysentery	10^3 CFU
V. fluvialis[a]	Enteritis	?
V. mimicus[a]	Enteritis	?
V. parahaemolyticus[a]	Enteritis	10^5 CFU
V. vulnificus[a]	Wound infections	?
Yersinia enterocolitica	Enteritis	10 CFU

[a] Found in the tropics.
[b] CFU, colony forming units (see Hazen et al., 1987; Dufour, 1986; Hutchinson and Ridgway, 1977; Hawkins et al., 1985).

Table 2.2 Other waterborne pathogens

Organism	Disease	Infectious dose
Viruses		
Adenovirus[a]	Enteritis, pharyngitis	1–10 PFU[c]
Calicivirus[a]	Enteritis	1–10 PFU
Norwalk virus[a]	Enteritis	1–10 PFU
Coronavirus	Enteritis	1–10 PFU
Coxsackievirus A & B	Meningitis	1–10 PFU
Echo virus	Enteritis, Meningitis	1–10 PFU
Hepatitis A virus[a]	Hepatitis	1–10 PFU
Poliovirus[a]	Poliomyelitis	1–10 PFU
Rotavirus[a]	Enteritis	1–10 PFU
Astrovirus	Enteritis	1–10 PFU
Cyanobacteria		
Cylindrospermopsis spp.[b]	Hepatoenteritis	Bloom
Fungi		
Candida spp.[a]	Candidiasis	?
Rhinosporidium seeberi[b]	Rhinosporidiosis	?
Protozoa		
Balantidium coli[a]	Balantidiasis	?
Cryptosporidium spp.[a]	Cryptosporidiosis	?
Giardia lamblia[a]	Giardiasis	1 cyst
Entamoeba histolytica[a]	Dysentery	1 cyst
Naegleria fowleri[a]	Meningoencephalitis	?
Acanthamoeba spp.[a]	Meningoencephalitis	?
Helminths		
Schistosoma mansoni[b]	Schistosomiasis	1 cercariae
S. haemotobium[b]	Schistosomiasis	1 cercariae
S. japonicum[b]	Schistosomiasis	1 cercariae
S. intercalatum[b]	Schistosomiasis	1 cercariae
S. mekongi[b]	Schistosomiasis	1 cercariae
Fasciola hepatica[a]	Fascioliasis	1 metacercariae
Fasciolopsis buski[b]	Fasciolopsiasis	1 metacercariae
Paragonimus westermani[a]	Paragonimiasis	1 metacercaria
Clonorchis sinensis[a]	Chinese liver fluke	1 metacercaria
Diphyllobothrium latum	Pernicious anaemia	1 pleurocercoid
Dracunculus mediensis[a]	Guinea worm	1 larvae
Ascaris lumbricoides[a]	Ascariasis	1 larvae

[a] Found in the tropics.
[b] Found exclusively in the tropics.
[c] PFU, plaque forming units (see Hazen et al., 1987; Dufour, 1986; Hutchinson and Ridgway, 1977; Hawkins et al., 1985).

Water temperature rarely gets below 15°C in the tropics and may be as high as 45°C in some areas, while temperate waters range from freezing to 20°C, and under rare instances may reach 30°C (Hill and Rai, 1982). Temperature differences over the course of the year are also much greater in temperate areas than in the tropics. In Puerto Rico, water temperatures in the rain forest are always between 18° to 24°C (Carrillo et al., 1985; López et al., 1987). Inland lakes and coastal waters in South America range from 23° to 33°C (Hill and Rai, 1982; Hagler and Mendonça-Hagler, 1981). In West Africa, the annual range in water temperature is 25° to 29°C (Wright, 1986), and in East Africa the annual range in river water temperatures is 23° to 34°C (Oluwande et al., 1983). Singapore water temperatures only vary from 28° to 30°C (Jen and Bell, 1982). Even in subtropical areas like Florida, water temperatures range from 8°C during the winter to more than 30°C during the summer (Tamplin et al., 1982). The effects that these differences in temperature regimes may have upon bacterial communities may be profound. Jen and Bell (1982) found that in tropical waters, significantly greater counts of bacteria were obtained when media were incubated at 30°C for 72 h. In contrast, Holden (1970) found that 22°C for 72 h gave the highest counts in temperate waters. Hill and Rai (1982) observed that bacterial activity in central Amazonian lakes were almost entirely due to mesophiles, whereas temperate lakes had a mixture of both psychrotrophs and mesotrophs.

Thermal stratification of reservoirs and lakes is also affected by seasonal temperature changes. Nearly all lentic habitats in the tropics are oligomictic, i.e. permanently stratified (Hutchinson, 1975). Because of the small variations in surface temperatures, the relative thermal distance to mixing is nearly 3 times greater in tropical lakes as compared to temperate lakes (Hill and Rai, 1982). Since the solubility of oxygen is inversely related to temperature, tropical waters also have less dissolved oxygen at saturation and become anoxic faster than temperate areas. Tropical lakes and rivers typically have large diurnal variations in dissolved oxygen concentrations. Rivers in Nigeria were observed to change from 0.5 to 7.5 mg l^{-1} dissolved oxygen in less than 12 hours (Oluwande et al., 1983). In Puerto Rico, dissolved oxygen concentrations also varied from 1 to 8 mg l^{-1} on a diurnal basis (López et al., 1987; Carrillo et al., 1985; Pérez-Rosas and Hazen, 1988). These large changes in dissolved oxygen mean that few organisms other than facultatively anaerobic bacteria can thrive. Indeed, Carrillo et al. (1985) found higher densities of anaerobic bacteria in a pristine tropical stream than did Daily et al. (1981) on the Anacostia River, Washington, D.C., a grossly polluted, temperate river. Unfortunately, few other studies have looked at anaerobes in tropical waters. The higher average temperature of the tropics and the more constant temperature during the year are going to have profound effects on the flora and fauna found in tropical waters, including the microbial community and allochthonous species, for example, pathogens and their indicators.

Annual variations in light intensity are much smaller in the tropics (Hill and Rai, 1982). It has been well documented that bacteria vary greatly in their

sensitivity to solar radiation, and some bacteria can be killed at high natural light intensities (McCambridge and McMeekin, 1981; Evison, 1988). Fujioka and Navikawa (1982) showed that the natural solar radiation in Hawaii could significantly reduce the density of coliforms and other indicators of biological pollution as well as cause injury in tropical source waters. Thus, the higher solar radiation of tropical areas could cause a significant underestimation of indicator bacteria in tropical source waters, as well as in areas receiving large amounts of solar radiation.

The constant high temperature and light intensity of tropical surface waters cause them to be in a state of hypereutrophy all year long. In temperate areas, light is quite often limiting to photosynthetic organisms during the winter, thus lowering productivity of natural communities and uptake of nutrients. In the tropics, light intensity changes vary little, with many areas having 12 h of darkness and daylight all year long. Light is usually only limiting in the tropics under dense rain forest canopies (Carrillo et al., 1985). In tropical source waters, concentrations of organics are high, while free forms of nutrients like phosphorus and nitrogen are low (Hill and Rai, 1982). More than 90% of nutrients like phosphorus and nitrogen may be tied up in the standing biomass at any given moment in tropical waters, leading to deficiencies if there are no significant allochthonous inputs (Hill and Rai, 1982). In contrast, temperate lakes vary widely in productivity during the year, owing to large changes in light intensity and temperature. In terms of nutrients and other physical chemical parameters, tropical waters are most similar to temperate waters during the late summer in eutrophic areas of the lower temperate latitudes. At this time, hypolimnetic waters are anoxic, temperatures are high, and free nutrient concentrations are low, even though standing biomass is high. Thus tropical source waters have higher densities of autochthonous bacteria than temperate waters. These higher densities of naturally occurring bacteria can cause severe limitations to assays which rely upon viable counts. This can be seen in the much greater tendency of allochthonous bacteria to overgrow standard media in tropical source waters (López et al., 1987; Santiago-Mercado and Hazen, 1987). In addition, several studies have shown that nonindicator background bacteria can produce inhibitory substances (bacteriocins) that affect the growth of indicator bacteria like *E. coli* (Means and Olson, 1981). High densities of other bacteria may also affect the differentiation of colonies on some media, for example, sheen of coliform colonies has been shown to be inhibited by high densities of nontarget bacteria (Burlingame et al., 1984). Thus, factors that influence community composition and trophic state can have a major effect on the survival and growth of indicator bacteria in tropical source water.

Water fluxes in wet tropical areas are much greater than in temperate areas. Rainfall in many tropical areas is catastrophic, with some watersheds receiving more than 61 cm in 24 h (Carrillo et al., 1985). This intensity of rainfall flushes microbes and nutrients from the soil and vegetation into the watershed, causing extreme changes in allochthonous nutrient and microbe input (Carrillo et al., 1985; Hill and Rai, 1982; Oluwande et al., 1983). In

addition, many rain forest streams are literally scoured clean during such rainfalls, due to the tremendous changes in volume and velocity of the water. Subsequently, the benthos is poorly developed in these streams. Turbidity is also quite high during these catastrophic rainfalls due to washing of soil into the stream. In tropical areas where rainfall is seasonal, some streams may dry up during the dry season and flood during the rainy season; however, these seasons usually are unrelated to temperate seasons and may vary greatly in length from year to year (Wright, 1986). Hill and Rai (1982) found that productivity and total bacterial counts were inversely related to water levels in Central Amazon lakes, yet studies in Africa (Barrell and Roland, 1979; Oluwande et al., 1983), Puerto Rico (Carrillo et al., 1985), and Hawaii (Fujioka and Shizumura, 1985) found that densities of total bacteria and coliforms increased with increasing rainfall. Rainfall also exerts a much greater influence on source water quality in tropical areas than it does in temperate regions.

2.2 Biological Characteristics of Tropical Waters

Wet tropical and subtropical forests alone account for more than 50.5% of the total gross production in terrestrial biospheres (Odum, 1971). As discussed above the higher light intensities and temperature increase the productivity of tropical source waters. Thus, most tropical source waters are hypereutrophic (Hill and Rai, 1982). The dominant photosynthetic species in tropical freshwaters are nearly always cyanobacteria or other photosynthetic bacteria. Since limiting nutrients are usually phosphorus or nitrogen, the faster adsorption rate of these smaller organisms provides a favorable characteristic. Temperate source water, on the other hand, is usually dominated by diatoms or green algae in the spring, green algae in the summer, cyanobacteria in the late summer and early fall, and diatoms in the winter (Hutchinson, 1975). These differences in dominant phytoplankton will also affect the type and quantity of grazing organisms and detritivores in the water. Thus, not only the quantity, but the quality of bacterial resources, that is, substrates and nutrients, are different in tropical waters. Indeed, the entire food chain is usually quite different.

The higher productivity and temperature of the tropics create an environment that is high in organics and dominated by mesophilic and thermotolerant microorganisms (Hill and Rai, 1982; Santiago-Mercado and Hazen, 1987). The psychrophilic microflora of temperate waters is virtually nonexistent in tropical waters. The microflora of tropical waters is thus much more similar to the microflora of animals. In fact, it has been well demonstrated that anthropogenic protozoa, fungi, and bacteria not only survive but may grow in tropical waters. Our own studies have indicated that *Escherichia coli*, including fecal biotypes, may be a naturally occurring bacterium in tropical rain forest watersheds (Hazen et al., 1988; Rivera et al., 1988; Bermúdez and Hazen, 1988). The finding of *E. coli* in pristine environments is extremely unusual because this bacterium inhabits the intestine of warm-blooded animals and its presence is only ex-

pected in environments that have been exposed to recent fecal contamination. Furthermore, it seems that this bacterium is capable of surviving indefinitely in tropical environments (Carrillo et al., 1985; Valdés-Collazo et al., 1987; López-Torres et al., 1987). This suggests that *E. coli* could be a natural inhabitant in these environments and that it may be part of a previously established community.

The lack of seasonality in many tropical areas means that reproduction by plants and animals occurs all year round (Whitaker, 1975). Thus input of degradative substances (e.g. pollen, seeds, embryos, etc.) is not seasonal as it is in temperate areas. Recruitment into tropical populations and senescence are also more constant than in temperate areas where the feast or famine environment causes dramatic changes in the degradative community structure of the water, and therefore the background flora that fecal bacteria must be contrasted to and/or compete with.

In general the microbial diversity of tropical source water is greater than temperate source water, as are the floral and faunal communities within the watershed. Thus, not only is there a greater array of autochthonous microorganisms, but there is also a larger variety of allochthonous microbes and substances entering tropical waters from the surrounding milieu (Hill and Rai, 1982). Tropical microbial flora, environmental characteristics, and survival and activity characteristics of allochthonous microbes are quite dissimilar from temperate waters.

Tropical environments, as would be expected, harbor a much greater array of water borne pathogens, including many of which are totally unknown in temperate areas (Tables 2.1 and 2.2). Yet, there are few temperate waterborne pathogens that are not also found in the tropics. Thus, indicator organisms must cover a wider range and diversity of pathogens in tropical waters than in temperate waters.

2.3 Social-Economic Characteristics of Tropical Waters

More than (65%) of the world population lives in tropical areas, yet these people have less than (10%) of the world's wealth (Odum, 1971). Research and development by these nations is unaffordable and virtually nonexistent. Tropical nations have accepted regulatory recommendations of the industrialized temperate nations without any program of verification, so that temperate recommendations for fecal coliform indices in potable and recreational water are used universally in the tropics. Seldom are the underlying assumptions of temperate water-quality assays tested or even considered before being put into legislation by nations in the tropics. Yet economic hardships may be exacerbated by attempts to meet unrealistic standards. Indeed, national pride and the opinion of other peoples and nations is being severely damaged by inability to meet legislated water quality standards. Thus as stated by Odum (1971), underdeveloped nations may become "never-to-be-developed nations."

The poorness of most tropical nations has also meant that large portions of their populations do not have access to sewage disposal and potable water. There is no alternative to proper water treatment, especially in areas where quantities of source water are limited. But, treatment is a costly enterprise. Many water-treatment plants have an inconsistent supply of chlorine (Toranzos et al., 1986; Carrillo et al., 1985). Since complete treatment is only achieved when there is continuous disinfection, it would be unrealistic to expect such water treatment plants to meet the current standards for potable water. Monitoring for the presence of bacteria in raw source water and finished water is prohibitive in most cases. In fact, water distribution systems are seldom found in many tropical areas. This creates a much greater reliance on point of use surface waters (Esrey et al., 1985; Blum et al., 1987). The lack of public education as well as the persistence of primitive cultures and taboos have also perpetuated the poor health of the people, by continued use of unprotected traditional water sources that are frequently heavily contaminated with human pathogens (Feachem, 1977; Esrey et al., 1985; Blum et al., 1987; Jiwa et al., 1981). Many preventable diseases that are virtually unknown in industrialized temperate nations (Table 2.1 and 2.2) exact a horrible toll on the peoples of the tropics (Feachem, 1977; Snyder and Merson, 1982).

2.4 Indicators of Biological Pollution in Tropical Source Waters

The literature is sparse with specific references to fecal contamination in the tropics, yet there are some notable exceptions. Evison and James (1973) reviewed literature from Ceylon, Egypt, India, and Singapore and reported that densities of *Escherichia coli* in water did not seem to coincide with known sources of fecal contamination. Feachem (1974), working in rural areas of New Guinea, found fecal coliform densities from 0 to 10,000 colony-forming units (CFU) per 100 ml and fecal streptococcus levels from 0 to 6,000 CFU per 100 ml, although the mean densities for both indicators were greater than 100 CFU per 100 ml. All sites sampled were totally unacceptable for drinking water, as far as levels of indicator bacteria were concerned, and Feachem concluded that all sites were grossly contaminated with fecal material. Yet he states that fecal coliform (FC) and fecal streptococci (FS) densities were more closely correlated with the number of domestic animals than the number of people in the watershed. In fact, FC and FS densities were lowest at sites where human population densities were high. In addition, as in most studies from tropical countries, Feachem did not confirm presumptive fecal coliform isolates. In Sierra Leone, Wright (1982) reported densities of fecal coliforms from 40 to 240,000 per 100 ml using a most probable number method (MPN) and fecal streptococci densities from 7 to 64,000 CFU per 100 ml for water sources used by 29 settlements. However, no correlation was found between any of the fecal indicators that Wright measured and the presence of *Salmonella* spp., which

would indicate a real health risk. Fujioka and Shizumura (1985) reported densities of fecal coliforms and fecal streptococci in tropical streams ranging from 100 to 10,000 CFU per 100 ml, including streams not known to be contaminated with sewage. Oluwande et al. (1983) found that rivers in Nigeria had total coliform counts ranging from 8 to 100,000 CFU per 100 ml. In this study it was also assumed that the high densities of fecal indicators meant that these waters were heavily contaminated with human feces, even though data showed that densities of total coliforms were quite often higher upstream from known contamination sources.

Thomson (1981) isolated *Salmonella* spp. from wells used for drinking water in Botswana and found no correlation between densities of either total coliforms, fecal coliforms, or *E. coli* and the presence of *Salmonella* spp. Lavoie and Viens (1983) reported that 95% of the traditional water sources in the Ivory Coast, West Africa had unacceptably high densities of fecal coliforms (11,421 CFU per 100 ml), yet fewer than 55% of the positive total coliform isolates were actually *E. coli* and less than 66% of the fecal coliform isolates were actually *E. coli*. However, when Lavoie (1983) examined the feces of local inhabitants he found that 92% of total coliform isolates were *E. coli* and 89% of the fecal coliform isolates were *E. coli*. Few, if any, of these studies have carefully examined and tested the underlying assumptions of the fecal-pathogen indicator being used. Bonde (1977) stated these criteria as follows:

1. The indicator must be present whenever pathogens are present.
2. It must be present only when the presence of pathogenic organisms is an imminent danger.
3. It must occur in much greater number than the pathogens.
4. It must be more resistant to disinfectants and to aqueous environments than the pathogens.
5. It must grow readily on relatively simple media.
6. It must yield characteristics and simple reactions enabling as far as possible an unambiguous identification of the group or species.
7. It should preferably be randomly distributed in the sample to be tested.
8. Its growth on artificial media must be largely independent of any other organism present.

In the late 1800's Houston proposed the idea of using three groups of bacteria (coliforms, fecal streptococci, and the gas-producing clostridia which are commonly found in the feces of warm blooded animals) as indicators of fecal pollution of waters (Hutchinson and Ridgway, 1977). He argued that since these groups could only come from fecal sources, their presence would indicate recent fecal pollution (Hutchinson and Ridgway, 1977). For nearly eighty years the coliform group of bacteria has been used as such indicators. The indicator

used universally to assess biological contamination of water is *E. coli*, in both tropical and temperate countries (Barbaras, 1986).

The first drinking water regulation for microbial contamination in the U.S. was published in 1914. This was the first Public Health Service Drinking Water Standard. Subsequently, this regulation was replaced by U.S. Public Service Acts of 1915 and 1962. The current U.S. regulation comes from the Safe Drinking Water Act (Public Law 93–523, 1974). The U.S. Environmental Protection Agency proposed changes that are now being implemented (Federal Register 48:45502–45521, October, 1983). The revised law was approved in July 1986 and is currently in its first phase of implementation. The new regulation requires that there should be 0 coliforms/100 ml by any method, for any sampling frequency for drinking waters. World Health Organization (WHO) (Barbaras, 1986) allows 10 coliforms/100 ml for small community water sources. For tropical nations and tropical parts of the United States, even the old regulations may be unrealistic. As observed in Botswana (Thomson, 1981) and Sierra Leone (Wright, 1982), no correlation could be found between the presence of *Salmonella* spp. and the presence of *E. coli* in tropical source water. In Puerto Rico, densities of pathogenic yeast (Valdés-Collazo et al., 1987), *Klebsiella pneumoniae* (López et al., 1987), *Legionella pneumophila* (Ortiz-Roque and Hazen, 1987), *Vibrio cholerae* (Pérez-Rosas and Hazen, 1988), *Yersinia enterocolitica* (Elías-Montalvo et al., 1988), and *Aeromonas hydrophila* (Hazen et al., 1981) were also found to be unrelated to densities of *E. coli* in source water. Thus pathogens could be present in the absence of *E. coli* in tropical source water. It has also been demonstrated that high densities of enteric viruses, a dominant cause of waterborne disease in both temperate and tropical areas, may be found in the complete absence of fecal coliforms of *E. coli* (Berg and Metcalf, 1978; Toranzos et al., 1986a; Toranzos et al., 1986b; Keswick et al., 1985; Rose et al., 1985; Cabelli, 1983; Hejkal et al., 1982; Herrero and Fuentes, 1977). However, a large number of diverse studies has also shown that fecal coliforms and *E. coli* may become injured, such that they do not grow on standard media and/or under standard incubation conditions (Bissonnette et al., 1975; Bissonnette et al., 1977; Stuart et al., 1977). The high light intensities found in the tropics could certainly contribute to this injury phenomenon (Fujioka and Navikawa, 1982).

In Nigeria (Oluwande et al., 1983), Hawaii (Fujioka and Shizumura, 1985), New Guinea (Feachem, 1974), Puerto Rico (Carrillo et al., 1985), Sierra Leone (Wright, 1982), and the Ivory Coast (Lavoie, 1983), high densities of *E. coli* were found in the complete absence of any known fecal source. The U.S. Geological Survey reported that 54 out of 67 water sampling stations on rivers in Puerto Rico exceeded the recommended MCL/s for recreational water (< 1,000 fecal coliforms per 100 ml) during 1984 (Curtis et al., 1984). Thus only 19% of all sites sampled met the recommended MCL for recreational waters, and none of these waters could meet raw source water standards (<2 fecal coliforms per 100 ml). These findings have resulted in condemnation of sewage treatment in Puerto Rico as a source of fecal pollution of natural waters by

regulatory agencies (Hazen et al., 1987). Yet, samples taken upstream from sewage-treatment plant outfalls had fecal coliform densities that were just as high as those of most downstream sites.

Recent studies have shown that *E. coli* can be isolated from pristine areas of tropical rain forests in Puerto Rico (Rivera et al., 1988; Bermúdez and Hazen, 1988). Plasmid profiles, antibiotic sensitivities, coliphage susceptibility, and physiological and biochemical characteristics confirm that even *E. coli* isolated from epiphytes in trees 15 m above the ground are very similar to clinical isolates of *E. coli* (Rivera et al., 1988). The DNA of these environmental isolates have identical mol% G+C and more than 85% DNA homology with *E. coli* B (Hazen et al., 1987; Bermúdez and Hazen, 1988). Thus, tropical source waters may not only have high densities of *E. coli* in the absence of pathogens or fecal sources, but *E. coli* may be naturally occurring in some tropical areas. This observation is further corroborated by the high densities of *E. coli* in diverse tropical countries in the absence of identifiable fecal sources. *Escherichia coli* is usually found in higher densities than other enteric pathogens. However, as noted in Botswana (Thomson, 1981) and Sierra Leone (Wright, 1982), *Salmonella* spp. may be found in the complete absence of *E. coli*. In addition, *Legionella* spp. could also be found in Puerto Rico in the complete absence of *E. coli* (Ortiz-Roque and Hazen, 1987).

Gordon and Fliermans (1978) demonstrated that *E. coli* could survive for much longer periods of time in a temperate lake receiving thermal effluent. Thus, environmental factors in the tropics might also significantly affect the survival of fecal coliforms in the environment. Bigger (1937) in India first reported the growth of coliforms in tropical waters. Ragavachari and Iyer (1939) showed that coliforms can survive for several months in natural tropical river waters. In Puerto Rico, several studies (Carrillo et al., 1985; López et al., 1987; Valdés-Collazo et al., 1987; Hazen et al., 1987) have shown that *E. coli* not only survives in rain forest streams but also proliferates. Thus, once introduced into the environment, *E. coli* could remain and/or become part of the normal flora. Recent studies by Xu et al. (1982), Colwell et al. (1985), and Roszak et al. (1984) have shown that enumeration on complex media like those used to culture *E. coli* may not be reliable. These studies have demonstrated that *E. coli* and pathogens like *Salmonella enteridis*, and *Vibrio cholerae* may be able to survive and remain pathogenic and yet be unculturable on standard media. Under in situ conditions in a tropical rain forest, *Yersinia enterocolitica* (Elías-Montalvo et al., 1988), *Klebsiella pneumoniae* (López et al., 1987), *Candida albicans* (Valdés-Collazo et al., 1987), *Aeromonas hydrophila* (Hazen et al., 1982), *Salmonella typhimurium* (Jiménez et al., 1989), and *Vibrio cholerae* (Pérez-Rosas, 1984), were all shown to have shorter survival rates than *E. coli*, their presumed indicator. The inability of *E. coli* to survive in a manner similar to pathogens would further impede its ability to indicate the presence of those pathogens in waters. The time of survival of *E. coli* in source waters of temperate climates has been shown under numerous circumstances to be days, with densities decreasing by more than 90% every 60 min (Bonde, 1977). Regrowth of *E. coli*

outside the intestinal tract in temperate areas has only been rarely observed (Bonde, 1977). As seen in Table 2.3, *E. coli* always survives significantly longer in situ in tropical waters as compared to temperate waters. The increased survival of *E. coli* in tropical waters suggests that temperate maximum contaminant levels based on fecal coliforms can not be justified for tropical waters.

Pagel et al. (1981) compared four fecal coliform assays in various types of freshwaters in Southern Canada. They observed that while these assays were somewhat variable in their abilities to detect fecal coliforms from environmental samples, they were all acceptable in terms of their specificity and selectivity. In similar studies, Santiago-Mercado and Hazen (1987) and Hazen et al. (1987) used the same methodology to detect fecal coliforms from freshwaters in Puerto Rico and found that the specificity of the media (determined by the ability of the medium to restrict growth of organisms other than the target bacterium) was at least 20% less than the specificity reported by the Canadian investigators (Table 2.4). Thus, all the methods gave significantly higher false-positive and false-negative errors with tropical water samples.

Table 2.3 Comparison of indicator and pathogen survival in *in situ* tropical and temperate source water

		Density	Survival (h)*	Reference
Indicators				
E. coli	Temperate	10^9	50	Gordon and Fliermans (1978)
		10^5	30.6	McFeters et al. (1974)
	Tropical	10^7	**	Carrillo et al. (1985)
		10^7	294	López et al. (1987)
		10^6	206	Valdez-Collazo et al. (1987)
S. faecalis	Tropical	10^7	226	Muñiz et al. (1988)
B. adolescentis	Tropical	10^7	92.6	Carrillo et al. (1985)
Pathogens				
V. cholerae	Temperate	10^5	13	McFeters et al. (1974)
	Tropical	10^7	198	Pérez-Rosas (1983)
Y. enterocolitica	Tropical	10^8	124	Elías et al. (1988)
C. albicans	Tropical	10^6	**	Valdés-Collazo et al. (1987)
A. hydrophila	Temperate	10^5	**	McFeters et al. (1974)
		10^7	75	Hazen and Esch (1983)
		10^8	100	Fliermans et al. (1977)
	Tropical	10^7	**	Hazen et al. (1982
S. typhimurium	Temperate	10^5	28.8	McFeters et al. (1974)
	Tropical	10^8	131	Jiménez et al. (1987)
K. pneumoniae	Tropical	10^6	125	López et al. (1987)
P. aeruginosa	Tropical	10^5	**	Cruz (1987)

* Survival time is the T_{90} time to reach 90% reduction of initial cell density.
** Survival time indefinite.

Table 2.4 Comparison of fecal coliform assay methods in tropical and temperate source waters

Performance parameter	MMB		mFC1		mFC2		mTEC	
	Temp*	Trop*	Temp	Trop	Temp	Trop	Temp	Trop
Accuracy[a]	59	67	89	84	100	105	94	93
Specificity[b]								
False positive	11	39	16	30	18	19	13	36
Undetected	4	25	1	20	2	11	2	21
Selectivity[c]	88	66	85	72	90	82	86	66
Comparability[d]								
FC recovery	26	41	41	75	48	94	45	73
Non-FC recovery	4	30	11	29	15	19	7	18
Overall rank[e]	2	4	3	2	1.5	1	1.5	3

* *Temp*, temperate (data of Pagel et al. (1981). *Trop*, tropical (data of Santiago-Mercado and Hazen, 1987). MMB = MacConkey membrane broth, mFC1 = Fecal coliform media with normal incubation, MFC2 = fecal coliform media with resuscitation at 35 °C for 2h, mTEC = mTEC media.

[a] Mean percentage number of colonies on test medium/mean number of colonies on a non-selective medium × 100, using *E. coli* (ATCC 10798 and ATCC 23848).

[b] Percentage false positive error = [false positive error + number of presumptive target colonies − number of verified target colonies]/[total presumptive target colonies] × 100. Percentage false negative error = false negative counts/[verified + undetected target counts] × 100.

[c] Selectivity Index = presumptive typical colonies/[presumptive typical or target colonies + presumptive nontypical or nontarget colonies] × 100.

[d] Percentage fecal coliform (FC) recovery and percentage nonfecal coliform (NFC) recovery.

[e] Best overall efficiency of the method is given by the lowest overall rank.

Controls using known strains of *E. coli* indicated the accuracy of the methods to be the same in both studies (Table 2.4). Identification of more than 300 fecal coliform isolates from various freshwater sites around Puerto Rico showed that less than 40% of these isolates were actually *E. coli* (Santiago-Mercado and Hazen, 1987; Hazen et al., 1987). Similar studies using the same methods in the continental U.S.A. (Dufour et al., 1981), South Africa (Grabow et al., 1981), Canada (Pagel et al., 1982), and England (Evison and James, 1973) have demonstrated that more than 90% of fecal-coliform-positive isolates are identified as *E. coli*. Wright (1982) found that in Sierra Leone waters, fewer than 10% of the positive isolates from fecal coliform assays were *E. coli*. Lavoie (1983) found that less than 66% of the fecal coliform isolates from Ivory Coast well waters were actually *E. coli*. It is not surprising that the thermotolerant *E. coli* encounter more mesophilic and thermotolerant background flora in the tropics, given the higher and more constant temperatures and productivities. Santiago-Mercado and Hazen (1987) showed that high densities of mesophilic and thermophilic background flora in tropical source waters can significantly reduce the numbers of *E. coli* detected on standard fecal coliform media.

Thus *E. coli*, the indicator used by most developed nations for determining biological contamination of source waters, does not fit most of the underlying assumptions of a good indicator in tropical source waters. It is not surprising, therefore, that developing nations in tropical areas are finding it difficult (if not impossible) to meet their own legislated standards for biological contamination.

2.5 Possibilities for Tropical Standards

Fecal coliforms, as discussed above, seem to be unacceptable as indicators of recent fecal contamination in tropical source water. Since the problems with the fecal coliform assay are due in part to *E. coli* being the target organism (because of its increased survival and/or natural occurrence), changes in media and incubation would not correct these problems for tropical analyses. Thus, alternative indicators or techniques will be required to correctly access the degree of fecal contamination and/or public health importance of tropical source waters.

Several investigations of tropical source water have looked at fecal streptococcus densities. Feachem (1974) found that streams in the New Guinea highlands had densities or fecal streptococci from 0 to 6,000 per 100 ml, and the FC/FS ratio ranged from 0.20 to 1.31. Feachem interpreted these results to mean that these waters were 25 times more likely to be contaminated by pigs than humans, based upon the studies of Geldreich and Kenner (1969). However, if *E. coli* is able to survive in these waters, the ratio could be inflated. Because of their high densities, the fecal streptococci would also seem to indicate fecal contamination when none may be present. Wright (1982) found that source water in Sierra Leone had densities of fecal streptococci (7 to 64,000 per 100 ml) that were an order of magnitude lower than densities of either fecal coliforms (40 to 240,000 per 100 ml) or presumptive *E. coli* (30 to 120,000 per 100 ml). When isolates of fecal streptococci were identified, Wright (1982) found that 14 to 100% were confirmed as *Streptococcus faecalis*, the target organism for the assay. In addition, Wright (1982) could find no correlation between the incidence of *Salmonella* spp. in these source waters and the densities of fecal streptococci. Fujioka and Shizumura (1985) also found 100 to 10,000 CFU per 100 ml of fecal streptococci in streams in Hawaii, even though many of these streams were not known to have any source of fecal contamination. Densities of fecal streptococci in Hawaiian streams were also quite similar to densities of fecal coliforms. Studies in Puerto Rico have demonstrated that *S. faecalis* can survive and remain active for long periods of time in diffusion chambers in a tropical rain forest stream (Muñiz et al., 1989). Even tropical marine waters with petroleum contamination could support high densities of *S. faecalis* (Santo Domingo et al., 1989). Monitoring of Puerto Rican waters by the U.S. Geological Survey has shown that like fecal coliforms, fecal streptococci exceeded recommended MCL for recreational waters more than 80% of

the time (Curtis et al., 1984). Also, as with fecal coliforms, densities of fecal streptococci were only slightly lower and occasionally higher at sampling stations upstream from sewage-treatment-plant outfalls. Evison and James (1973) suggested that *E. coli* is in much lower proportions, relative to *S. faecalis*, in human feces from tropical countries. McFeters et al. (1974) demonstrated that even in temperate waters fecal coliforms and fecal streptococci have different rates of survival. This would also invalidate the use of the FC/FS ratio for determining the origin of the fecal contamination, as would the higher survival rates of both *E. coli* and *S. faecalis* in the tropics. Though fewer studies have been done in tropical water with the fecal streptococci than with fecal coliforms, the fecal streptococci seem to satisfy only one of Bonde's eight criteria for an ideal indicator.

Evison and James (1975) suggested that *Bifidobacterium* spp. might be a more appropriate indicator for the tropics. Bifidobacteria are obligate anaerobes and thus are not likely to grow outside the intestinal tract. In addition, this genus is always found in the human gut, quite often in higher densities than *E. coli*, and is seldom observed in other animals (Resnick and Levin, 1981). With the advent of the YN-6 medium (Resnick and Levin, 1981), bifidobacteria would seem to be an excellent candidate for a fecal indicator organism in tropical source waters. Carrillo et al. (1985) showed that *Bifidobacterium adolescentis* decreased one order of magnitude each day during in situ exposure in a tropical rain forest stream in Puerto Rico. Gyllenberg et al. (1960) and Evison and James (1975) showed that densities of bifidobacteria were always greater than densities of *E. coli* in contaminated waters in temperate areas. In the Puerto Rico rain forest, however, densities of bifidobacteria-like organisms were greater than *E. coli* at all sites except those at a sewage outfall (Carrillo et al., 1985). In addition identification of isolates from the rain forest stream using the YN-6 medium showed that less than 80% were actually bifidobacteria. Bifidobacteria show promise as an indicator of recent fecal contamination in terms of lack of survival in situ and specificity as a human fecal indicator. Unfortunately, the currently available medium for enumeration (YN-6) is hampered by a lack of specificity and insufficient resolution when background anaerobic bacterial densities are high (Carrillo et al., 1985).

Clostridium perfringens, an anaerobe, has also been suggested as a suitable alternative indicator to fecal coliforms and fecal streptococci in tropical source waters. Fujioka and Shizumura (1985) found that in uncontaminated streams densities of *C. perfringens* were from 0 to 46 CFU per 100 ml, while discharge sites had from 56 to 2,100 CFU per 100 ml. The same study showed that 91% of Hawaiian isolates from membrane *C. perfringens* (mCP) medium were confirmed as *C. perfringens*. This compares favorably with the results of a study by Bisson and Cabelli (1980) in temperate waters. However, densities of *C. perfringens* in human fecal material from the tropics are much lower than any of the previously mentioned indicators (Wright, 1982). This raises serious doubts as to the ability to easily detect *C. perfringens* when pathogens are an immanent danger. Carrillo et al. (1985) found that sites which received heavy

rainfall had high densities of total anaerobes (>500,000 per 100 ml). Fujioka and Shizumura (1985) made the observation that densities of *C. perfringens* increased significantly in uncontaminated sites after rainfall. This suggests that *C. perfringens* might live outside the intestinal tract under some conditions. Wright (1982) found that source waters in Sierra Leone had densities of *C. perfringens* from 40 to 1,500 per 100 ml and that they compared favorably with densities of fecal streptococci. He also found that like the fecal streptococci they were unrelated to the isolation of *Salmonella* spp. Like the bifidobacteria, *C. perfringens* seems to satisfy more, but not all, of Bonde's criteria for an ideal indicator than the fecal coliforms or the fecal streptococci. The inability of enteric nonspore-forming anaerobes to proliferate in most source waters makes them more suitable indicators of recent fecal contamination. Unfortunately, techniques involving anaerobes also require more sophisticated and expensive equipment and confirmation methods.

Coliphages have been suggested as indicators that would be more specific and not subject to the cultivation problems of *E. coli* (Berg and Metcalf, 1978; Funderburg and Sorber, 1985; Wentsel et al., 1982). Coliphages can be concentrated from large volumes of water with relative ease and high efficiency. Studies in our laboratory (Toranzos et al., 1988) have shown that when *E. coli* C3000 (ATCC 15597) is used, coliphages are detected only in water receiving sewage effluent. In the latter experiments, one liter volumes of the water samples were concentrated by the viradel technique (Goyal and Gerba, 1983) and the eluate assayed using the above strain of *E. coli*. Pristine as well as sewage-contaminated waters were sampled where indigenous *E. coli* had been isolated previously. Coliphages were consistently isolated from sites receiving sewage effluents whereas none of the other sites contained coliphages. In addition, coliphages (such as MS2, F2, T4, and T7) would not grow on more than 20 indigenous *E. coli* strains. Evison (1988), however, showed that coliphage survival in microcosms simulating temperate environmental conditions were quite variable, depending on the environmental conditions and the coliphage being used. Since the coliphage assay is specific for a particular strain of *E. coli*, it could eliminate the tropical background microflora problem seen when using standard media assays for *E. coli*. However, since the host organism for these viruses is a specific strain of *E. coli*, the viruses might reproduce in situ since this bacterium appears to survive and grow in tropical source waters. Thus, coliphage assays would be appropriate for tropical source waters only if a specific host strain of *E. coli* were demonstrated not to survive outside the intestinal tract. This seems unlikely, since all strains of *E. coli* tested to date survive in tropical freshwaters (Elías-Montalvo et al., 1988; López et al., 1987; Valdés-Collazo et al., 1987; Hazen et al., 1981; Jiménez et al., 1988; Pérez-Rosas, 1984; Carrillo et al., 1985). Coliphages that infect *E. coli* would probably be no better than *E. coli* as an indicator of fecal contamination in the tropics.

As discussed above, nonsporeforming obligate anaerobes that are only found in the intestinal tract of man or similar warm-blooded animals appear not to survive for long periods of time or proliferate in most tropical source

waters. The inherent difficulty with assaying for these organisms is their requirement for complex media, anaerobic culture conditions, and differentiation from indigenous anaerobes (Carrillo et al., 1985). Assays might be developed for enumeration of bacteriophages of these bacteria that would have fewer problems than any of the assays discussed so far. Recent studies with bacteriophages of *Bacteroides fragilis* have shown that this approach is plausible (Tartera and Jofre, 1987). There is no reason to believe this approach would not also work for *Bifidobacterium* spp., a group that has already been shown not to survive for extended periods in tropical water and have better human fecal specificity than coliforms or fecal streptococci. Since these methods are hypothetical, or only at an early experimental stage, a major research and testing effort would be required before any of these techniques could be implemented in tropical source waters.

2.6 Perspective and Recommendations

The problems associated with determining fecal contamination of tropical source waters are many and varied (Table 2.5). Certainly the standard fecal indicator, *E. coli*, is unacceptable. Because few studies have reported the use of fecal indicators (other than *E. coli*) in tropical source water, objective evaluations of the efficacy of these alternate indicators is difficult. At present, ob-

Table 2.5 Problems assessing tropical microbiological source water quality

1. Regrowth of indicators in freshwater.
2. Thermotolerant and mesophilic background flora overgrow assays which rely upon the thermotolerant nature of indicators and which assume a psychrophilic background flora.
3. High false-positive natural flora using viable culture techniques.
4. Pathogens found in the absence of indicators, eg, *Salmonella* spp.
5. Higher natural productivity of environments allows grater survival and regrowth of indicators and pathogens.
6. Different flora and fauna of tropics creates different interactions and environments for indicators and pathogens.
7. Torrential rainfall in some areas causes extreme changes in allochthonous input of nutrients and microbes from surrounding environments.
8. Greater number of waterborne diseases.
9. Greater reliance on local surface waters as traditional drinking water sources.
10. Few sewage treatment and water treatment facilities and inadequate monitoring and enforcement.
11. Possible autochthonous origin of indicators.
12. Because of higher annual temperatures people spend more time in direct contact with water.

ligate anaerobes or their phages seem the best candidates for a better indicator for tropical source water, primarily due to their inability to survive outside the intestinal tract. However, all of these indicators have the inherent difficulty that they or their host may survive under some conditions and that the media used for bacterial indicator enumeration may allow the growth of false-positive background flora (Table 2.5). The viable but nonculturable phenomenon reported for many pathogens in both temperate and tropical waters suggests that indicators may only rarely be correlated with disease risk in source waters (Colwell et al., 1985; Hazen et al., 1987; Baker et al., 1983). Thus, the best indicator may be no indicator, that is direct enumeration of selected resistant pathogens. This would allow a more realistic estimation of health risk.

Immunofluorescent staining can detect densities of pathogenic bacteria as low as 10 cells per ml, a density which may give no culturable counts (Fliermans et al., 1981; Colwell et al., 1985). The use of monoclonal antibodies makes this technique specific at even the strain level for some organisms. However, as a result of cross-reactivity when using immunofluorescence (even with monoclonal antibodies), the most specific and sensitive method for detecting pathogens may be nucleic acid probes (DNA or RNA). DNA probes have already been developed and tested for enterotoxigenic *E. coli* (Bialkowska-Habrzanska, 1987; Hill et al., 1983; Moseley et al., 1982) and *Salmonella* spp. (Fitts et al., 1983). Thus, direct detection of pathogens is currently possible. Common enteric pathogens which could be enumerated are poliovirus and *Salmonella typhimurium*. Detection of either one of these in tropical source water would indicate risk of human disease. Instead of enumeration, maximum contaminant levels could be based on detection only. One potential problem with this approach is that the presence or absence of one pathogen may have little bearing on other pathogens. A multi-species test for two or more of the more resistant and common pathogens found in tropical source waters may be necessary. The public health of people living in the tropics and their economic development is dependent upon a more suitable microbiological standard being developed for tropical source waters. The currently used fecal coliform assays are unacceptable for indicating fecal contamination of tropical source waters.

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