EVALUATION OF CURRENT INDICATORS OF WATER SAFETY FOR COASTAL RECREATIONAL WATERS

by

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1. Introduction

As summer months approach, excitement for the warm sun and water trigger families to solidify plans for a trip to the beach. Parents worry about packing for the kids, affording travel expenses, finding lodging, and making sure there is enough sunscreen for everyone. Does anyone ever worry about the condition of the ocean water they will be swimming in? In America, the Environmental Protection Agency monitors the health of coastal recreational waters and has the authority to close beaches that do not meet their safety standards.

Bodies of water may contain pathogenic bacteria, protozoa, and viruses found in animal waste. These fecal pathogens contaminate our waterways through coastal and shoreline development, wastewater collection and treatment facilities, septic tanks, urban runoff, disposal of human waste from boats, bathers themselves, animal feeding operations, and natural animal sources like wildlife. Humans that swim in these infected waters risk diseases as mild as ear infections and sore throats, to more serious diseases such as dysentery, typhoid fever, and Hepatitis A. However, instead of testing for a variety of diseases, the EPA uses indicator organisms, *E. coli* and enterococci, to monitor fecal contamination in coastal recreational waters. If *E. coli* counts in freshwater rise above 126 organisms per 100 ml or if *Enterococci* counts in saltwater rise above 35 organisms per 100ml, a sign posting or beach closure is necessary.

E. coli and *Enterococci*, referred to as indicator organisms or fecal indicator bacteria (FIB), are two of the many organisms that live in the intestinal tracks of mammals and birds. Everyday one human will pass approximately 100 billion to 10 trillion individual *E. coli* bacteria in their feces. These indicators do not normally harm

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humans. However, there has been a lot of press about *E. coli* infecting the public through food. This particular strain of *E. coli*, *E. coli* 0157:H7, is a rare but dangerous strain which causes hemorrhaging in the intestines.

Because these indicators are relied on so heavily to determine safety of water, it is imperative to determine if these are suitable indicators, understand the environmental factors that allow them to thrive, and ways to eliminate them from the waters. Some of these factors include temperature, light, salinity, rainfall, predation, available nutrients and environmental pollutants.

2. History of Water Quality Policies

2.1 Federal Water Pollution Control Act/Clean Water Act

The enactment of the Federal Water Pollution Control Act (FWPCA) of 1972 follows many attempts at creating an organized act addressing water pollution. The original act was approved in 1948 and subsequent amendments broadened the federal government's authority. In 1969, a federal study found half of the public supply systems were substandard, creating urgency for a solution. This realization resulted in the 1972 amendment to the FWPCA, which restructured the authority for water pollution control and consolidated authority in the administrator of the EPA.

The FWPCA, better know as the Clean Water Act after the 1977 amendments, set out to accomplish two goals: to eliminate the discharge of all pollutants into the navigable waters of the United States by 1985 and to reach water quality levels that protect fish, shellfish, wildlife, and recreation by July 1, 1983. This required enforcers to regulate the amount of pollutants being discharged from particular point sources; a change from regulating the amount of pollutants in a body of water. Regulating effluents identifies the source of pollution, keeping an eye on pollution hot spots. This act delegated responsibility to the EPA to publish reports setting pollutant discharge limits and identifying the best available technology to reduce or eliminate pollutant discharge. Two published documents fulfill this requirement and address bacteria levels in waterways: *Quality Criteria for Water of 1976* and *Ambient Water Quality Criteria for Bacteria of 1986*.

2.2 Marine Protection Research and Sanctuaries Act of 1972

During the restructuring of the FWPCA in 1972, Congress passed the Marine Protection Research and Sanctuaries Act of 1972, prompted by the realization that the ocean has a limit to the amount of sludge waste and junk it can safely absorb. This act regulates all materials dumped into ocean waters and prevents or strictly limits dumping any material which would adversely affect human health, welfare, or amenities; or the marine environment, ecological systems; or economic potentialities.

2.3 Quality Criteria for Water of 1976

The *Quality Criteria for Water of 1976* was the first required publication of up-todate scientific knowledge concerning water quality—a responsibility the FWPCA delegated to the EPA administrator. This document identifies fecal coliform as the standard indicator of water health. Fecal coliform bacteria exist in the intestines of warm-blooded animals and include the genera *Escherichia*. At this time, *E. coli* was not recommended due to complicated testing methods and scarcity of experienced microbiologists. The *Quality Criteria for Water of 1976* also considered, but did not recommend, enterococci as an indicator because biochemical testing needed more standardization.

To set a safe limit of fecal coliform (FC) allowable in recreational waters, its presence must be compared to probable occurrence of waterborne pathogens. According to the *Quality Criteria for Water of 1976*, the relationship between concentrations of FC and *Salmonella* was the only information used to set the maximum limit at 200 organisms of FC per 100ml. When tests showed fecal coliform densities in freshwater above 200 organisms per 100ml, the frequency of *Salmonella* increased sharply—recovered from 85-95% of samples. At the set limit of 200 org/100ml, 28.4% of estuarine water samples contained *Salmonella*. Above this limit, sixty percent of estuarine samples contained *Salmonella*. Due to natural factors, such as shifts in wind direction, current flow, and tidal fluctuations, the mean count of samples taken over a thirty-day period (5 samples minimum) is used to test contamination.

2.4 Ambient Water Quality Criteria for Bacteria of 1986

Ten years later, the EPA used a quantitative approach to determine which indicator of water quality correlates best with swimming-associated health effects. The results, published in *Ambient water Quality Criteria for Bacteria of 1986*, established *E. coli* and enterococci as better suited indicators of pathogen load than fecal coliform.

The EPA conducted epidemiological surveys at selected beach sites (saltwater and freshwater), selecting a polluted (from sewage) beach and paired it with an unpolluted recreational beach from five locations. Each visitor participating in the survey was to send back information about any illness they came down with in the week after swimming at these sites. Information was also collected from non-swimmers which was

subtracted from the reported swimming illnesses. Illness were divided into four categories—gastrointestinal; respiratory; eye, ear, and nose; and other. Due to the variability in self-diagnoses by participants, some symptoms were categorized in a new category called "highly credible gastrointestinal symptoms". These symptoms include: vomiting, diarrhea with fever or a disabling condition, and stomachache or nausea accompanied by a fever. Individuals reporting these symptoms were said to have acute gastroenteritis.

The results showed that only gastroenteritis symptoms were significantly different between the polluted and unpolluted beaches. Forty-one percent of the trials resulted with gastroenteritis illnesses at the polluted beaches with zero reports at the unpolluted beaches. Reports of the other three categories—respiratory; eye, ear, and nose; and other—did not show an excess of illnesses at either of the paired beaches at each study location. Shockingly enough, up to this study in 1984, this was the only available evidence linking sewage contaminated water with a health risk for bathers.

This studies concluded that the best indicator for fecal contamination in saltwater was enterococci and for freshwater it was *E. coli*, with enterococci having a lower correlations but not significantly different. Having identified suitable indicators, the EPA decided not to change the stringency of the bacterial criteria. According to the *Quality Criteria for Water of 1976*, a geometric mean of 200 fecal coliform bacteria per 100ml would result in 8 illness per 1,000 swimmers at fresh water beaches and 19 illnesses per 1,000 swimmers at marine beaches. These numbers are only approximate but are still used today to develop criteria for *E. coli* and enterococci, since they are the EPA's best estimates of accepted illness rates up to 1986.

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Since fecal coliform is a general term, encompassing all coliforms found in human intestines, and the new government standards use the more specific organisms *E. coli*, the limit was lowered. Regression analysis, using the accepted illness rates mentioned in the previous paragraph, was used to determine the new limits for these indicators. The *Ambient Water Quality Criteria for Bacteria of 1986* establishes a new limit for freshwater set at 126 organisms of *E. coli* per 100ml of water. Enterococci was re-evaluated as an indicator organism and passed the test. It can be used as a freshwater indicator—limit set at 33 organisms per 100ml using the same equation to determine *E. coli*—and is established as the recommended indicator for saltwater—limit set at 35/100ml. The testing procedures still follow the mean levels over the 30 day testing period and these standards are still used today.

2.5 BEACH Act of 2000

Currently, states with coastal areas including the Great Lakes, must adopt water quality standards stated in the Beaches Environmental Assessment and Coastal Health Act (BEACH) of 2000. The BEACH Act amends the Clean Water Act by requiring states, having coastal waters or Great Lakes shoreline, to adopt water quality criteria that are as protective of human health as the levels proposed in the *Ambient Water Quality Criteria for Bacteria -1986* by April 10, 2004. If a state fails to comply by the deadline the EPA administrator will propose regulations. States can opt to set levels, but EPA approval is still needed to confirm the states proposed regulations are as protective as the federal standards. If the EPA approved the states' proposed criteria, federal standards were withdrawn. There are thirty-five coastal states and territories that must follow these requirements. As of April 10, 2004, 14 complied for all their coastal recreational waters, 13 are in the process of adopting the criteria, 5 have adopted requirements for some of their coastal recreational waters, and 3 have taken no action.

3. Economic Importance of Our Nation's Beaches

The federal government saw it was important to set the deadline and make states adopt protective limit for bacterial concentration in recreational waters because our beaches have been degrading over the years and they are so important to the nation's economy. Over half the U.S. population lives in coastal watershed counties and roughly one-half of the nation's gross domestic product (\$4.5 trillion in 2000) is generated in these coastal counties. Coastal recreation and tourism are estimated to contribute over \$640 billion annually to the U.S. economy (85% of all U.S. tourist revenues). Unfortunately, in 2003, 18,000 beach closings were reported, a rise of 51% from 2002.

This value is also of great importance to states and counties. North Carolina's 320 miles of coastline generated \$3.0 million in economic value and created 50,000 jobs in 2000 (Marlow & Co. 2004). In Brunswick and Carteret counties, beaches contributed \$200 million in tourist related revenues. Without revenue from the beaches in Carteret County, the property taxes would rise 75% (Marlow & Co. 2004).

4. Life Span of Indicators

When determining a general life span of *E. coli* and enterococci outside the intestines, many factors must be taken into consideration. High salinity, heavy metals, sunlight, temperature, competition for nutrients, predation by other microorganisms, lysis

by bacteriophage, aggregation, and adsorption to particulate matter have all been reported to be the primary mechanism by which coliform bacteria are killed or the numbers are reduced in the marine environment (Fujioka 1981). The source of fecal contamination wastewater, dog feces, sewer water—and species strain also affect the persistence of the indicators (Anderson 2005).

Results from experiments, which discussed indicator life span, showed varied results. One experiment shows fecal coliform survived in saltwater for one to three days (Fujioka 1981). Another experiment reported FC concentrations in water for two weeks and in sediment for four weeks (Anderson 2005). Other laboratory studies demonstrated *E. coli* survival in marine water samples for three years (Griffin 2001). All these experiments involved different experimental designs, but they showed *E. coli* populations can survive one day to three years outside the intestine.

5. Reaction of Indicators to Different Stressors

5.1 Saltwater

Many studies exist on the affects of saltwater on fecal indicator bacteria (Anderson 2005, Anderson 1979, Barcia-Lara 1991, Ferguson 2005) and scientists are in agreement that saltwater eliminates fecal indicator bacteria. Further research showed a combination of the biological, chemical and physical makeup of saltwater all affect the presence of indicator bacteria (Anderson 2005).

A study conducted by Iris Anderson shows that the higher the salinity, the more stressed the sample of *E. coli* becomes (Anderson 1979). The seawater used was 45°C,

sterilized seawater from the Atlantic, and the experiments were not exposed to sunlight.

Table 1 shows the results of *E. coli* concentrations in different salinities (Anderson 1979).

Salinity (‰)	Exposure (days)	% Survival
10	2 5 8	100.6 87.6 53.5
15	2 5 8	27.9 11.7 7.1
25	2 5 8	8.6 5.1 4.3
30	2 5 8	1.7 0.7 2.0

<u>**Table 1**</u> – Percent survival of *E. coli* in seawater at different salinities (Anderson 1979).

This experiment shows, without sunlight or potential predators in the water, the salinity level and the time of exposure negatively affect the presence of *E. coli*.

Other studies indicate that it is not just the presence of salt in the water but also the presence of predators that affect the elimination of *E. coli* (Enzinger 1976, McCambridge 1981). Enzinger observed a large reduction in numbers of antibioticresistant strains of *E. coli* in bay water (35%c) that had been sterilized—predators removed—and seeded with untreated seawater containing protozoa. The protozoa used in this experiment (amoebae and microflagellates from seawater) survived for 6 months in a 3.0% Rila salt solution by adding *E. coli* (10^9 cells/ml) as the sole nutrient source. The protozoa had a lytic (destructive) affect toward the *E. coli* (Enzinger 1976). However, *E. coli* numbers declined gradually in completely sterile waters (Enzinger 1976), signifying that saltwater with protozoa predators eliminate *E. coli* more efficiently than just saltwater alone (see graph 1). In this experiment, day two to day four exhibits a ten-fold increase of the number of protozoan predators resulting in the most sever reduction of *E. coli* (see graph 1) (Enzinger 1976).



FIG. 2. E. coli removal and predator growth in samples containing penicillin and streptomycin (500 mg of each per liter). Symbols: $(\bigcirc \ \bigcirc \bigcirc)$ E. coli in unseeded autoclaved bay water; $(\bigcirc \ \bigcirc)$ E. coli survival in autoclaved water seeded with 1.0 ml of freshly collected untreated bay water; $(\blacktriangledown \ \neg \)$ predator growth in autoclaved water seeded with 1.0 ml of freshly collected, untreated bay water.

<u>**Graph 1.**</u> *E. coli* populations in waters with and without protozoa present (Enzinger 1976).

Before day two, deficient protozoa populations accounted for the slow die-off of *E. coli*. However, once the protozoa multiplied, *E. coli* populations declined logarithmically. This lag time reduced when a less concentrated sample of *E. coli* was added (Enzinger 1976). *E. coli* concentrations in samples lacking predators (unseeded), remained high. This study shows that the presence of protozoa leads to significant coliform reduction (Enzinger 1976).

The organism *Bdellovibrios* was abundant in each of the samples where protozoa eliminated *E. coli* (Enzinger 1976). This organism preys upon 70 to 85% of recovered bacteria in an estuarine environment (Yair 2003) and 40-76% of bacterial strains and

isolates from ocean waters (Rice 1998). More information on this protozoan is found later in the report.

5.2 Sunlight

The effects of sunlight factor heavily on the survival of *E. coli*. As discussed above, seawater affects the population of *E. coli*. When adding sunlight to *E. coli* in natural seawater, the combination of saltwater predators and sunlight destroys *E. coli* more efficiently (see Graph 2) (McMambridge 1981). When adding *E. coli* to sterilized seawater incubated in the dark, the numbers remain virtually unchanged (McMambridge 1981). Exposing the *E. coli* sample in sterile water (no predators) to sunlight reduces *E. coli* numbers to levels similar to samples exposed to predators in the dark. The combined action of predators and solar radiation produces a significantly greater reduction in the *E. coli* numbers than each factor acting independently (McMambridge 1981). For example, a sample of *E. coli* with protozoa incubated in the dark reduced the numbers from 5 x 10^8 to 6 x 10^2 organisms per ml after 10 days of incubation. With sunlight and predators, *E. coli* numbers were reduced from 6 x 10^8 to 0 organisms per ml in 8 days.



Graph 2 – Effect of Sunlight and Predators on concentrations of E. coli

This research indicates that saltwater predators, salinity and sunlight decrease the populations of *E. coli* in water. Roger Fujioka conducted an experiment investigating whether freshwater predators and sunlight affect *E. coli* populations (Fujioka 1981). All samples of water were taken from natural water sources (both fresh and salt) and were not sterilized, leaving natural predators in the samples. The results show that the seawater sample with no light took 2-4 days to reach T_{90} (the amount of time for 90% of *E. coli* population to inactivate). Seawater exposed to natural light took 90 min to reach T_{90} (Fujioka 1981). When conducting this experiment with predators in freshwater, the population of *E. coli* remained stable when incubated in the dark and died off slightly in freshwater exposed to natural light (see graph 3). Fujiokas study also showed that visible

light, not UV light, kills bacteria and visible light can penetrate glass, linear polyethylene, and at least 3.3m of clear seawater (Fujioka 1981).



<u>**Graph 3**</u> – Fecal Coliform (FC) and Fecal Streptococci (FS) concentrations exposed to freshwater and saltwater in sunlight

5.3 pH

The pH level of water affects *E. coli* survival. Fecal coliform elimination peaks at pH values greater than 9. When exposed to pH values ranging from 7.5 to 8.75 little dieoff was observed. (Pearson 1987).

I5.4 Predation

As mentioned earlier, the protozoa bdellovibrios (belonging to genus *Bdellovibrio*, was present in all saltwater samples of Enzinger's experiment where *E. coli* was successfully eliminated (Enzinger 1976). A closer look at these organisms explains how. Bdellovibrio inhabits fresh and brackish water, sewage, water reservoirs, and seawater (Kadouri 2005). The marine forms require NaCl for its growth (Williams 1981, Sutton 1994). Optimal range for halophilic, estuarine bdellovibrios is 20-30° C and 0.6 – 1.6% salinity (Rice 1998). Bdellovibrios, unlike most bacteriophages recovered in marine or brackish waters, typically prey on a broad range of gram-negative species (Rice 1998, Wilkinson 2001, Nunez 2005) including fecal indicators *E. coli* and enterococci.

Bdellovibrio invade host cell (gram-negative bacteria) (Varon 1969, Yair 2003, Kadouri 2005) and increase in size until they divide into four to six daughter cells leaving behind the ghost of the host cells (Varon 1969, Kadouri 2005) (see Figure 1). The new generations of bdellovibrio cells move actively and can immediately infect other host cells (Varon 1969). Survival depends on locating and successfully penetrating a prey cell before starvation (Straley 1977). Bdellovibrio require a minimum density of 1.5×10^5 host cells/ml to have a 50% survival rate over a 10h period and a minimum of 10^7 host cells/ml for population growth (Rice 1998). If prey populations fall below the required concentrations the protozoa can survive if suitable sources of carbon and energy are present (Straley 1977).

Host concentration is important because bdellovibrios hunts by random collisions, not by chemical signals (Straley 1977). Hunting in this manner requires speed, which earned bdellovibrio the title of the fastest motile bacteria (Yair 2003). Efficiency of bdellovibrio predation increases at the water surface compared to the water column (Rice 1998, Nunez 2005) because surface associated bacteria may be less mobile and therefore easier targets for the predators (Rice 1998). Surface bacteria may also be larger, providing more room for a greater number of daughter cells to develop (Rice 1998). This protozoa can even multiply using dead *E. coli* cells, killed by temperature (above 70° C) or sunlight, thereby not relying on host viability for its preproduction (Rice 1998, Varon 1969).



Figure 1 – Life cycle of *Bdellvibrios* (Tudor 2006)

A true test of bdellovibrios accomplishments is the effect it has on biofilms. Biofilms are complex microbial communities that are resistant to attack by bacteriophages and to removal by drugs and chemicals (Nunez 2005). The extent of damage brought about by *Bdellovibrio* on *E. coli* biofilms was visualized by SEM imaging. The protozoa destroyed the bulk of biofilm cells, leaving behind cell residue and matrix (Kadouri 2005). Nutrient concentration also has an affect on the ability of bdellovibrio to destroy *E. coli* biofilms. Bdellovibrio completely eliminated *E. coli* biofilms in a diluted medium, even if the biofilm is allowed 24 hours to establish itself before adding the predator (Nunez 2005). In a richer medium, bdellovibrios diminished the number of cells on the surface to half the concentration in a predator-free environment (Nunez 2005).

Natural bdellovibrio populations studied in the Chesapeake Bay and around the Great Barrier Reef fluctuate seasonally (Williams 1981, Sutton 1994). The highest populations occur in the summer months and deacrease throughout the fall, until they reach undetectable levels in the winter months (Williams 1981, Sutton 1994). This pattern correlates with water temperature (Sutton 1994). However, they can be recovered in the benthic sediment in the winter months even when absent from the water column above (Williams 1981). This observation leads scientists to believe that the benthic sediments serve as a habitat for bdellovibrios throughout the winter months (Williams 1981).

5.5 Multiplying indicators

Previously, scientists believed that fecal indicators do not have the ability to multiply outside of a host (Anderson 2005, Meyers 2006). However, recent findings prove otherwise (Anderson 2005, Solo-Gabriele 2000, Davies 1995, Desmarais 2002, Byappanahalli 2005, Ferfuson 2005). In Solo-Gabrielles experiment, *E*. coli contaminated soil samples were submerged into *E. coli* contaminated water and dried in cycles (Solo-Gabriele 2000). *E coli* concentrations of the water and soil were measured during each cycle. The sediment samples cycled in and out of the water sample as follows: 6h wet, 6h dry, 12h wet, 6h dry, 6h wet, 12h dry, etc. For each test, initial moisture of soil, concentration of *E. coli* in soil, and concentration of *E. coli* in the water were taken. Results showed that soil moisture affects the ability of *E. coli* to multiply in sediments. Soil that had dried to 0.8% moisture exhibited the highest change in

concentration of *E. coli* in the soils and in the overlying water after twelve hours, rising from 15 organisms/gram to over 4.8×10^4 organisms/gram in soil samples and 200 organisms/100ml to greater than 1.5×10^4 organisms/100ml in water.

Cultured *E. coli* has survived for 68 days in sediment, suggesting that sediments provide a favorable environment for the bacteria (Davies 1995). Solo-Gabrielle's experiment clearly shows the ability of *E. coli* to thrive in soils collected from shorelines of the tidally influenced tributary of the new river in Ft. Lauderdale, FL, especially when subjected to periodic wetting and drying cycles, such as tides, and the experiment showed that the initial soil moisture of the sample plays a crucial role in the ability of *E. coli* to grow within the soils tested (Solo-Gabriele 2000).

There are two possible reasons *E. coli* thrives in the soil. It is likely that *E. coli* can survive at lower soil moisture than its predators (Solo-Gabriele 2000, Davies 1995) and bacteria adsorbed to sediment particles may be protected from the influence of such factors as UV radiation, high salinity, heavy metal toxicity, and attack by bacteriophage (Davies 1995). Therefore, upon soil drying, conditions are suitable for *E. coli* growth and survival (Solo-Gabriele 2000) and show that the changing environmental conditions in tidally influenced soils help support elevated population of enteric bacteria (Desmarais 2002). Areas that experience the most extreme drying conditions—outer fringes of channel banks—will contact water from high tide, have time to dry out during low tide, and dominate the contribution of *E. coli* to the water column (Solo-Gabriele 2000). This tidal influence results in the highest levels of *E. coli* concentrations during high tides and lower concentrations during low tides (Solo-Gabriele 2000).

Depth of *E. coli* in the soil also plays a factor in its survival. When analyzing core samples taken from sediment inhabited by *E. coli*, the bacteria was only found in the top 5 cm and does not survive below this (Desmarais 2002).

In the tropics and subtropics *E. coli* proliferates in soil and natural vegetation in the absence of fecal contamination (Desmarais 2002). Studies conducted in Hawaii and other tropical environments show the soil as the source of concentrations of fecal coliforms and *E. coli* in streams (Byappanahalli 1998) especially where organic content in the soils is high (Desmarais 2002). Under natural soil conditions, microorganisms obtain nutrients more efficiently and it is hypothesized that fecal bacterial grow sporadically in response to available nutrients (see table 2 below) (Byappanahalli 1998).

	Log ₁₀ CFU/g dry soil					
Time (d)	Sterile soil (w/ nut	rients added)	Natur	al soil		
	Fecal Coliforms	E. coli	Fecal coliforms	E. coli		
0	2.75	2.75	3.05	3.05		
1	4.76	4.29	3.34	3.34		
2	5.03	4.99	Not Determined	Not Determined		
3	5.19	4.95	3.22	3.22		
5	5.57	5.45	2.74	3.04		
7	5.85	5.55	5.99	5.29		
8	6.96	6.57	5.99	5.21		
9	5.80	5.45	5.79	5.23		

<u>**Table 2**</u> – Concentration changes of fecal coliforms in natural sediments and sterile sediments with nutrients added (Byappanahalli 1998).

Sediments may contain 100 to 1,000 times as many fecal indicator bacteria as the overlying water (Davies 1995, Ferguson 2005). Thus, the calculated bacterial concentrations in sediments were not because of overlying water (Ferguson 2005). This information also supports reports that fecal coliforms can exist without a steady source of accumulation from pollution or storm drains. It can be present from an isolated incident and then over time multiply in the sediment and be reintroduced back into water sources.

When tested, these waters will show high concentrations of fecal bacteria even if there is currently no consistent addition of bacteria.

5.6 Storms and Rain Events

Storms also affect the concentration of *E. coli* in the water column. Studies conducted at the North Fork River in Fort Lauderdale, FL showed a cyclical pattern in *E. coli* concentrations two days after a storm event (Solo-Gabriele 2000). Storms may flush *E. coli* from the soil banks and then *E. coli* takes roughly two days to noticeably increase to levels that affect the water column (Solo-Gabriele 2000). However, at high tide after a storm, concentrations are significantly higher than values observed two days immediately after the storm (Solo-Gabriele 2000).

6. E. coli vs Enterococci

E. coli (a fecal coliform member (FC)) and Enterococci (ENT) are two indicator organisms recommended by the EPA to use to asses the microbiological safety of water, but they react differently to different surroundings (see table 3) (Anderson 2005). Saltwater increases the decay rates of fecal coliforms and enterococci more than freshwater (Anderson 2005). Comparing the different indicators in each of the habitats shows a greater persistence of fecal coliform in freshwater than enterococci and enterococci has a greater persistence in saltwater than fecal coliform (Anderson 2005). With this information it is important to see that the use of one regulatory standard for diverse bodies of water may prove difficult.

Water Type	Location	Decay rate from:						Overall Decay Pate	
		Dog Feces		Wastewater		Soil inoculum		Overall Decay hale	
		FC	ENT	FC	ENT	FC	ENT	FC	ENT
Fresh	Water	-0.37	-1.49	-0.27	-0.31	-0.08	-0.39	-0.24	-0.73
	Sediment	-0.03	-0.29	-0.03	-0.21	-0.02	-0.16	-0.02	-0.22
Salt	Water	-3.8	-4.2	-4.2	-1.05	-0.83	-0.99	-2.9	-2.1
	Sediment	-0.65	-3.1	-3.1	-0.22	-0.28	-0.01	-1.3	-1.1

<u>**Table 3**</u> – Decay rates for fecal coliforms and *Enterococcus* spp. from various sources (Anderson 2005). Rates are calculated as log_{10} (CFU – colony forming units 100/ml) per day for water columns and log_{10} (CFU 100/g) per day for sediments.

With information suggesting that these indicator organisms can multiply in sediments, is the public really at a higher risk of catching a swimming-related illness if levels are above the EPA limits? If environmental conditions favor indicator multiplication, *E. coli* and enterococci concentrations may rise above the limit, warranting a beach closure or warning sign. However, the existing pathogens dangerous to human health will not exceed the acceptable risk values set by the EPA. Therefore, closing a beach due to high fecal indicator concentrations may cause unnecessary concern about the beach's pollution.

In some cases the opposite scenario has been proven. An analysis of coastal areas surrounding the Florida Keys detected enteroviruses at sites contaminated with human wastes although at a majority of these sites indicator bacteria suggested good water quality (Griffin 2001).

7. Case Study

7.1 Setup

Historically, the runoff from heavy rainfall events of Unites State coastal communities has been piped to the local shorelines and released through a series of

outlets (Grace 2006). Some outfall onto the sand and create little streams to the ocean or lake while other are piped a few hundred meters off shore. This water may contain high concentrations of pathogens and indicator bacteria from sources including septic systems, animal waste, and anything else dumped into sewers—some RV owners see sewers as a dump for their waste. The water that runs through this system should be treated, but the volume of water depends on rainfall events and can become very expensive to treat. Therefore a process that is efficient and cheap must be considered. Simple structural changes to the systems would work efficiently. Initial expense may be high but the upkeep is fairly inexpensive.

One location of concern involves four beach outfalls. Holding tanks before the outfalls contain the stormwater runoff until a certain volume is reached, triggering a switch to pump water onto the beach (see Figure 2).



Figure 2 – cross-section of outfall layout on beaches.

7.2 Problem

These holding tanks may create a favorable environment for fecal indicator bacteria to sustain their populations. Levels of *E. coli* and enterococci have exceeded the limits, reaching over 10,000 for both indicators. Tests are conducted throughout the grid of storm water pipes, including the holding tanks. This system does not allow in sunlight, which would create a stressful environment for the bacteria. The water, mostly from rainfall and runoff, may not possess necessary predators to prey on the indicator organisms. As this water is pumped onto the beach, the sediment and tides create an environment conducive to multiplying.

Some of the areas along the beach exhibit *E. coli* and enterococci counts that exceed the EPA limit. Policy states these beaches should be closed or a sign should be posted. However, there may not be associated pathogens present since the high counts could be from multiplication of the indicators, not high fecal contamination. To avoid closing the beach and possibly causing economic hardship for the area, curbing the multiplication of indicators in the system may be enough to avoid summer beach closures.

7.3 Potential Solutions

To alleviate the multiplication of these organisms a few alterations to the system could be made. Incorporating transparent tops to the holding tanks, allowing in sunlight, may reduce the population of *E. coli* in the holding tanks. Even on cloudy days this should help significantly (Fujioka 1981). Experiments should also be done with culturing organisms such a bdellovibrios to add to these holding tanks to prey on the indicator organisms. With these additions the number of indicator organisms in the outfall to the beach should be lowered significantly and these changes do not use chemical or other products that may be harmful to the surrounding environment.

However, these changes may also eliminate both indicators, creating a disjoint relationship between the indicators and harmful pathogens, resulting in the need to find alternative methods to test for fecal contamination. Experiments will need to be conducted on that topic.

8. Conclusion

The EPA has authority to keep the coastal recreation areas healthy, keeping the public safe from water-borne pathogens which can cause diseases such as Hepatitis A, viral and bacterial gastroenteritis, typhoid fever, and dysentery. This is done by testing the waters for fecal contamination. In 1986, the EPA established *E. coli* and Enterococci as the recommended indicators of water contamination. These bacterial indicators do not harm humans, but high levels suggest a high probability of dangerous pathogen contamination from feces.

Information gathered should be used to create a model to predict the life span of the indicator bacteria. So far research has shown that different sources of fecal indicators, amount of sunlight, type of sediment, height of tides, rainfall events, salinity, present predators, different strains of indicator bacteria, and pH are a few of the factors that affect the life span of these relied-on bacteria in the water. The current system takes salinity into consideration and uses the mean from at least 5 counts over 30 days which allows for slight variations but because our nation's ecosystems are so diverse, problems may ensue by using the same limits for sub-tropical waters (Florida and Hawaii) as for temperate waters (Virginia and Maine). Variation is allowed if individual states propose different limits, which need approval by the EPA.

Over the last thirty years, the EPA has done a commendable job noticing the importance of water quality along coastal recreational areas. They have made it easy for the public to keep tabs on their favorite beaches through a program called Beach Advisory and Closing Online Notification (BEACON) system. The template is up and running with small bits of information entered, but this will be an extremely useful site in the years to come as information is filled in.

The main question is still whether or not the EPA is monitoring our beaches enough to keep our public healthy. There is an acceptable risk to everything we do and at this point it is up to an individual's opinion of what acceptable is. With the numbers in place now, freshwater beaches will be closed if *E. coli* concentrations rise above 126 organisms per 100 ml of water or enterococci concentrations rise above 33 organisms per 100 ml of water. For saltwater beaches, readings must be below 35 organisms per 100 ml of water. This equates to approximately 1.9% of people getting sick from freshwater beaches and 0.8% of people getting sick form saltwater beaches. Since experiments show indicators are capable of multipling in sediments, potentially influencing indicator concentration in overlying water, beaches may be closed unnecessarily. If beaches are routinely closed, coastal areas across the nation may experience economic hardships.

Current limits, based on experiments conducted in the eighties to keep the public safe, use bacteria to approximate the presence of viruses, which may be risky. The EPA should conduct more research on the correlation between fecal indicator bacteria and enteric viruses that are present in waterways. Illness rates associated with current limits are approximate at best. With faster, more accurate testing techniques and the

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availability of the internet, new information can be shared and analyzed from many sources, hopefully unveiling a new method for beach health monitoring.

References:

- Anderson, I. C., Rhodes, M., and Kator, H. 1979. <u>Sublethel Stress in</u> <u>Escherichia coli: a Function of Salinity</u>. Applied and Environmental Microbioloby. 38:1147-1152.
- 2. Anderson, K.L., Whitlock J. E. and Harwood, V. J. 2005. <u>Persistence and</u> <u>Differential Survival of Fecal Indicator Bacteria in Subtropical Waters and</u> <u>Sediments</u>. Applied and Environmental Microbiology. 71:3041-3048.
- **3.** Barcia-Lara, J., Menon, P., Servais, P and Billen, G. 1991. <u>Mortality of Fecal</u> <u>Bacteria in Seawater</u>. Applied and Environmental Microbiology. 57:885-888
- Byappanahalli, M. N. and Fujioka. 1998. Evidence that Tropical Soil Environment can Support the Growth of *Escherichia Coli*. Water Science and Technology. 38:171-174.
- 5. Clean Beaches Council. 2005. <u>2005 State of the Beach Report: Bacteria and Sand.</u>
- 6. Davies, C. M., Long, J. A., Donald, M. and Ashbolt, N. 1995. <u>Survival of Fecal</u> <u>Microorganisms in Marine and Freshwater Sediments</u>. Applied and Environmental Microbiology. 61:1888-1896.
- 7. Desmarais, T. R., Solo-Gabriele, H. M. and Palmer, C. J. 2002. <u>Influence of Soil on Fecal Indicator Organisms in a Tidally Influenced Subtropical Environment.</u> Applied and Environmental Microbiology. 68:1165-1172.
- 8. Enzinger R. M. and Cooper, R. C. 1976. <u>Role of Bacteria and Protozoa in the</u> <u>Removal of *Escherichia coli* from Estuarine Waters.</u> Applied and Environmental Microbiology. 31:758-763.
- 9. Ferguson, D., Moore, D., Getrich, M. and Zhowandai, M. 2005. <u>Enumeration</u> and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. Journal of Applied Microbiology 99:598-608.
- 10. Fujioka, R. S., Hashimoto, H. H., Siwak, E. B., and Young, R. 1981. Effect of Sunlight on Survival of Indicator Bacteria in Seawater. Applied and Environmental Biology. 41:690-696.
- **11. Gomez, M., de la Rue, A, Garralon, G., Plaza, F., and Hontoria, M. A.** 2006. <u>Urban wastewater disinfection by filtration technologies.</u> Desalination. 190:16-28.
- **12. Grace, R**. 2006. <u>Considerations for Storm-Water Outfalls off Exposed Coasts</u>. Journal of Irrigation and Drainage Engineering. 132:609-618.

- **13. Griffin, D.** 2001. <u>Marine Recreation and Public Health Microbiology: Quest for</u> the Ideal Indicator. BioScience. 51:817-825.
- **14. Kadouri, D. and O'Toole, A.** 2005. <u>Susceptibility of Biofilms to *Bdellovibrio bacteriovorus* Attack. Applied and Environmental Microbiology. 71:4044-4051.</u>
- 15. Mara, D. D., and Johnson, M. J. 2006. <u>Aerated Rock Filters for Enhanced</u> <u>Ammonia and Fecal Coliform Removal from Facultative Pond Effluents</u>. Journal of Environmental Engineering. 132:(4) 574-577
- **16. Marlow & Company**. 2004. <u>The Value of America's Beaches to the Economy</u>. American Shore and Beach Preservation Association.
- **17. McCambridge, J. and McMeekin, T. A.** 1981. <u>Effect of Solar Radiation and</u> <u>Predacious Microorganisms on Survival of Fecal and Other Bacteria.</u> Applied and Environmental Microbiology. 41:1083-1087.
- **18. Mitchell, M and Stapp, W.** <u>Field Manual for Water Quality Monitoring: An</u> <u>Environmental Education Program for Schools.</u> 89-91.
- 19. Myers, J. 6/29/2006. Bacteria in sand baffle scientists. Duluth News Tribune.
- **20.** Nunez, M., Martin, M., Chan, P., and Spain, E. 2005. <u>Predation, death, and survival in a biofilm: *Bdellovibrio* investigated by atomic force microscopy. Biointerfaces. 42:263-271.</u>
- **21. Oasis Design.** 2006. <u>Fecal Coliform Bacteria Counts: What They Really Mean</u> <u>About Water Quality.</u> www.oasisdesign.net/water/quality/coliform.htm
- 22. Pearson, H., Mara, D., Mills, S. and Smallman, D. 1987. <u>Physico-chemical parameters influencing faecal bacterial survival in waste stabilization ponds.</u> Water Science Technology. 19:145-152.
- 23. Rhodes, M. W. and Kator, H. 1988. <u>Survival of E. coli and Salmonella spp. In</u> <u>Estuarine Environments.</u> Applied and Environmental Microbiology. 54:2902-2907
- **24. Rice, T., Williams, H. and Turng, B.** 1998. <u>Susceptibility of Bacteria in</u> <u>Estuarine Environments to Autochthonous Bdellovibrios.</u> Microbial Ecology. 35:256-264.
- **25. Richardson, S. D. and Rusch, K.** 2005. <u>Fecal coliform Removal within a</u> <u>Marshland Upwelling System Consisting of Scatlake Soils.</u> Journal of Environmental Engineering. 131:60-70.
- **26.** Sanders, B. F., Arega, F. and Sutula, M. 2005. <u>Modeling the dry-weather tidal</u> cycling of fecal indicator bacteria in surface waters of an intertidal wetland. Water Research. 39:3394-3408.

- **27. Solo-Gabriele, H. M et al.** 2000. <u>Sources of *Escherichia coli* in a Coastal Subtropical Environment. Applied and Environmental Microbiology. 66:230-237</u>
- **28. Straley, S. and Conti, S.** 1977. <u>Chemotaxis by *Bdellovibrio bacteriovorus* Toward Prey.</u> American Society for Microbiology. 132:628-640.
- **29. Sutton, D.**, and Besant, P. 1994. <u>Ecology and characteristics of bdellovibrios</u> from three tropical marine habitats. Marine Biology. 119:313-320.
- **30. Tudor, J.** 2006. Research Interests. http://www.sju.edu/biology/jtudorweb/ tudorresearch.html
- **31. U.S. EPA.** <u>Monitoring and Assessing Water Quality: Volunteer Stream</u> <u>Monitoring.</u> www.epa.gov/cgi-bin/epaprintonly.cgi
- **32. Varon, M and Shilo, M.** 1969. <u>Interaction of *Bdellovibrio bacteriovorus* and <u>Host Bacteria</u>. Journal of Bacteriology. 99:136-141.</u>
- **33. Wetz, J., Lipp, E., et al.** 2004. <u>Presence, infectivity, and stability of enteric</u> <u>viruses in seawater: relationship to marine water quality in the Florida Keys.</u> Marine Pollution Bulletin. 48:698-704.
- **34. Wilkinson, Michael.** 2001. <u>Predation in the Presence of Decoys: An Inhibitory</u> <u>Factor on Pathogen Control by Bacteriophages or Bdellovibrios in Dense and</u> <u>Diverse Ecosystems</u>. Journal of Theoretical Biology. 208:27-36.
- **35. Williams, H., Falkler, W. Jr., and Shay, D.** 1981. <u>Seasonal distribution of bdellovibrios at the mouth of the Patuxent River in the Chesapeake Bay</u>. Canadian Journal of Microbiology. 28:111-116.
- **36.** Yair, S., Yaacov, D., Susan, K. and Jurkevitch, E. 2003. <u>Small eats big:</u> ecology and diversity of *Bdellovibrio* and like organisms, and their dynamics in predator-prey interactions. Agronomie. 23:443-439.