

# Pathogen Occurrence and Analysis in Relation to Water Quality Attainment in San Francisco Bay Area Watersheds

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## **Introduction**

### PROBLEM STATEMENT

Microorganisms occur in every niche of the environment and play important roles in ecological processes. They are essential to decomposition and to the release of nutrients needed for life in both aquatic and terrestrial systems. However, a small proportion of microorganisms cause human diseases (USEPA 2001). Disease-causing organisms (pathogens) in water can infect humans through skin and mucous membrane contact or ingestion of contaminated water, and through the consumption of contaminated food, especially fish and shellfish. Water-borne and seafood-borne infection have been known to cause skin rashes, sore throats, ear, eye and respiratory infections, stomach aches, minor or serious cases of nausea and dysentery, fever, paralysis and even death.

### PATHOGENS ISSUES IN WATERSHEDS OF THE BAY AREA

Contamination of surface waters with disease causing organisms is of great concern to environmental managers and human health workers in the San Francisco Bay region. The local Region 2 Regional Water Quality Control Board (RWQCB) under authority from the California EPA is responsible for addressing pathogen and other water quality issues through the Total Maximum Daily Load (TMDL) process conforming to the expectations prescribed by the Clean Water Act.

Section 303(d) of the Clean Water Act requires that impaired water bodies be identified. Impaired water bodies are those where water quality standards are not expected to be met after implementation of the best available technological controls, with respect to permitted wastewater. Water quality standards are generally based on: (1) designated uses (such as fish and wildlife habitat and recreational use); (2) any narrative or numeric water quality objectives; and (3) anti-degradation or maintenance of ambient water quality. The 303(d) actions provide the RWQCB with a planning tool to identify waters where regulatory programs are not addressing water quality issues of concern to the public.

Once a water body is listed under Section 303(d), the State is required to determine the amount that the pollutant of concern must be reduced to meet the applicable water quality standard and eliminate beneficial use impairment. This allocation of allowable pollutant discharge from various sources is called a Total Maximum Daily Load, or TMDL. The U.S. EPA specified in its 1991 guidance that a TMDL have two essential functions:

- 1) That the TMDL process be used for implementing state water quality standards; that is, it be a planning process that will lead to the goal of meeting water quality standards; and

- 2) That the TMDL be a numerical quantity determining the present and near future maximum load of pollutants from point and nonpoint sources as well as from background sources to receiving water bodies that will not violate the state water quality standards (with an adequate margin of safety). The permissible load is then allocated by the state agency among point and nonpoint sources.

On the 1998 list of impaired water bodies, there were four watersheds in the Bay Area that were listed for pathogens. Lagunitas Creek flows into the head of Tomales Bay and is current listed for nutrients, sediments, and pathogens. TMDLs for Lagunitas Creek are being developed by the RWQCB and its local partners in conjunction with TMDL development for Tomales Bay. Napa, Sonoma, and Petaluma are also listed for sediments, nutrients, and pathogens. The RWQCB is addressing these TMDLs through local partners, SFEI (nutrients and pathogens), and Stillwater Sciences Inc. (sediments) (Stillwater Sci. Inc. 2002; Pearce et al., 2002). The TMDLs for pathogens are due in 2005 (Napa, Sonoma, and Petaluma) and 2007 (Lagunitas).

Existing (contact) recreational water quality standards are for fecal coliform (log mean < 200 most probable number/ 100 ml or 90 percentile of samples < 400 MPN) and total coliform (a median < 240 most probable number/ 100 ml or no sample > 10,000 MPN) (California Regional Water Quality Control Board 1995). These may be supplemented by EPA's recommended 1986 criteria for water quality recreation.

## OBJECTIVES OF THIS REVIEW

The regulatory framework for water quality attainment of water bodies in the Bay Area was the impetus for the following brief review of the occurrence and measurement of pathogens in the environment (with an emphasis on freshwater). In that context the objectives were:

- 1) To review the occurrence, measurement and regulatory framework of pathogens in freshwater environments; and
- 2) To note cautionary issues with current measurement methods and make recommendations on possible innovative techniques.

## **General occurrence, properties and sources**

### OCCURRENCE AND PROPERTIES

Water-borne bacterial illnesses such as cholera, typhoid, campylobacteriosis, and parasitic diseases (e.g., those caused by amoebas and tapeworms) are common throughout the world although viruses cause the majority of water-borne and seafood related illnesses (National Research Council 2000). In the United States, many water-borne diseases, especially the non-viral ones are relatively rare (Laws 1993) due to water

treatment, food sanitation, and other measures. Instead, in the U.S. we are concerned with pathogens like the protozoans, giardia and cryptosporidium, that are resistant to our conventional drinking water treatment practices (Laws 1993). In the U.S we are also concerned about the risks to the public posed by shellfish consumption and by contact with water-borne pathogens through recreational activities (Table 1). The National Research Council (2000) cites a 1984 GAO report that attributes the largest occurrences of water-borne and shellfish-borne illnesses to the Norwalk viruses.

**Table 1.** Some of the important pathogens in the U.S. and their health effects (modified from U.S. EPA 2001- TMDL protocol).

<b>Bacteria</b>	<b>Effects</b>
<i>Escherichia coli</i> 0157:H7 (enteropathogenic)	Vomiting, diarrhea
<i>Salmonella</i>	Diarrhea, dehydration
<i>Shigella</i>	Bacillary dysentery
<b>Protozoan</b>	
<i>Cryptosporidium</i>	Diarrhea, death in susceptible populations
<i>Giardia lamblia</i>	Mild to severe diarrhea, nausea, indigestion
<b>Virus</b>	
<i>Adenovirus</i> 48 serotypes; 40 and 41 are of primary concern	Respiratory disease, gastroenteritis
<i>Enterovirus</i> (68 types, e.g., polio, echo, encephalitis, conjunctivitis, and Coxsackie viruses)	Gastroenteritis, heart anomalies, meningitis
<i>Hepatitis A</i>	Jaundice, fever
<i>Reovirus</i>	Vomiting, diarrhea
<i>Rotavirus</i>	Vomiting, diarrhea
<i>Calicivirus</i> (e.g., Norwalk-like and Vomiting, diarrhea Sapporo-like viruses)	Vomiting, diarrhea
<i>Astrovirus</i>	Vomiting, diarrhea

## SOURCES OF PATHOGENS

The primary sources of water-borne pathogens are thought to be fecal pollution from humans and other animals (National Research Council 2000), with transmission of disease determined by the concentration of the pathogen and an individual's natural resistance (Laws 1993). Common sources of water-borne pathogens include outfalls from sewage treatment plants, septic tanks, meat packing plants, and waste lagoons from dairy operations. Other more diffuse sources of fecal contamination include storm runoff from streets (cat and dog feces) and farm fields (cow, sheep, horse and other farm animal feces), wildlife, and boat waste-discharge. Given the range of possible sources, it is clear that the sources and processes that transfer contamination into the receiving waters will be watershed specific. An important part of investigations in the TMDL listed watersheds of the Bay Area will be to determine what combination of sources and processes are

causing impairment. This information will increase the likelihood of implementation of sound management solutions. Water-borne pathogens may be found in the water column and in bed sediment. Pathogens present in bed sediment can be reintroduced to the water upon disturbance during periods of high water flow in the winter season, or may be a direct cause of a loss of a beneficial use if these pathogens enter the tissue of animals such as bivalves.

Public health studies and regulatory monitoring tend to focus on human waste contamination of waters, though there are known human pathogens whose origins are not fecal contamination. *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio cholerae* (pathogen of the cholera bacteria that continues to cause large pandemics) occur naturally in the environment (National Research Council 2000; Jiang 2001; Griffin et al. 2001) and cause illness through contaminated food or water. In recent years, the bilges of international ships have been recognized as possible sources for human pathogens, as well as other exotic organisms (National Research Council 2000; National Research Council 1996).

## **Origins of current regulations – history and science**

The earliest national recommendations for (recreation) water quality criteria for fecal coliform were established in 1968 by the National Technical Advisory Committee of the Department of the Interior (U.S.EPA 1986). They were based on national Public Health Service studies from 1948 to 1950, which used total coliform as the indicator. Data for these epidemiological studies were collected at beaches on Lake Michigan, the Ohio River, Long Island Sound, and New Rochelle, New York. Participating families throughout a summer recorded swimming behavior and illnesses. Water quality was measured, and illness risks were correlated with indicator counts. Later the researchers transposed the total coliform index into a fecal coliform index in the belief that the latter would be a more precise measure of gastroenteritis risk. Subsequent researchers faulted the study on its design, its methodology, and the basis by which the total coliform index was created and transformed into a fecal coliform index.

A series of studies that begun in 1972 and lasted into the early 1980s set out to correct the deficiencies of the previous research (Cabelli 1983; Dufour 1984). One goal was to show quantitative relationships between specific indicator(s) and a health risk for bathers in marine and fresh waters (several indicators were used to monitor water quality). These “Bathing Beach Studies” showed a statistically significant excess of gastroenteritis for people who swam in waters impacted by treated sewage. As a result, the 1986 EPA *Ambient Water Quality Criteria* recommends the use of E.coli or enterococci in fresh waters and enterococci alone in marine waters instead of fecal coliform (U.S.EPA 1986).

Despite the 1986 EPA recommendations, only eight states used recommended average and single maximum sample standards for recreational marine waters as of 2000, and only ten states used them for fresh waters (Natural Resources Defense Council 2001).

Monitoring agencies use different bacterial indicators, measure them at different frequencies, and have varied “action” thresholds and responses to exceedences. Many public beaches are monitored either infrequently or not at all. Monitoring, however, has increased over time, as have responses to threshold exceedences. According to the Natural Resources Defense Council, there were 11,270 beach closings in 2000 across the U.S., indicating a major public health problem at public beaches. Beach closings nearly doubled from 1999 to 2000, but it is not clear whether this reflects an increased incidence of water pollution at beaches or improved monitoring and action. Ironically, 56% of pollution problems at U.S. beaches were reportedly from unknown sources.

Many current water quality studies look at potential risks presented by swimming or eating seafood caught in marine waters possibly at the expense of studies on fresh waters. This could be due to the tremendous number of people potentially exposed to polluted marine recreation waters, such as the 600,000 that reportedly visit Santa Monica Bay on a typical summer weekend, and also in recognition of the fact that seafood consumption is still a main source and potentially deadly route of disease. Nevertheless, scientists have gained important knowledge about indicators and pathogens in both marine and fresh water systems.

For example, organisms have different rates of survival or productivity under distinct environmental variables, such as salinity, UV light, and temperature. The EPA made use of this finding in its 1986 recommendations for water quality criteria of recreational waters that advocate distinct bacterial indicators for fresh waters and for marine waters (Dufour 1984). Current literature indicates that this was an improvement over previous coliform standards but still insufficient to protect the public from the risk of water-borne illness (which is not limited to gastroenteritis). And, despite the information gap with respect to recreation in fresh waters, it may be prudent in the future to further refine the water quality standards for all impaired water bodies (fresh, estuarine and marine) to include only scientifically supported bacterial/ viral indicators and pathogens (i.e., dropping fecal and total coliform from criteria and replacing them with others).

## **Evolution in investigation and analysis: techniques for analysis**

### APPROACHES TO PATHOGEN INVESTIGATION

Over the years, scientific study has shown that many different organisms are responsible for human illness (with viruses being responsible for most), however scientific advances have not eliminated worldwide illness and mortality due to contaminated food and water (National Research Council 2000). Monitoring for all potential pathogens is expensive and impractical because pathogens have variable characteristics (Laws 1993). That is, sources vary and the pathogens themselves respond distinctively to factors such as time, temperature, salinity, UV radiation, and susceptibility to standard water treatment.

Perhaps due primarily to cost and tradition, the most widely practiced water quality monitoring for human pathogens to date, has been the use of bacterial indicators. The bacterial indicator strategy has been used in conjunction with epidemiological studies (Cabelli 1983; Dufour 1984; Prüss 1998; van Asperen et al. 1998) and most recently, it has been used as a point of departure for testing alternative methods for pathogen detection.

Indicators are used to signal the presence of fecal contamination in water and therefore an impairment of water quality. To protect human health from water-borne pathogens, the EPA recommends monitoring marine recreational waters for *enterococci* (recommended threshold: geometric mean of 35 /100mL), and in fresh water, *E.coli* (126 /100 mL threshold) or *enterococci* (33/100mL) (U.S.EPA 2001). For shellfish harvesting, total coliform (not exceeding a geometric mean of 70 MPN per 100 mL, with not more than 10 percent of the samples taken during any 30-day period exceeding 230 MPN per 100 mL) and fecal coliform monitoring (median concentration should not exceed 14 MPN per 100 mL with not more than 10 percent of the samples taken during any 30-day period exceeding 43 MPN per 100 mL) is recommended (U.S.EPA 2001).

According to Griffin and colleagues (2001), the historical concept of the ideal water quality indicators (first used in relation to drinking water) is that they should be organisms that are only present where pathogens are, though in much larger numbers. The indicator density should relate to the degree of contamination and to the health hazard. The ideal indicator organisms should not be pathogens themselves and they should be more resistant to common treatment practices than pathogens, and should not exist in an unpolluted environment.

The most common indicators to date have been “total coliform” bacteria, containing a subset of fecal coliform. Using total coliform includes counting fecal and non-fecal coliform, which may result in data that are misleading and do not relate to the risk of water-borne illness (National Research Council 2000). Fecal coliform is thought to be a better indicator of fecal contamination because fecal bacteria tolerate higher environmental temperatures; hence, they are more similar to the fecal-oral pathogens (Griffin et al. 2001). The indicator analyses used today require culturing the bacteria until numbers of “colony forming units” can be enumerated.

Unfortunately, there are numerous limitations to the conventional indicator practice. The National Research Council (2000) and Griffin and colleagues (2001) name several as they relate to total and fecal coliform, *enterococci*, and *Clostridium perfringens*:

- Non- coliform bacteria such as *vibrio* and *pseudomonas* may mimic coliform colonies and be counted as such.
- Non-fecal coliform bacteria such as *Klebsiella pneumoniae* are erroneously counted in the fecal coliform analysis.

- Fecal coliform can be long-lived in aquatic habitats, especially in estuaries and in shellfish.
- Fecal coliform have little if any quantifiable relationship with human pathogens.
- Bacterial indicators do not accurately indicate direct fecal pollution over a range of geographic locations and distinct environments (i.e., tropics versus temperate; high saline versus brackish versus fresh).
- The sources of fecal coliform and *enterococci* are not necessarily human but often presumed to be so.
- Current media-based assays require a minimum of 12-18 hours.
- Current assays have a problem with false negatives and false positives, they also do not count bacteria that are viable but dormant phase (non-culturable).
- Occurrence or high concentration of indicator bacteria does not necessarily indicate public health risk, and low concentration of these do not necessarily indicate a low public health risk.
- Alternatively, low bacterial counts in the water column, do not assure that disturbance of sediment will not resuspend pathogens that are stored there.

Studies that link cause and effect with respect to disease outbreaks, or better yet, a dose- response relationship for a disease-causing agent provide a solid scientific foundation for public health policy. The EPA preference for *E.coli* and *enterococci* as indicators for recreational water is based on epidemiological studies showing a correlation between these bacteria and an increased rate of gastroenteritis in exposed bathers (Cabelli 1983; Dufour 1984).<sup>1</sup> Prüss points out the difficulty of conducting epidemiological studies and their limitations in her 1998 survey (Prüss 1998). For example, there is little if any consistency among epidemiological studies in such factors as identification of symptoms, forming control groups, and illness reporting techniques. And within each study, these and other aspects of research methodology were found to be ambiguous. The EPA studies are not above some of these common problems. The methodological challenges are probably some of the reasons, along with expense and ethical issues, that so few epidemiological studies have been reported for the period covered by this review (1998 – 2002), and why few are likely to be pursued in the near future.

In contrast, progress in laboratory technologies and an improved understanding of microbiology have opened up new doors for the identification of disease-causing agents and alternative indicator organisms. Current research often compares densities of traditional indicators to those of water-borne pathogens. It demonstrates that indicator methodology is particularly problematic in tracking viruses, but some bacterial pathogens have also been missed by traditional strategies. A study in Santa Monica Bay (Noble and Fuhrman 2001), for example, collected water samples near typical urban discharge sources (natural and manmade drainages) and tested for total coliform, fecal coliform, *enterococci*, and for enteroviruses. Results showed no correlations between single

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<sup>1</sup> . The earlier water quality recommendations for recreation water by the NTAC in 1968 were not adopted as criteria by the National Academy of Sciences- National Academy of Engineers because of insufficient data (Dufour 1984). These recommendations, however, are what many states use today as water quality standards, including California (California Regional Water Quality Control Board 1995).

bacterial indicators and enteroviruses, though there was a weak correlation between a composite of the bacterial indicators and enteroviruses. Doré and colleagues (Doré et al. 2000) collected market-ready oysters that met European Community standards for *E.coli*, but found high levels of the viral indicator, FRNA bacteriophage, which was strongly correlated with harvest area fecal pollution and shellfish associated disease outbreaks in the UK. Researchers in Spain found inconsistent relationships between polluted water samples (high in standard fecal indicators) and the existence of salmonella in those samples. Salmonella was positive in marine and reservoir samples with high geometric means of fecal coliform, total coliform and/ or *enterococcus*, but in river samples with high indicator levels, salmonella was absent. And, in Boston Harbor, which has a constant stream of fecal contamination, no indicator (neither bacterial nor viral) correlated well with any other (Ricca and Cooney 1999).

In the context of this review, it is important to consider whether the traditional indicators are adequate predictors of disease risk given the pathogen sources and the relevant pathways of human disease in Bay Area watersheds. Many of the urban and rural land uses in the San Francisco Bay Area watersheds (industrial uses, low to high density residential, pasture, crop lands, and concentrated livestock operations) potentially contribute pathogens to local waters. Pathogens from streets and rural areas are introduced to watercourses during storm events, and treatment facilities and private septic systems release waste to the Bay and streams on a daily basis<sup>2</sup>. Sediment is also an important reservoir of bacteria, which can be released into the water column upon disturbance (Crabill et al. 1999; Obiri-Danso and Jones 2000).

Although recreation and shellfish water quality research has not been carried out for pathogens in the Bay Area watersheds, research elsewhere does provide useful information. We now know that the indicator methodology does not provide the whole story of potential risks to public health. This suggests that there may be benefits if the EPA recommendations are supplemented (or even replaced) in the Bay Area watersheds by a strategy that includes a baseline survey of environmental conditions, and a monitoring program that includes assays for relevant pathogens and indicators. Only in this way could we be completely assured to protect local recreationists and seafood consumers from human and animal- derived pathogens.

## INNOVATIVE MONITORING STRATEGIES

Numerous authors have used alternative monitoring strategies for pathogens and/ or indicators. Using implanted bivalves, for example, was a strategy use by one research team to differentiate human and animal waste sources and the associated pathogens, information commonly lacking in traditional monitoring programs (Donnison and Ross

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<sup>2</sup> According to the U.S. EPA, typical treatment plants reduce but do not eliminate bacterial loads of wastewaters (USEPA 2001). An average of 80 million gallons per day of treated wastewater enters the Bay from the EBMUD treatment plant (EBMUD 2002). Larger volumes of wastewater with lower treatment levels enter the Bay during large storms when this and other facilities around the Bay exceed storage capacity.

1999). Another team found that zebra mussels retained peak *E.coli* levels longer than conventional water samples and therefore could be used to produce more effective monitoring strategies in streams (Selegan et al. 2001). Crabill and colleagues monitored bacterial indicators in sediment and water in an Arizona recreation area (Crabill et al. 1999). They looked at the seasonal trends and found reservoirs of bacteria in sediment influencing water quality when direct human sources were no longer present.

Traditional monitoring virtually ignores viruses, most probably due to laborious techniques necessary to culture them. Recent work illustrates important strides in tracking viruses through alternative indicators or direct pathogen detection. The presence of F-RNA bacteriophage, a viral indicator, demonstrated a lingering health risk in oysters after *E.coli* had been flushed to meet consumption standards (Doré et al. 2000). One limitation of this cultured assay is that it does not distinguish between human and animal viruses so it would be most valuable if used in seafood harvest areas that are known to receive human fecal waste. Reverse-transcriptase polymerase chain reaction (RT-PCR) assays have been used in numerous investigations and could be an effective monitoring device in the future. Thirty-two percent of samples collected by Noble and colleagues in Santa Monica Bay during 2001 were positive for viruses using this laboratory technique. The advantage of RT-PCR is that it can rapidly detect a specific organism at very low concentrations, and it has detected viruses in fresh and marine water settings. RT-PCR has been used successfully in Florida, detecting human waste at sites where bacterial indicators had negative results (Griffin et al. 2001). One limitation, however, is that inhibitory compounds can confound results, as was the case in Mamala Bay, Hawaii, where viral cell culture techniques were more effective than RT-PCR (Reynolds et al. 1998).

## **Conclusions and Recommendations**

Decades of marine and fresh surface water monitoring have focused on bacterial indicators in order to advise public health officials regarding risks of disease outbreaks due to fecal contamination. Research since the 1970s, however, has shown that densities of traditional indicator bacteria, such as *E.coli*, *enterococci*, total coliform, and fecal coliform are not reliably correlated with the incidence of water-borne pathogens. In this survey of recent studies, alternative monitoring strategies have been presented that could improve the ability of regulatory agencies to protect the public from health risks posed by pathogens in recreational and seafood harvest waters.

Although we currently have no scientific analyses of Bay Area pathogens and their sources, there is much that can be gleaned from existing studies in other watersheds. Current water quality standards in the San Francisco Basin Plan, for example, are not adequate to completely protect the public. The supplemental recommendations for *E.coli* and *enterococci*, though more specific indicators for recreational waters, are probably not sufficient to protect people from what are thought to be the principle water-borne pathogens: viruses.

That is why we recommend that the RWQCB consider the following steps to create a pathogen monitoring/ pathogen reduction program that would dovetail with the TMDL process for the San Francisco watersheds. The program should:

- Survey each impaired watershed for probable human and pathogen sources.
- Initiate long term monitoring for several relevant indicators and pathogens, considering water, sediment, and bivalve samples.
- Establish baseline conditions for organisms and significant environmental variables (e.g. conductivity, temperature, wind, rainfall).
- Establish new water quality standards that represent the best available knowledge of health risks.

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