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Assessment of the Extra-Enteric Behavior of Fecal Indicator Organisms in Ambient Waters

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1. Executive Summary

The U.S. Environmental Protection Agency (hereafter EPA) is charged with the development of criteria and regulations to protect the public from exposure to microbiological contaminants of fecal origin in recreational water. In 1986, EPA promulgated recreational ambient water quality criteria based on a series of epidemiological studies that were conducted in the late 1970s and early 1980s and apply to all waters of the United States that have a recreational use designation. The studies were conducted in temperate fresh and marine recreational waters that were impacted by point sources of human sewage. The criteria correspond to the increased risk of acute gastroenteritis (gastrointestinal [GI] illness) resulting from exposure to contaminated water as measured by the presence of *E. coli* or enterococci—both of which are "fecal indicator" bacteria that grow in the gastrointestinal tract of humans and other warm-blooded animals and are excreted in large numbers in feces—in fresh recreational waters or measures of enterococci in marine waters. Several other epidemiological studies, most of which were conducted in temperate regions of the world, have shown a consistent and often strong correlation between traditional bacterial indicators and human illness. The illnesses are usually some form of acute GI illness resulting from recreation in water contaminated by point sources of human sewage. Nonpoint microbial pollution also represents a major source of contamination of marine and fresh recreational waters, and other epidemiological studies have concluded that there is a poor correlation between levels of the traditional bacterial indicators and nonpoint sources of microbial pollution.

While the relationship between the presence of traditional fecal indicators in ambient water and human illness has been quantified in many temperate regions, numerous researchers have reported the presence of fecal indicator bacteria in the aquatic environment in the absence of human fecal contamination. Although these exceptions were originally noted in tropical and subtropical regions, they have been documented increasingly in temperate areas. Collectively, these studies suggest that under particular conditions, fecal indicator organisms from a variety of point and especially nonpoint sources (e.g., stormwater runoff, domestic pets, birds) can colonize and proliferate in ambient waters and associated sand and sediment. As a consequence, routine monitoring of fecal indicator bacteria may detect fecal indicator concentrations in excess of federal or state water quality standards—possibly resulting in unnecessary action being taken, such as beach advisories and closings (and also listings of ambient waters on the Clean Water Act (CWA) 303(d) list of impaired waters).

In 2001, a group of 18 national and international experts convened the "Tropical Water Quality Indicator Workshop" to discuss the growing body of literature and research on potential limitations of the continued reliance on fecal indicator bacteria for assessing the microbial quality of recreational waters in tropical and subtropical regions. The focus of the workshop was to evaluate the problems associated with appropriate water quality standards in tropical locations, as described and reported by experts/scientists from Hawaii, Guam, Puerto Rico, and south Florida. The participants agreed that reliable interpretations of the current recreational water quality standards in (sub)tropical locations may be compromised due to environmental sources of fecal indicator bacteria. Such consensus statements represent agreements in understanding regarding how environmental factors can control the behavior of traditional indicator microorganisms in ambient waters and how these factors can affect the development and use of

water quality criteria and standards in recreational environments. Given the importance and relevance of that workshop, details of its objectives, goals, and other consensus statements are summarized in Appendix A of this draft report.

Various environmental parameters can affect the behavior of fecal indicator microorganisms in the aquatic environment. The parameters that can affect the concentration of fecal indicator organisms include the following: temperature, rainfall, light, runoff, suspended solids, turbidity, water depth, stratification, mixing, resuspension, pH, alkalinity, and salinity. Because these environmental parameters may have different and potentially significant effects on the presence of indicator microorganisms depending upon the climate (tropical, subtropical, or temperate) and the combination of parameters, they are reviewed in this draft report. The resulting effects can be extreme; for example, blooms of *E. coli* in inland water reservoirs in Australia may be classified incorrectly as a result of recent fecal contamination. Misclassification of fecal associated organisms as evidence of human fecal contamination as opposed to indigenous populations may result ultimately in beach closings and unneeded, expensive, and ineffective mitigation efforts.

A comprehensive review of the literature indicates that the most important general features of environments influencing the growth or persistence of indicator organisms are, in order of importance, sunlight, salinity, and temperature. Synergy between the effects of temperature and sunlight and temperature and salinity were observed. Other factors playing important roles in the persistence of indicator organisms are soil type and properties (for organisms in soils and sediments or for particle-associated bacteria) and competition and predation from the indigenous population. Growth of indicator organisms has been reported or inferred in all climate zones (tropical, subtropical, and temperate) of concern in this draft report; in all water types (marine, estuarine, and fresh); and in soils and the water column—with the exception of growth in the marine water column. Absence of observations of growth in the marine water column does not necessarily indicate there is no potential for growth. Site extensive properties (rainfall, mixing, and watershed characteristics) play important roles in determining the occurrence of indicator organisms and are related to extreme variability of indicator counts in the water column and sediments.

There are several opportunities for further analyses of data in the literature survey, though data gaps must be filled before these analyses can be conducted. First, studies exploring the kinetics (growth and decay) of indicator organisms could be analyzed together in an attempt to ascertain optimal conditions for growth or persistence. This analysis will require quantification of the role of predation on observed decay rates and assembly of additional data collected in experiments in soils and sediments, and in samples collected in regions where fecal impacts from agricultural sources are expected. Other analyses could seek to relate indicator organism occurrence and temporal variations to rainfall, watershed characteristics, and mixing. Challenges to the development and execution of these analyses will be that the literature are characterized by a number of well-studied environments and relatively few studies on other environments such as riverine environments, and that most studies in the literature provide only qualitative data on watershed hydrologic characteristics and fecal indicator organism loadings.

Due to the documented limitations of culturable methods to detect and enumerate traditional fecal bacteria as indicators of waterborne pathogens in tropical and subtropical regions of the United States and abroad, a variety of alternative indicators and indicator approaches have been proposed and assessed in recreational areas. Such alternative approaches can be divided broadly into those that are microbiologically-based and those that involve the use of chemical markers. This draft report reviews many of the more promising indicators and indicator approaches. Much of the research into alternative indicators of microbial water quality is closely tied to microbial (fecal) source tracking efforts. Notably, whether an approach is chemically- or microbiologically-based, these alternative methods are used often in conjunction with traditional bacterial indicators to assess their validity and utility in assessing microbial water quality.

Another promising approach to assessing microbial water quality involves the development and use of water quality notification models—most commonly simple heuristic models that relate precipitation or land use to water quality. These models are often used in conjunction with the collection and enumeration of traditional microbial indicator organisms. However, a discussion of modeling approaches and applications as related to the use of alternative indicators for assessing microbial water quality is beyond the scope of this draft report.

Until the last decade or so, most alternative microbiological indicators to assess recreational water quality criteria have been culturable bacteria. With the growing development and use of molecular detection techniques (e.g., polymerase chain reaction [PCR]), a broader variety of bacteria and now viruses (especially viruses of bacteria, called bacteriophage or phage) are now available as potential alternatives to traditional fecal indicator bacteria. Notably, these techniques have also given rise to the science needed to develop robust approaches to identify and track the sources of microbial contamination, using both traditional indicator bacteria as well as many of the novel bacteria and viruses that are discussed in this draft report. Given these technological advances, some researchers have advocated approaches to detect so-called indicator bacteria (e.g., *Staphylococcus aureus*, *Vibrio vulnificus*) and viruses (e.g., enteroviruses) that are actually or potentially pathogenic, especially to immunocompromised persons. However, a discussion of the direct detection of pathogens to assess microbial water quality is also beyond the scope of this draft report.

Researchers have been documenting issues and limitations associated with the use of traditional indicator bacteria for assessing the presence of fecal contamination in recreational waters for decades. Many of the limitations noted in this draft report were reported initially in first tropical, then subtropical regions of the United States and abroad. It is becoming clear that sand, sediment, and soil can serve as reservoirs of fecal indicator bacteria in many tropical, subtropical, and temperate recreational waters. Generally, studies agree that such media provide microenvironments that are more suitable to the survivability and (re)growth of fecal indicator bacteria—and thus potentially for some waterborne pathogens—than the water column. However, current EPA recreational water quality criteria and state water quality standards do not typically require monitoring of beach sand for fecal indicator bacteria. Thus, many researchers have questioned the validity of continued reliance on the periodic measurement of fecal indicator bacteria in the water column as the sole means of determining the microbial quality of ambient waters.

To help address and potentially resolve these limitations, researchers have evaluated and reported on the use of a wide variety of alternative (and increasingly sophisticated) microbiological and chemical indicators of fecal contamination in ambient waters in a variety of regions. Despite the limitations of current fecal indicator bacteria, there is no clear support for the widespread use of an alternative indicator to replace current fecal indicators in ambient waters in tropical, subtropical, or temperate regions. The 2004 National Research Council (NRC) report, *Indicators for Waterborne Pathogens*, concluded "indicator approaches will still be required for the foreseeable future because it is not practical or feasible to monitor for the complete spectrum of microorganisms that may occur in source waters for drinking water and **recreational waters** [emphasis added], and many known pathogens are difficult to detect directly and reliably in water samples." This fact has not changed in the six years since the NRC committee of experts issued this key conclusion. Confidence in the development and use of alternative indicators to supplement or potentially replace current indicators could increase, however, as EPA and others conduct health studies that demonstrate a statistically valid correlation between the presence of one or more alternative indicators with an increased incidence of illness in exposed persons, thus ensuring that the use of a new indicator is based on health risk.

2. Background and Introduction

2.1 Background

The EPA is charged with the development of criteria and regulations to protect the public from exposure to microbiological contaminants of fecal origin in recreational water. The overall goal of the current ambient water quality criteria (AWQC) for bacteria in the United States is to provide public health protection from gastroenteritis (GI illness) associated with exposure to fecal contamination during water-contact recreation. Periodic review of the scientific literature and re-evaluation of emerging scientific issues including detection and enumeration methodologies are required to meet these requirements effectively. Since EPA's most recent release of recreational water quality criteria in 1986, there have been significant advances, particularly in the areas of molecular biology, microbiology, and analytical chemistry. EPA believes that that these and other scientific and technical advances need to be considered and evaluated for feasibility and applicability in the development of new or revised CWA Section 304(a) criteria for recreation by 2012. To this end, EPA has been conducting research and assessing relevant scientific and technical information to provide the scientific foundation for the development of new or revised criteria.

The enactment of the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 (which amended the CWA) required EPA to conduct new studies and issue new or revised criteria—specifically for Great Lakes and coastal marine waters. To help address and fulfill the requirements of the BEACH Act, in March 2007, EPA convened a group of 43 national and international technical, scientific, and implementation experts from academia, numerous states, public interest groups, EPA, and other federal agencies at a formal workshop (referred to as the Airlie Workshop) to discuss the state of the science on recreational water quality research and implementation issues (USEPA 2007a). In addition to the written report of the Airlie Workshop, EPA developed a *Critical Path Science Plan for Development of New or Revised Recreational Water Quality Criteria* (USEPA 2007b) by soliciting and considering the feedback, detailed input, and recommendations from the larger group of stakeholders who represented the general public, public interest groups, state and local government, industry, and municipal wastewater treatment professionals. One key question posed in the Science Plan is the extent to which indicators of waterborne pathogens perform differently in a tropical environment compared to temperate or subtropical environments. The Science Plan emphasizes the need to better understand the underlying causes for any differences.

The purpose of this draft report is to survey the existing knowledgebase to determine what the available data say with regard to indicator behavior in ambient waters and what differences exist in currently used fecal indicators when they are applied in tropical, subtropical, and temperate regions. Additionally, potential alternative indicators will be described and their applicability in tropical and subtropical environments will be discussed.

2.2 Introduction

Millions of people swim and recreate in coastal and inland waters in temperate, tropical, and subtropical locations throughout the United States and its territories every year (Dorfman and

Stoner 2007). These polluted waterbodies can contribute to waterborne illness, especially in children and people with compromised immune systems. Annually, an estimated 120 million gastrointestinal illnesses are experienced globally due to contact with polluted coastal waters, resulting in $12 billion in public health costs (Shuval 2003). It is believed that the majority of the illnesses are caused by exposure to pathogenic bacteria and viruses of human fecal origin (NRC 2004, USEPA 2009). The World Health Organization (WHO) has identified at least 20 pathogens found in recreational water that can cause severe health effects, including adenoviruses, hepatitis viruses, *Giardia lamblia*, *Cryptosporidium parvum*, pathogenic *Escherichia coli*, *Campylobacter* spp., and *Salmonella* spp. (WHO 2005).

Public health can be effectively protected by monitoring and controlling the microbial pathogens that cause illness. Theoretically, regular monitoring of these microorganisms could identify where and when concentrations reach a level associated with risk of human illness. Monitoring results could be used to post of warnings of water contamination or to make decisions about the closing of beaches by public health officials. However, many pathogens are difficult or costly to collect and detect (Griffin et al. 2001). Because widespread monitoring of recreational waters directly for all disease-causing microorganisms remains infeasible, public health and environmental protection agencies have relied on the detection of fecal indicator organisms to indicate the presence and magnitude of fecal material. This approach assumes that waterborne pathogens co-occur with the fecal material and that greater quantities of fecal contamination yield greater quantities of pathogens (NRC 2004).

The properties of ideal indicators are presented in Chapter 4 of the NRC report titled *Indicators of Waterborne Pathogens* (NRC 2004). Effective indicator organisms have the following characteristics:

- a demonstrated correlation with health risk;
- similar or greater survival time than the target pathogen;
- similar or greater transport than the target pathogen;
- presence in greater numbers than the pathogen; and
- specificity to a fecal source or an identifiable source of origin.

Thus, the development of an indicator for use in recreational waters includes both the identification of the organism that will reliably predict the presence of fecal contamination as well as the method used to analyze the indicator. Consequently, effective indicator analysis methods should have the following characteristics (NRC 2004):

- be specific to the desired target organism;
- have broad applicability;
- be precise;
- have adequate sensitivity;
- produce rapid results;
- be quantifiable;
- measure viability and/or infectivity; and
- be logistically feasible.

These indicator characteristics are supported by the World Health Organization (WHO), which has a similar set of criteria for effective indicators of fecal contamination. Accordingly, indicators should (WHO 2005):

- be universally present in large numbers in the feces of humans and animals;
- not multiply in natural waters;
- persist in water in a similar manner to fecal pathogens;
- be present in water in higher numbers than fecal pathogens;
- respond to treatment processes in a similar fashion to fecal pathogens; and
- be readily detected by simple, inexpensive methods.

The "classic" indicators include culturable total coliforms, fecal (thermotolerant) coliforms, *E. coli* (an important member of the coliform group), and enterococci, all of which have been used for decades (Field and Samadpour 2007, Fujioka 2001, Schwab 2007). However, because the total coliform group is often found in vegetation and soil, yielding variable correlation with public health, it is no longer widely used in recreational waters of the US and cannot be used by Great Lakes states (Cabelli et al. 1983, USEPA 2004).

Though states set their own water quality standards, in 1986 the USEPA recommended enterococci as recreational water fecal indicator bacteria in marine water and *E. coli* for freshwater (USEPA 1986). Both bacteria are facultative anaerobes, though enterococci are Gram positive while *E. coli* are Gram negative. These bacteria are highly concentrated in fecal material and easy and inexpensive to detect. Furthermore, a series of epidemiological studies conducted in the 1970s and 1980s (Cabelli et al. 1982, Cabelli et al. 1983, Dufour 1984) demonstrated these indicators were correlated with gastrointestinal symptoms in swimmers at temperate marine and freshwater beaches affected by point source fecal pollution.

The epidemiological data were generated at a small number of temperate locations with known sources of fecal pollution and then generalized for use in all US ambient waters, regardless of climate, geography and source of pollution. There is growing concern that these indicators may not be effective in all climatic zones and geographic locations (for definitions of climates, refer to Text Box 1); for example, traditional fecal indicator bacteria are observed at levels exceeding EPA criteria in Hawaii even in the absence of fecal contamination (Fujioka et al. 1997). Particular concerns have been raised in other tropical and subtropical regions of the US and its territories because designated indicators appear to flourish and reproduce in many soils, sediments, algal wrack, and other benthic systems (Solo-Gabriele et al. 2000, Desmarais et al. 2002, Byappanahalli et al. 2003, Byappanahalli and Fujioka 2004, Yamahara et al. 2007). These inconsistencies in the behavior of *E. coli* and enterococci in ambient waters suggest that using fecal indicators that have not been validated widely in diverse geographic regions could result in significant numbers of false positive results, which could lead to warnings, violations, and potential beach closings in the absence of actual fecal contamination of recreational waters. The estimates for annual costs of beach closures in California alone range from $17,000,000 to $179,000,000 per year (Pendleton 2008).

There are a number of reasons to expect that the efficacy of *E. coli* and enterococci as fecal indicator bacteria may not be appropriately extrapolated from one geographic region across the

Text Box 1. Definitions of Climates

Temperate climate—Very generally, the climatic zone of the "middle" latitudes; the variable climates between the extremes of tropical climate and polar climate.

Subtropical climate—In general, a climatic zone with a climate typical of the subtropics, with warm temperatures and meager precipitation.

Tropical climate—In general, a climatic zone with a climate typical of equatorial and tropical regions; that is, one with continually high temperatures and with considerable precipitation, at least during part of the year.

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Definitions of climates come from the Glossary of Meteorology:
http://amsglossary.allenpress.com/glossary/.

entire United States. As mentioned previously, indigenous populations of these traditional fecal indicator bacteria have been found in tropical soil (Fujioka et al. 1997), which is problematic because the use of fecal indicator organisms is predicated on the presumption that there are no significant environmental sources of these microorganisms. There are also likely differences in sources of fecal contamination among geographic areas and climates. While *E. coli* and enterococci may be appropriate proxies for health effects in water directly affected by sewage, epidemiological studies have shown that there is a poor correlation between levels of fecal indicator bacteria and nonpoint sources of microbial pollution (Colford et al. 2007). Nonpoint pollution sources to recreational water areas are diffuse and much more difficult to identify, regulate, and mitigate. They typically include agricultural and stormwater runoff, malfunctioning septic tanks, and fecal waste from domestic pets and wildlife, especially birds. Although animals shed high numbers of traditional fecal indicator bacteria that can enter recreational waters, there has been a long-held presumption that animal sources of pathogens (including zoonotic pathogens, which infect humans but may be carried by animals) and fecal indicator bacteria are of less public health concern than those released by humans. Nevertheless, epidemiology data demonstrate a relationship between adverse health effects and swimming in nonpoint source-affected waters (Haile et al. 1999).

Physical and biological factors that affect the fate and transport of traditional fecal indicator bacteria also vary across climates. Meteorological factors such as temperature and light have been shown to limit traditional fecal indicator bacteria persistence (Fujioka and Byappanahalli 2003, Solic and Krstuvolic 1992). Rainfall has also been associated with increased traditional fecal indicator bacteria concentrations in surface waters (Lipp et al. 2001). Biological factors that may differ between climates and geographic zones also affect traditional fecal indicator bacteria survival. Predation can eliminate up to 86% of enteric bacteria released into seawater (Iriberri et al. 1994), but predator populations, including zooplankton and protists, have spatial variation (Piontkovski and Williams 1995). Available nutrients, which also vary spatially and temporally, also affect fecal indicator bacteria survival in surface waters (Korhonen and Martikainen 1991).

The EPA has long recognized these potential weaknesses of *E. coli* and enterococci as fecal indicator bacteria in diverse and particularly subtropical and tropical regions. A "Tropical Indicators Workshop" was convened by the EPA Office of Water, the Department of Health of the State of Hawaii, and the Water Resources Center of the University of Hawaii in 2001 to address these weaknesses. Experts from this workshop agreed that the environmental characteristics of the tropics affect the relationship between indicators of fecal contamination and health effects observed in bathers, compromising the efficacy of the EPA recreational water quality guidelines. (For full treatment of this workshop, refer to Appendix A). According to the *Critical Path Science Plan for Development of New or Revised Recreational Water Quality Criteria* (USEPA 2007b), EPA intends to conduct epidemiological tests to determine whether temperate-derived fecal indicator bacteria standards are appropriately extended to tropical regions. However, because indicator persistence and behavior in tropical waters has not been well-characterized, the EPA first requires a comprehensive literature review to determine the extent to which current indicators may perform differently in tropical, subtropical, and temperate environments to better understand the underlying causes for any difference (USEPA 2007b). This is the purpose of this draft report.

This draft report reviews and summarizes research on the applicability and behavior of fecal indicators in ambient waters and related environments in which the indicators may fail to meet NRC (2004) and WHO (2005) criteria for effectiveness outlined above. This paper does not discuss direct linkages or correlations between the indicators and the pathogens that ultimately cause human illness due to exposure to fecal matter. Literature was reviewed to identify evidence that geography, climate, and various environmental parameters influence the effectiveness of fecal indicators. The specific search strategies and databases employed are provided in Appendix B. Because the purpose of using indicators is to discriminate between recreational waters that are contaminated with feces that could pose risks to humans and water that is safe for recreational use, conditions that could result in either an indicator not being detected when there is a risk (false negative), or in the detection of indicators in the absence of pathogens when there is not a risk (false positive) are highlighted and explored. Persistence, growth, and regrowth of indicators in soils, sands, and sediments are of particular concern because the literature suggests that false positives are often the result of regrowth in sediments (e.g., Grant et al. 2001). The rates and extent of fecal indicator (re)growth for different conditions are compared and contrasted to determine whether differences exist based on locations, climate classifications, salinities, and other environmental parameters.

The focus of most of the literature presented is on characterizing results of monitoring for fecal indicators in specific climates and environments, with particular emphasis on tropical, subtropical, and temperate climates and regions and areas characterized by different levels of fecal impact (expected or observed significant impacts from human sewage, expected or observed impacts from human agriculture/animal operations, and expected or observed limited impacts from human sources). Temperate, subtropical, and tropical climates were previously defined in Text Box 1. These definitions are very broad, and it is unlikely that the biological and ecological processes associated with the effective use of these indicators can be attributed to such general climate categories. It is more likely that specific processes occur at particular sites that are related to the large-scale climatic conditions, but they may not be generalized across all recreational waters in a particular climate category. Ashbolt et al. (1997) asserted that the

concentration of organisms and the relationship between indicator occurrence and concentration to human illness are highly site-specific.

Subsequent sections of this paper document the behavior, including fate and transport, of indicator organisms in a variety of aquatic environments. The focus is on summarizing evidence that monitoring for indicators of fecal contamination can lead to erroneous conclusions regarding exposure to pathogens that can cause human illness and to identify data gaps and research opportunities to address these critical issues.

3. Ambient Factors and their Effects on Indicator Presence and Behavior

3.1 Introduction

The literature suggests that traditional fecal indicator bacteria (fecal coliforms, *E. coli*, and enterococci) may persist and multiply independent of human fecal contamination in various geographic locations. Studies conducted in the tropics and subtropics show proliferation of *E. coli*, enterococci, and/or fecal coliforms in sediment and sand associated with inland water (Alm et al. 2003 and 2006, An et al. 2002, Davies et al. 1995, Fujioka et al. 1999, Whitman and Nevers 2003, Whitman et al. 2003) and coastal waters (Bonilla et al. 2007, Brownell et al. 2007, Craig et al. 2003, Davies et al. 1995, Ferguson et al. 2005, Ghinsberg et al. 1994, Lee et al. 2006, Oshiro and Fujioka 1995 Yamahara et al. 2007). Studies show proliferation of fecal indicator bacteria in coastal (Grant et al. 2001) and inland vegetation (Byappanahalli et al. 2007, Rivera et al. 1988, Whitman et al. 2003 and 2005) and that changing environmental conditions in tidally-influenced sediments help support elevated populations of fecal indicator bacteria in water (Bonilla et al. 2007, Desmarais et al. 2002, Solo-Gabriele et al. 2000). Recent research in the Great Lakes region (Alm et al. 2003 and 2006, Byappanahalli et al. 2006, Whitman and Nevers 2003) demonstrates that traditional fecal indicator bacteria can also persist and possibly (re)grow in the sand, soil, and sediment. Bacteria harbored in the sand may persist longer than in the water because they adhere to sediment particles, unlike free bacteria in the water. Sand acts as a natural filter that traps environmental particulates and organic matter, providing a habitat for growth of bacteria including fecal indicator organisms. Microorganisms in the beach sand can then be mobilized during rising tides or from storms and transported to the water column (Yamahara et al. 2007).

To facilitate a discussion of the efficacy of indicators for waterborne pathogens in recreational waters, it is useful to understand the effects of environmental parameters on indicators in different geographic regions. Relevant research has been conducted over the past 20 years in tropical, subtropical, and temperate regions to evaluate the effects of geography and climate on indicators. Some of the most relevant environmental parameters that affect survival and persistence of fecal indicator bacteria are temperature, salinity, and ultraviolet (UV) irradiation (Fujioka and Byappanahalli 2003, Solíc and Krstuvolic 1992). The survival of these microorganisms may also be enhanced due to deposition or adsorption once they are introduced to coastal waters (Gerba and McLeod 1976). Rainfall has been associated with increased concentrations of fecal indicators, and extreme wet weather events may overwhelm wastewater treatment plants and result in runoff from urban and rural areas, leading to increased loading of fecal associated microorganisms (Fujioka and Byappanahalli 2003, Lipp et al. 2001). Storms may also result in the reintroduction of microorganisms due to resuspension (Lipp et al. 2001).

This section includes a review of studies conducted in tropical, subtropical, and temperate climates in which the effects of environmental parameters on fecal indicator bacteria were evaluated. The ability of fecal indicator bacteria to survive and multiply in the environment independent of human contamination is described and followed by a short section on the effects of environmental parameters on target pathogens. A summary section emphasizing key points concludes this section.

3.2 Environmental Parameters that Potentially Affect the Behavior of Fecal Indicators in Different Geographic Regions

Various environmental parameters can affect the behavior of fecal indicator bacteria in ambient waters and the benthic environment. These parameters may have differing effects on the indicator microorganisms depending upon the climate (e.g., tropical, subtropical, or temperate). Section 4 and Appendix C include information and summary tables of both general and context-specific parameters that may affect the occurrence, persistence, or growth of fecal indicator organisms in environmental waters.

This paper does not exhaustively discuss the effects of environmental parameters on target pathogens and focuses for the most part on traditional fecal indicator bacteria and other well studied alternative indicators and pathogens. However, it is important to review some key environmental waterborne zoonotic pathogens for the scope of this paper. For a detailed discussion of this topic, please refer to *Review of Zoonotic Pathogens in Ambient Waters* (USEPA 2009). Some of the most commonly studied environmental parameters affecting pathogen persistence and survival in water include pH, salinity, light exposure, and temperature. However, additional factors such as UV light (duration, intensity), rainfall, runoff, dispersal, suspended solids, turbidity, nutrients, organic content, organic foams, water quality, biological community in water column, water depth, stratification, mixing (e.g., wind and waves), presence of aquatic plants, biofilms, and predation.

Because fecal indicator bacteria are considered proxies for the pathogens of concern, it is important to point out some of the environmental conditions relevant to the six key pathogens of concern. Although waterborne outbreaks of *E. coli* are not as common as foodborne (Boczek et al. 2007), it is estimated that from 1971 to 2000 approximately 30% of waterborne diarrheal illness was due to contact with untreated recreational water (Craun et al. 2004). One of the more common forms of pathogenic *E. coli*, Enterohemorrhagic (EHEC) O157:H7 and other EHEC variants, are zoonotic pathogens associated with severe human illnesses. Ruminants such as cattle are considered the dominant natural reservoir; however, water has also been implicated as a means of transmission. Due to methodological limitations, evaluation of the persistence of EHEC in the ambient environment has remained elusive (Muniesa et al. 2006).

Campylobacter spp. has been implicated in 3% of waterborne outbreaks (1991 to 2002) and *Salmonella* spp. was responsible for 0.9% of waterborne disease outbreaks (1971 to 2000) according to Craun et al. (2004). Illness cause by *Leptospira* infection has been related to unusual rainfall events (Bolin et al. 2004, Craun et al. 2004), as well as the rainy season, and in temperate climates, peak concentrations are seen during summer and fall, due to the pathogen's preference for warm, humid conditions (Levett 2001). *Cryptosporidium* is one of the leading causes of waterborne diarrheal outbreaks in the United States and increases in disease incidence are correlated with run-off events (CDC 2007, Tate et al. 2000). *Giardia* infections have an association with increased bather density and higher turbidity due to resuspension of cysts in the sediments by bathers (Graczyk et al. 2007, Sunderland et al. 2007). While the risk and severity of human illness associated with exposure to treated and untreated sewage remains the greatest threat, the implications of human illness due to contamination with animal feces and zoonotic pathogens should not be underestimated. It is important to remember that the aforementioned

waterborne pathogens are most likely to have the most debilitating effects on the elderly, children, and immunocomprised individuals. Pathogenic *E. coli* has the greatest implications for severe illness and been implicated in several deaths (USEPA 2009).

3.3 Effects of Environmental Parameters on Fecal Indicators in Different Geographic Regions

As discussed previously, fecal indicator bacteria are present in large numbers in the feces of humans and animals, however in order to be effective, they must, persist in water in a similar manner to fecal pathogens, be present in water in higher numbers than fecal pathogens, and respond to treatment processes in a similar fashion to fecal pathogens (NRC 2004, WHO 2005). Fecal indicator bacteria that persist or grow in the aquatic environment are no longer specific to recent fecal contamination events and thus may not be protective of public health (WHO 2005). Effects of environmental parameters on persistence and (re)growth of indicator organisms in tropical, subtropical, and temperate regions are discussed in subsequent subsections.

3.3.1 The Great Lakes Region

The Great Lakes Region has a temperate climate, with well-defined seasons. Areas around the Great Lakes are moderated in temperature by the surrounding water body, but can also create snow belts in the winter. The evaporation from the Great Lakes also increases the amount cloud cover and thus incident solar irradiation. (http://www.city-data.com/states/Michigan-Climate.html).

The occurrence of autochthonous (indigenous) populations of fecal indicator microorganisms is not limited to tropical and subtropical regions. Although temperate coastal regions such as the Great Lakes experience some environmental conditions different than sub-tropical regions, traditional fecal indicator microorganisms including *E. coli* and enterococci have been shown to persist and grow in ambient waters and associated benthic environments. Moist sand provides a presumably suitable environment for many microorganisms including protection from sunlight, a large surface area for biofilms, buffered temperatures, a steady supply of organic material, and microhabitat protection from predation (Whitman and Nevers 2003). As in tropical and subtropical regions, populations of fecal indicator microorganisms do not necessarily relate directly to a recent fecal contamination event. The EPA Environmental Monitoring for Public Access and Community Tracking (EMPACT) study investigated fecal indicator microorganisms on five beaches, including four located in temperate climates: a Massachusetts ocean beach, a Maryland estuarine beach, a Lake Michigan beach, and a Detroit riverine beach (Wymer et al. 2005). The EMPACT researchers identified several physicochemical parameters that affected the persistence and regrowth of fecal indicator microorganisms, including but not limited to the following: tides, sunlight, time of day of sampling, precipitation, water temperature, and air temperature. The EMPACT study showed that the amount of sunlight can impact the water and air temperatures, thus emphasizing the complexity involved in predicting how these physicochemical parameters may affect the persistence and regrowth of fecal indicator microorganisms in recreational waters.

Temperature has been shown to have an effect on persistence of fecal indicator microorganisms. Historically, *E. coli* were thought to be unable to survive long outside of human or animal bodies, in part due to the cooler temperatures of temperate waters—especially winter temperatures. Wang and Doyle (1998) found that the pathogen *E. coli* O157:H7 can survive up to 91 days at 8º C. Under these cooler temperatures, the bacteria entered a viable but nonculturable (VBNC) state until temperatures warmed again, when the bacteria regained metabolic activity. Whitman and Nevers (2003) determined that *E. coli* can survive winter temperatures, including freezes, in the sands on a Lake Michigan beach. Samples of beach sand showed a significant increase in *E. coli* counts between April and May. After May, *E. coli* counts in the sand remained relatively stable for the rest of the summer. In addition, Vigness et al. (2006) demonstrated that *E. coli* found in Lake Michigan beach sands could tolerate temperatures as high as 44.5º C. These studies suggest that fecal indicator bacteria such as *E. coli* can persist in sand in low and freezing temperatures during winter months in temperate regions and then regrow when temperatures increase.

Humidity and water availability can also play a role in fecal indicator microorganism survival. Vigness et al. (2006) showed that humidity affects the ability of *E. coli* to grow in beach sands. Samples from a Lake Michigan beach were sterilized and inoculated with *E. coli*, which was then allowed to dry under natural conditions. Upon desiccation (humidity of <5 percent), *E. coli* entered a metabolically inert state but remained viable. The bacteria resumed active growth after rehydration. Byappanhalli et al. (2006) demonstrated that higher humidity encouraged growth of *E. coli* in soils of the Dunes Creek watershed on the southern shore of Lake Michigan.

Beach sand and sediments have been shown to act as habitats and reservoirs for *E. coli* (Ishii 2007). Whitman and Nevers (2003) examined the foreshore sand at a Chicago beach on Lake Michigan. The sand acted as a nonpoint source of *E. coli* and could support an indigenous population of *E. coli* for extended periods of time, regardless of additional input from humans, animals, or lake waters. The bacteria adhered to sand particles, thus providing physical protection to the bacteria. Beach sand replacement studies demonstrated that *E. coli* populations decreased immediately after replacement but *E. coli* populations were re-established to prereplacement levels in as little as two weeks. Byappanhalli et al. (2006) investigated the occurrence of *E. coli* in a pristine environment in the Dunes Creek watershed on the southern shore of Lake Michigan. Forest soil plots were covered with mesh to exclude animal and human input. Higher concentrations of *E. coli* were recovered from the covered plots than from surrounding soils. Because the plots were covered, the higher concentrations were not due to human or animal input but were more likely due to the microclimate of the enclosure such as higher humidity and shading. The authors suggested that higher humidity and shading are similar to environments caused by forest canopies near river shores. Genetic analysis of the *E. coli* recovered from the soils indicated that although the initial source of the *E. coli* was from animals (such as birds and deer), over time lasting and genetically diverse populations in the soils were established. Further genetic analysis suggested that the soil-borne strains of *E. coli* belong to a different group than *E. coli* of animal origin found in the temperate coastal environment. Therefore, soils, sediments, and sand can act as a reservoir for fecal indicator microorganisms that are not associated with point source fecal contamination events.

Other biological interactions can influence the persistence and regrowth of fecal indicator microorganisms in ambient waters. Whitman et al. (2003) investigated 10 Lake Michigan beaches in Wisconsin, Illinois, Indiana, and Michigan and found that *E. coli* and enterococci were present in up to 97% of *Cladophora* samples. *Cladophora* is considered to be a nuisance macrophytic, green alga commonly found in the Great Lakes during the summer months. To investigate the survival ability of *E. coli* and enterococci in these algae, *Cladophora* mats were harvested, sun-dried, and kept at 4ºC for 6 months. Samples from the mats were then rehydrated and incubated at 35º C—a temperature similar to exposed beach or shallow waters in summer months. Regrowth of *E. coli* and enterococci occurred within 24 hours of rehydration, and stable populations were established within 72 hours. Byappanhalli et al. (2007) studied the genetic diversity of *Cladophora*-borne *E. coli* from the Indiana Dunes National Lakeshore of Lake Michigan. Over 800 strains of *E. coli* were isolated from *Cladophora* mats. The *E. coli* isolates represented a high degree of genetic diversity but were clearly genetically distinct from *E. coli* strains of fecal origin. These studies suggest that *Cladophora* may be source of recurring *E. coli* and enterococci for nearshore beaches.

In 2008, Zehms et al. conducted a study that looked at the seasonal variations and patterns of *E. coli* in both the sand and adjacent beach water. The highest number of *E. coli* were observed in the swash zone, with the highest numbers seen in the summer month, however the numbers of *E. coli* varied greatly spatially which altered the relationship of the *E. coli* seen in the sand and that of the overlying beach water in different locations.

3.3.2 Florida

Considered a subtropical climate with a mild temperatures and sunny days, Florida is dominated by high humidity and abundant rainfall due to its proximity to the Atlantic and the Gulf of Mexico, and the state's many inland lakes and ponds (http://www.city-data.com/states/Florida-Climate.html).

A number of studies have documented the effects of various environmental parameters on fecal indicator bacteria in Florida. Lipp et al. (2001) examined the effects of seasonal variability and weather on fecal indicators, including fecal coliform bacteria, enterococci, *Clostridium perfringens*, and coliphages in southwestern Florida. (Note, the latter two indicator organisms are considered to be alternative in this draft report and are discussed in Sections 5.1.1 and 5.1.4, respectively). Samples were collected from Charlotte Harbor in recreational and shellfish harvesting waters and from sediment to determine which environmental factors affected fecal indicators. With regard to chemical parameters, sampling sites with more freshwater influence, and therefore lower salinity, were associated with higher levels of all fecal indicators. Concentrations of fecal coliform bacteria, enterococci, and *C. perfringens* indicators were negatively correlated with salinity in the water column, and concentrations of fecal coliform bacteria and coliphages in sediment were also negatively correlated with salinity. In addition negative correlations with pH and concentrations of *C. perfringens* and enterococci in the water column were observed. Decreases in temperature were responsible for decreased concentrations of fecal indicator bacteria in the water column, whereas rainfall and increased stream flow coincided with increased abundances of fecal coliform bacteria, enterococci, and coliphages. Sediment concentrations of enterococci were also positively correlated with rainfall. Turbidity

was positively correlated with concentrations of *C. perfringens* in the water column and sediment.

Harwood and Rose (2004) observed sources and fate of fecal indicator organisms in Florida and found that saltwater significantly increased decay rates of fecal coliform and enterococci compared to freshwater. The study also found that fecal coliform decay rates were lower in sediments than in the water column in both freshwater and saltwater, but the difference was only significant in freshwater. Enterococci decay rates were not significantly different in sediments compared to the water column in saltwater or freshwater, but persistence in sediments tended to be greater posing the possibility that sediments provide refuge for bacteria from predation and UV irradiation.

Shibata et al. (2004) studied the effects of multiple parameters on enterococci, *E. coli*, fecal coliform, total coliforms, and *C. perfringens*. Contrary to Lipp et al. (2001), environmental parameters such as rainfall, temperature, pH, and salinity did not appear to be significantly correlated with change in most fecal indicator bacteria concentrations. The exception was total coliforms for which the concentration increased significantly in warmer weather with increasing rainfall. The authors also noted that increased concentrations of bacteria were related to close proximity to the shoreline with the greatest concentrations found at high tide when the water level peaks along the shore. In addition, *C. perfringens* concentrations were positively correlated with turbidity. The highest concentrations of *C. perfringens* and total coliform were found below seaweed, which was hypothesized to be the source of shelter from UV light, nutrients, and moist conditions.

Desmarais et al. (2002) investigated the parameters that affect the number of *E. coli*, enterococci, and *C. perfringens* in soil and sediments in the North Fork of the New River in Fort Lauderdale. This study was spurred by previous research conducted in the same river that found that *E. coli* numbers were elevated at high tide due to bacterial regrowth within tidally impacted soil embankments and contamination from storm events (Solo-Gabriele et al. 2000). Desmarais et al. (2002) documented the effects of water content in soil, amount of sediment in soil, and soil content on fecal indicators. The highest numbers of *E. coli* and *C. perfringens* were observed within 50 cm from the edge of the water where the moisture content was the highest. Sediments further from the water edge had lower numbers of indicator microbes, suggesting that water content affects sustainability of indicator populations. Changing moisture content, the addition of sediment, and increased inactivation of indigenous microorganisms in the sediment and thus increasing nutrient availability, and eliminating predation promoted the growth of *E. coli* and enterococci. In addition, soil with higher organic content, and a greater fraction of fines, stimulated growth of *E. coli*.

More recently Bonilla et al. (2007) examined the prevalence of fecal indicators (fecal coliforms, *E. coli*, somatic coliphages, F+ specific coliphages) in tidally affected beach sand with fecal indicator bacteria counts in the overlying water. Bacteria were consistently higher in beach sand, particularly moist sand, than corresponding water samples and both coliphage types were routinely detected in sand, however F+ coliphages were detected less often. Interestingly, the researchers showed that the seeding of a single sample of gull feces affected an area of the beach

sand of 3.1 m^2, indicating that there is great heterogeneity of fecal indicator detection in beach sand.

Another unlikely source of fecal indicator bacteria contamination actually comes from bathers themselves. Elmir et al. (2007) studied the effects of human shedding of enterococci and *Staphylococcus aureus*, a common skin pathogen, both directly from their skin and indirectly by adherence of sand to skin. Of particular interest, the researchers found that on average humans shed enterocci and *S. aureus* on the magnitude of 6×10^5 to 6×10^6 per person during the first 15 minutes of "bathing". The amount of bacteria that adhered to sand from the skin was small in comparison to direct shedding from the skin. This study showed that human microbial bathing load should be an important consideration as a non point source for recreational water quality models.

Abdelzaher *et al.* (2010) recently published the first known study to examine all three classes of pathogens (viral, protozoan, and bacterial) as well as fecal indicator bacteria (fecal coliforms, *E. coli*, and enterococci) in both water and sand at a sub-tropical non-point source beach. Due to the short study period (2 days) no significant relationship could be established between indicator organisms and pathogens, even though the opportunistic pathogen *Vibrio vulnificus* and *Giardia* spp. were detected as well as the presence of Human Polyomaviruses (HPyVs),which is a human source tracking marker indicative of human urine or sewage source. Notably, these researchers also determined that tidal height impacted microbe concentration in surface water and that sand proved to be a reservoir of microbes that contributed to the water column, which is conclusive with that of Bonilla et al. (2007).

3.3.3 Mississippi

Mississippi, like Florida, is considered subtropical with short winters and long, humid summers. Carr et al. (2010) examined to what extent *Salmonella* spp. existed in coastal waters and sediments and how *Salmonella* spp. correlated with enterococci, as well as salinity, turbidity, and sunlight. They found that as salinity and turbidity increased, the likelihood of detecting *Salmonella* spp. decreased. Likewise, as with other studies, when solar intensity increased the chance of detecting *Salmonella* spp. decreased. Enterococci concentrations were also suggestive of the levels of *Salmonella* spp. present. Carr and colleagues suggested that since specific pathogen examination in environmental waters has been limited, and since fecal indicator bacteria are used as proxies of pathogens of concern, that the rationale of Field and Samadpour (2007) should be utilized. That rationale suggests the identification of the pathogen of concern based upon epidemiological data, and then subsequent monitoring of those pathogens along with fecal source tracking methods in order to permit evaluation of the existence of pathogens in that water source.

3.3.4 California

Dependent on latitude, elevation, and proximity to the coast, California's climate can vary widely—primarily from arid to mediterraenean. In coastal and southern parts of the state, the influence of the ocean moderates temperature extremes, with somewhat rainy winters and dry summers, producing a Mediterranean climate.

Several studies have been conducted in California investigating the effects of environmental parameters on fecal indicator bacteria. Yamahara et al. (2007) collected beach sand along the California coast and found that 91% of samples had detectable enterococci and 62% had detectable *E. coli*. Physical parameters such as degree of wave shelter and percent fines and chemical parameters such as moisture and organic carbon content affected fecal indicator bacteria concentrations. For example, *E. coli* were more commonly found in fine sands with high moisture and organic carbon content and at wave-sheltered beaches. Increases in enterococci densities were found in the presence of a presumed source of fecal indicator bacteria and were associated with the degree of human development surrounding beaches

He et al. (2007) observed the effects of ponded waters versus flowing waters, temperature, conductivity, salinity, and nutrients on total coliform, fecal coliform, and enterococci in streams in southern California. Fecal indicator bacteria concentrations were higher in ponded water and sediment compared to flowing water likely due to higher nutrient levels and lower dissolved oxygen levels in the ponded water. Increasing temperature resulted in higher concentrations of all bacteria, whereas higher conductivity (i.e. increased salinity), had negatively impacted fecal indicator bacteria concentrations.

Other researchers (Evanson and Ambrose 2006, Lee et al. 2006) have also found that California beach sediments provided favorable environments for survival and growth of fecal indicator bacteria. Evanson and Ambrose (2006) hypothesized that increased indicator bacteria concentrations in sediment may be attributable to rainfall-induced changes in environmental parameters such as lower salinity, increased moisture and nutrient inputs.

Santoro and Boehm (2007) measured *Bacteroides* (an alternative indicator; see Sec tion IV.1.2), total coliforms, fecal coliforms, and enterococci at four coastal locations in Orange County, California to determine whether tidal variability affected microbial pollution at beaches. Abundance of fecal indicator bacteria (total coliforms, fecal coliforms, and enterococci) was significantly affected by tide level, with more fecal indicator bacteria being detected at low tide at some sampling locations. Abundance of the human-specific *Bacteroides* molecular marker did not appear to be affected by tidal variability.

Rosenfeld et al. (2006) examined effects of tidal variability on fecal indicator bacteria concentrations. The researchers observed that 50% of fecal indicator bacteria exceedances occurred within two days of the spring tide and that bacterial concentrations were highest at night as opposed to during the day concluding that total and fecal coliform levels were controlled primarily by tide and that levels of enterococci were controlled primarily by the day-night cycle.

A study by Noble et al. (2004) observed the effects of sunlight on total coliforms, *E. coli*, enterococci, and F+ coliphages (an alternative indicator; see Section 5.1.4) in fresh and saltwater. Temperature and solar irradiation significantly affected indicator organism inactivation. Sunlight was observed to increase inactivation rates by a factor of five and was found to inactivate enterococci more quickly than *E. coli* and F+ coliphages. Total suspended solids and nutrient levels did not significantly affect inactivation rates of the indicators. Inactivation rates were similar in salt and fresh waters when tested at similar temperatures.

Boehm et al. (2002) also observed increased sensitivity of total and fecal coliforms and enterococci to inactivation by sunlight which induced bacterial die-off or injury. In addition, the effect of total rainfall on fecal bacteria concentrations was evaluated. The highest mean concentrations of total coliforms were observed during the period with the most rainfall. This agrees with Dwight et al. (2002) who also observed a relationship between total coliform levels and rainfall in California. The researchers found that precipitation was significantly correlated with water discharged from rivers, and the latter was significantly correlated with bacteria levels at most of the beaches that were sampled Sampling sites closest in proximity to the river discharge point had consistently higher concentrations of total coliforms than sites further from the river discharge point

Lee et al. (2008) examined whether sediment concentration of fecal indicator bacteria persisted longer following a storm than overlying water and whether quiescent sediments at enclosed beaches retained higher fecal indicator bacteria than those at open beaches. At the open ocean beach sites, although both surface water and sediments contained increased levels of enterococci and *E. coli*, both returned to pre-storm levels within a week. At an enclosed beach, however, high levels of fecal indicator bacteria persisted in the sediments regardless of antecedent precipitation events. Even though high levels of fecal indicator bacteria were observed at the open ocean beach, high energy outward transport of microbial contaminants in the sediments would be facilitated by wave action, wheras the low energy environment found at the enclosed beach may promote bacterial sorption and biofilm formation on sediment surfaces, leading to intermittent exceedences due to resuspension events.

3.3.5 Hawaii

Although located near the tropical equator, the climate of Hawaii is more subtropical than tropical, due to the moderating effect of the surrounding sea. There is little temperature variation and the weather conditions tend to be fairly consistent, however, the climate of each Hawaiian island can differ according to whether they fall on the windward side or not. Those on the windward side experience more rain and cloud cover caused by prevailing north-easterly trade winds.

As early as 1988, Fujioka and colleagues documented the occurrence of high fecal indicator bacteria levels in mountainous streams originating in undeveloped areas and whose source was purely rainwater. The early Fujioka studies explored the plausible cause of elevated fecal indicator bacteria in these undeveloped streams and hypothesized that since there are no large animals in the area that the bacteria were most likely from the feces of birds and rats and that these bacteria must be persisting and multiplying in the warm, humid soil/water environment of Hawaii. There are several studies documenting the presence, persistence, and proliferation of fecal indicators such as *E. coli* and enterococci in Hawaii. Moisture, nutrient content, and pH levels of Hawaiian soil provide a favorable environment for indicators (Fujioka and Byappanahalli 2003). Fujioka et al. (1981) observed the effects of sunlight and salinity on fecal

coliforms and fecal streptococci (i.e., enterococci[1]) in coastal water in Oahu. The study found that sunlight dramatically inactivated fecal indicator bacteria. Bacteria survived for days in the absence of sunlight, but in the presence of sunlight, 90% of bacteria were inactivated within 30 to 180 minutes, depending on the type of bacteria. In addition, increasing salinity levels have been widely documented to negatively affect the persistence of fecal indicator bacteria.

Hardina and Fujioka (1991) conducted a study to determine the source of fecal indicator bacteria in Hawaii's pristine streams. They concluded that soil was the primary source of fecal indicator bacteria in the environment in Hawaii and that soil bound fecal indicator bacteria were transported by precipitation events into the pristine streams and rivers. These results were based on the fact that the fecal indicator bacteria, *E. coli* and enterococci, were found in all soil samples and high concentrations of these same bacteria were found in stream water. The concentrations of fecal indicator bacteria increased as the stream flowed from the mountain to the ocean in the same manner as does the land mass area, which also had greater concentrations of fecal indicators as the land mass increased. The warmer temperature and higher nutrient concentration of the soils served to create a microenvironment which favored the persistence and regrowth of fecal indicator bacteria. Fujioka and Byappanahalli (2003) also observed that rainfall was the mechanism of transport for soil-associated fecal bacteria. Rainwater falls onto small areas of land and the excess water forms streams. Soil-associated fecal bacteria are transported from the streams to storm-drains. Rainfall is a crucial environmental parameter whose effects on indicator organisms have been documented in Hawaii.

3.3.6 Guam

Guam is a tropical pacific island and due to its close proximity to the equator, it has higher humidity and warmer temperatures than the Hawaiian Islands (Hardina and Fujioka 1991. One of the studies conducted in Hawaii (Hardina and Fujioka 1991) was replicated in Guam to determine if those same standards found in Hawaii would be applicable to Guam or other tropical islands. In the Guam study (Fujioka et al. 1991), the researchers found that 66% and 88% of fresh water samples exceeded the EPA single sample standard for *E. coli* and enterococci, respectively in fresh river water, supporting the earlier of work of Hardina and Fujioka (1991). In addition, soil was also found to have high levels of both indicators and is consistent with the findings in the Hawaii studies (Hardina and Fujioka 1991). Like Hawaii, rain was the source water for streams and therefore fecal indicator bacteria bound to soil was transported to fresh water streams and rivers through runoff during precipitation events. This study also documented that several of Guam's marine beaches also exceeded the EPA recommended recreational water quality standards. This study showed that although some similarities did exist with earlier Hawaii studies, there were some notable differences. First, Guam contains few birds, which were implicated as a source of fecal indicator bacteria in Hawaii and second, the climatic conditions of Guam are more tropical than Hawaii which is located much further north.

[1] The terms fecal streptococci, enterococci, intestinal enterococci, and *Enterococcus* are often used to refer to essentially the same environmental and fecal species of bacteria (see NRC 2004 for further information). Thus, for convenience, this draft report uses the term enterococci unless otherwise noted.

3.3.7 Puerto Rico

Studies conducted in Puerto Rico also contribute to the literature on influences of environmental parameters on fecal indicators in subtropical and tropical regions. As early as 1939, Ragavachari and Iver reported that coliforms survived for several months in natural tropical river waters. Since then there have been numerous studies in Puerto Rico documenting the survival and regrowth of *E. coli* in rain forest streams, becoming part of the natural flora (Carillo et al. 1985, López-Torres et al. 1987, Valdéx-Collazi et al. 1987, Hazen et al. 1987). This work is supported by work in other laboratories in Puerto Rico and the fact fecal coliforms and enterococci have become part of the natural flora of rainforests, devoid of human and large mammalian impacts, has become "dogma" (Toranzos 1991).

Carillo et al. (1985) observed that counts of indicators such as fecal coliforms and *E. coli* were correlated with water temperature, alkalinity, nitrates plus nitrates, and total phosphorus. The authors concluded that nutrient concentration had the most influence on the density of fecal bacteria in the watershed. Further evidence is supported by one of the first studies to show that fecal coliforms could grow in and on vegetation and was conducted in Puerto Rico by Rivera et al. (1988). Samples collected from water accumulated in leaf axilae of bromeliads (an abundant epiphyte in tropical rainforests but also native to regions throughout the Americas) at pristine sites in a tropical rainforest were unexpectedly found to consistently contain fecal coliforms. Subsequent identification of positive isolates demonstrated the presence of *E. coli*, which was also isolated from leaf surfaces. The investigators noted that birds were not a likely source for the presence of *E. coli* and hypothesized that that these bacteria may at some time have originated from fecal sources and became capable of surviving indefinitely in tropical environments (thus becoming part of the normal flora of some vegetation); alternatively, *E. coli* could have always been part of the natural microflora of tropical regions. The authors concluded that ambient and water temperatures and nutrient concentrations created a favorable environment for fecal indicator bacteria.

Hartel et al. (2005) studied the survival and regrowth of enterococci in sediments in Puerto Rico. In addition to Puerto Rico, the researchers (Hartel et al. 2005) also sampled sediments from Georgia (sub tropical) and New Hampshire (temperate) and compared survival and regrowth of enterococci in marine and estuarine sediments from the three regions. The locations were chosen due to their differences in latitude, annual seasonal temperatures, and soil characteristics. The study methodology included desiccating and rewetting sediment samples after 0, 2, 30, and 60 days to determine whether enterococci could survive and regrow. The researchers found that all moist sediments had large concentrations of enterococci and enterococci counts decreased with increased length of drying. Sediments from Georgia and New Hampshire contained high concentrations of enterococci after 60 days, whereas sediment from Puerto Rico had low numbers after 60 days. The poor survival rate in the Puerto Rican sediment was attributed to the texture of the sediment, which contains a higher percentage of sand than sediments from New Hampshire and Georgia as sediments with a higher percentage of clay than dry sand are expected to have higher bacterial survival rates.

3.3.8 Australia and New Zealand

3.3.8.1 Sydney, Australia

The temperature in Sydney is moderated by its proximity to the ocean having warm summers and mild winters, and rainfall spread throughout the year. Sydney is considered to have a temperate climate. Because Sydney and Queensland have different climate types, they are considered separately in this section.

Ashbolt and Bruno (2003) performed a study on the influence of environmental parameters (rainfall, sunlight, and tidal variation) on thermotolerant (fecal) coliforms and enterococci counts at three beaches. The authors found that precipitation alone was a sufficient predictor of enterococci counts Sunlight hours and tidal variation were also sufficient predictors of enterococci counts during dry periods with increasing number of sunlight hours contributing to a decrease in enterococci counts. Higher fecal indicator counts were associated with rising or high tide 70% of the time.

Craig et al. (2001, 2004) investigated the influence of water versus sediment, sediment content, and temperature on *E. coli*, enterococci, and coliphages at three coastal sites in Australia. Notably, the studies reported that in all cases, indicator decay was greater in water compared to decay rates in sediment. *E. coli* had a higher decay rate than enterococci and coliphages in overlying water and sediment at all temperatures. In sediment samples, small particle size and high organic content were conducive to survival of indicator organisms. An inverse relationship was observed between survival of indicator organisms and temperature.

A study by Ferguson et al. (1996) examined the influences of rainfall, sewage overflow (resulting in increased organic matter in sediment), and salinity on concentrations of a number of fecal indicators including fecal coliforms, fecal streptococci (a surrogate for enterococci measurements) and *C. perfringens* spores in water and sediment samples from six sites in an urban estuary in Sydney. The study found that rainfall and sewage overflow were associated with significant increases in concentrations of all indicator organisms in water. The lowest concentrations of most indicator microorganisms were detected at the sampling site with the highest salinity. Fecal coliforms were the only microorganisms that increased significantly in sediment concentrations with rainfall. Sewage overflow resulted in increased concentrations of all indicator organisms measured in sediment.

Davies et al. (1995) studied the survival of fecal coliforms, fecal streptococci (as a surrogate for enterococci measurements), and *C. perfringens* spores in marine and freshwater sediments in Sydney. The environmental parameters investigated included predation and growth. Sediments were collected from the bottom of a pool located below the discharge point of a storm water drain at one of Sydney's northern beaches and from a marine site adjacent to a deepwater treated sewage outfall outlet. The researchers found that fecal coliforms may be capable of regrowth in both marine and freshwater sediments. When predators were present, a net die-off of indicators was observed. The difference between concentrations of fecal coliforms in the presence of predators compared with concentrations in the absence of predators was greater for fecal coliforms than for fecal streptococci. Notably, the survival of *C. perfringens* in sediments did

not appear to be affected by the presence of predators. The authors concluded that *C. perfringens* can persist in sediments for undefined periods of time whereas other fecal coliforms and fecal streptococci decayed more rapidly. An observed overall die-off effect of fecal coliforms and fecal streptococci suggested an imbalance between predation and growth in sediment, but this was not empirically tested.

3.3.8.2 Queensland, Australia

The climate along the Queensland coast ranges from hot and humid in the far north, to comfortable cool temperatures in the southeast and is considered tropical/subtropical (http://www.qldbeaches.com/climate.html). A study by Mill et al. (2006) examined the effects of spatial and tidal variation on concentrations of total coliforms, *E. coli*, and enterococci. The investigators found that tidal variation limited indicator levels in an estuarine creek where lower tidal areas of the creek were observed to reduce the contamination of point and nonpoint source contributions of fecal material and thus were associated with decreased concentrations of indicator bacteria. While solar radiation was not explicitly measured, the researchers hypothesized that solar radiation was a primary stressor at the sampling site and thus contributed to the observed decrease in fecal indicator bacteria concentrations.

A study by Sinton et al. (2002) documented inactivation by sunlight of fecal indicators (fecal coliforms, enterococci, *E. coli*, somatic coliphages, and F+ RNA coliphages) and the effects of salinity in effluents from waste stabilization ponds and raw sewage. Over the course of two years, the researchers observed that sunlight inactivation rates were 10-times higher than dark inactivation rates. The overall pattern from greatest to least inactivation was enterococci, fecal coliforms, *E. coli*, somatic coliphages, and F+ RNA phages. Fecal coliform and enterococci inactivation rates were more similar in winter months than summer months. The researchers also observed that sunlight inactivation of all indicator organisms were greater with increasing salinity.

3.4 Indigenous Populations of Fecal-Associated Organisms

As demonstrated by the regional studies discussed previously in this section, under favorable environmental conditions, fecal indicator organisms can persist and (re)grow outside of the gastrointestinal tracts of humans and other warm-blooded animals. The literature presented is consistent in demonstrating that these microorganisms are endemic to tropical, subtropical, and temperate regions and thus, their presence in water is not necessarily an indication of recent fecal contamination. There is evidence that the presence of traditional fecal indicators such as *E. coli*, fecal coliforms, and enterococci in tropical and subtropical waters originate in soils rather than fecal sources (Roll and Fujioka 1997, Hardina and Fujioka 1991, Fujioka et al. 1999.). The ability of *E. coli* to multiply in soil and sediment was also documented in Florida and was determined to be a function of soil moisture content as opposed to human fecal contamination (Solo-Gabriele et al. 2000).

Interestingly, studies in colder climates have also shown fecal indicator bacteria can persist and proliferate in the absence of fecal contamination. Gauthier and Archibald (2001) showed that total and fecal coliforms (which include *E. coli*) and enterococci are commonly found in most or

all pulp and paper mills. Conditions in the pulp and paper production process allow coliforms and enterococci to grow rapidly and extensively throughout the water system in the absence of fecal matter inputs. The authors proposed that the high temperatures typical of mill water systems likely select for thermotolerant (fecal) coliforms. *Klebsiella pneumoniae*, a naturally-occurring microorganism found commonly on wood and bark, is also a total coliform bacterium making its detection in pulp and paper mill water ineffective for assessing fecal contamination. Sjogren (1995) showed that when a multiple antibiotic resistant strain of *E. coli* was applied as a tracer to field plots of rye grass in northern Vermont, it penetrated the upper soil layers and could be isolated at depths >30 inches and in groundwater for the duration of the 13-year study. The strain serotype and its multiple antibiotic resistance pattern allowed it to be tracked and identified as the original strain applied.

In addition to soils, tropical canopies can act as a natural environment for fecal indicator bacteria. Rainwater that cascaded through the canopy of a tropical rainforest in Puerto Rico and collected in the leaf axilae of bromeliads was sampled and found to contain *E. coli* and fecal coliforms (Rivera et al. 1988). The occurrence of fecal indicator bacteria in this pristine environment indicates that these organisms are indigenous to the native vegetation and are not of fecal origin (Toranzos 1991). Similarly, Carillo et al. (1985) observed that *Bifidobacterium* spp., fecal coliforms, and *E. coli* survived and possibly became normal flora in tropical freshwater environments in Puerto Rico. Diffusion chamber studies showed that *E. coli* could survive, remain physiologically active, and regrow at rates that were dependent on nutrient levels in ambient waters. Whitman et al. (2005) observed that fecal indicator bacteria are common in northern pitcher plants, further establishing that *E. coli* and enteroccoci can survive and thrive in temperate, terrestrial, and aquatic environments.

Inland lakes may also provide a favorable environment for fecal indicator bacteria to grow in the absence of human fecal contamination. Researchers in Australia found that it was highly unlikely that fecal contamination could account for massive *E. coli* levels in inland lakes (Ashbolt et al. 1997, Power et al. 2005). Blooms were dominated by three encapsulated *E. coli* strains, and the presence of the same three strains in bloom events in different geographical regions of a temperate climate and at different times indicates that free-living *E. coli* strains are able to persist in these water reservoirs (Power et al. 2005).

Most recently, Badgley et al. (2010) and others (Byanppanahalli et al. 2003, Craig et al. 2004, Anderson et al. 2005, Ishii et al. 2007, Englebert et al. 2008) have shown that submerged aquatic vegetation are reservoirs allowing for the persistence of environmental populations of fecal indicator bacteria by providing secondary habitat, allowing for the formation of biofilms and protection from UV irradiation. Badgley et al. (2010) found that the dominating population of enterococci at the study site (a freshwater lake in Florida) was *E. casseliflavus*, which is known to inhabit the guts of waterfowl and that surround the lake.

3.5 Summary

The scientific literature suggests general trends regarding the effects of environmental parameters on fecal indicators in subtropical and tropical regions. For over three decades,

studies in Hawaii and Guam (Fujioka and Roll 1997, Fujioka et al. 1988, 1999) and Puerto Rico (Carillo et al. 1985, Hazen et al. 1987, López-Torres et al. 1987, Toranzos 1991, Valdéx-Collazi et al. 1987) have shown that even in the absence of fecal contamination, beach sands and sediments, and even vegetation are known to have high concentrations of fecal indicator bacteria. Some physical parameters such as rainfall, tidal cycle, and turbidity appear to be positively correlated with concentrations of fecal indicator bacteria, while other physical parameters such as temperature and sunlight are negatively correlated with fecal indicator bacteria concentrations. Chemical parameters such as pH levels and salinity appear to be negatively correlated with indicator concentrations, whereas other chemical parameters such as nutrient and organic content of sediments and soils appear to be positively correlated with indicator concentrations. The environmental parameters that are positively correlated with indicator concentrations may create favorable conditions for indicators to persist and multiply in tropical and subtropical areas. Due to such favorable conditions, fecal indicator bacteria may be indigenous to many of these regions. However, it has been shown that these same environmental parameters that are positively correlated with indicator concentrations in the tropical and subtropical regions may also be characteristic of temperate regions such as the Great Lakes area. Therefore, indigenous fecal indicator bacteria populations may also exist in some temperate regions.

The literature also shows that currently used fecal indicator bacteria, fecal coliforms, *E. coli*, and enterococci, consistently do not meet two key criteria for fecal indicators identified by WHO (2005). Specifically, many studies have observed proliferation of these indicators in ambient waters, violating criterion 2. In addition, current fecal indicators may also persist, regrow, and recover in water, violating criterion 3. For these reasons, measurements of current fecal indicators may exceed levels associated with health risks in the absence of actual fecal contamination. Therefore, current fecal associated bacteria and viruses may not be effective indicators of fecal contamination and associated health risks, especially at non-point source beaches (Colford et al. 2007). Discussion of alternative fecal indicators that could potentially fulfill EPA and states' needs and WHO criteria—particularly in tropical and subtropical regions—is provided in the following section.

4. The Role of Physical, Chemical, and Biological Factors in the Extra-Enteric Behavior of Fecal Indicator Organisms in Ambient Waters, Sediments, and Soils

4.1 Background

Numerous studies (e.g., Boehm et al. 2002, Carillo et al. 1985, Hardina and Fujioka 1991, Ishii et al. 2007) have called into question the extent to which the traditional fecal indicator bacteria are specific to a fecal source of identifiable origin, do not grow outside the intestines of humans and animals, and persist relative to pathogens of human and animal origin. Using either direct measurement of persistence and growth or inference of persistence and growth, these studies demonstrate that, in some settings, stocks, flows, and growth of the traditional fecal indicator bacteria within the natural environment can be significant contributors to the populations of fecal indicator organisms. As discussed below, growth of fecal indicator bacteria has been demonstrated in controlled experiments conducted with natural marine water, fresh water, estuarine water and sediment samples and in soils in all climate zones. Growth of fecal indicator bacteria has also been inferred from *in situ* measurements of fecal indicator bacteria at numerous sites. These extra-enteric sources of indicator organisms hamper the ability of public health professionals and researchers to link suspected fecal contamination to identifiable and recent sources of fecal contamination, thereby hampering direct coupling to human health outcomes that have been determined empirically against exposure to wastewater effluents.

In this section, the defining characteristics of factors contributing to extended occurrence, persistence, or growth of fecal indicator bacteria are assessed. The objectives of this assessment are two-fold. First, this assessment intends to quantify, to the extent possible, the environmental conditions under which extended occurrence, persistence, or growth may occur. Second, it attempts to assess whether certain combinations of environmental conditions lend themselves to extended occurrence, persistence, or growth of indicator organisms. The environmental conditions assessed were defined based on the climate zone (tropical, subtropical, or temperate), media (water column, sediments, or soils) and anticipated level of impact from fecal pollution (impacted by human fecal pollution, impacted by fecal pollution from human agricultural activities, or no expected human fecal pollution impacts). Although differences in survival of indicator organism in different climate zones has been suggested by researchers in the past (e.g., Carillo et al. 1985, Fujioka et al. 1997), there is a growing consensus that, as stated by Whitman and Nevers (2003), "Most conditions outlined for *E. coli* growth in tropical soil are met in the temperate United States during summer, and differences in thermal seasonality are ecologically limiting and quantitatively distinctive only during cooler months."

Available scientific literature was reviewed to identify specific factors that have been observed to influence survival of fecal indicator bacteria in aquatic systems. Data gaps in the understanding of the influence of the parameters on fecal indicator bacteria survival were identified and, to the extent possible, the relative influences that the various parameters and combinations of parameters have on survival were noted. Although a meta-analysis of the available literature might be a desired outcome of such work, it has become clear that the designs of the numerous studies that have been conducted are generally vastly different from one another. It is useful to summarize data that has been generated to date, but within this document,

there has not been a broad scale attempt to generalize across all studies. Instead, summaries of like findings are presented, particularly for those studies that are well constrained (often those conducted in the most controlled conditions).

4.2 Section Contents

Sections 4.3 and 4.4 provide an overview of the studies and describe how the studies were classified. Sections 4.5 to 4.7 review and assess the chemical, biological, physical, and site-specific features believed to influence extra-enteric fecal indicator bacteria survival. In Section 4.5, the factors influencing extra-enteric behavior of fecal indicator bacteria are discussed and distinctions are drawn between "general" factors (i.e., properties that may be measured at a particular location and time) and factors that are specific to sites (location-specific).

Section 4.6 summarizes the conditions under which fecal indicator bacteria growth has been observed or inferred. The media and climatic zone in which growth occurred are identified and media and climate zones for which no growth has been reported are also noted. The absence of reported growth for climate zone-media combinations is not considered evidence that growth is not possible under those conditions. Rather, the absence of reported growth indicates a data gap and controlled experiments assessing the potential for growth under those conditions are recommended.

Section 4.7 describes and, where possible, quantifies the influence of general and context-specific factors on extra enteric fate of fecal indicator bacteria. First, in Section 4.7.1, the influence of individual growth conditions on indicator organism occurrence, persistence, and growth are assessed. Data describing the influence of growth conditions on indicator growth are found in three types of studies, (1) systematic studies conducted in laboratory or controlled environments; (2) *in situ* studies conducted in microcosms or mesocosms deployed in soils, sediments, or waters; and (3) analyses of indicator organism concentration and ecological parameters measured concurrently. Next, Section 4.7.2 reviews the occurrence, persistence, and growth of indicator organisms in particular contexts. It is hypothesized that growth in any particular ecology occurs when conditions are favorable and that the occurrence of organisms in a particular environment reflect the extent to which that environment provides favorable growth conditions and the fluxes of organisms in that particular environment. The roles that context-specific site features such as land use, runoff, and mixing have on indicator organism occurrence are reviewed and data gaps are identified.

Section 4 concludes with an assessment of the most important factors in determining extra-enteric fecal indicator bacteria survival, a listing of major data gaps precluding a full understanding of extra-enteric survival, and an assessment of the data needs for determining the alternative indicators that merit further evaluation for use in criteria setting.

4.3 Description of Studies Examining Extra-Enteric Occurrence and Survival

Broadly, the studies identified in the literature may be classified as follows:

A. studies in which the persistence, decay, or growth of fecal indicator bacteria were measured directly under controlled environmental conditions;
B. studies in which the persistence, decay, or growth of fecal indicator bacteria were measured *in situ* in diffusion chambers or other apparatuses allowing equilibration with the local environment;
C. studies in which the occurrence of fecal indicator bacteria was measured *in situ* concurrent with observations of environmental factors that might contribute to indicator organism occurrence;
D. microbial source tracking studies in which the presence of specific organisms, strains, or markers are related to specific sources of fecal pollution;
E. studies describing techniques for improved detection of fecal indicator bacteria in environmental samples or detection methods for alternative indicators;
F. studies comparing indicators;
G. modeling studies;
H. epidemiology studies; and
I. other studies.

The studies of greatest relevance to the evaluation of the extra-enteric fate of fecal indicator bacteria are those classified as A, B, and C. The number of studies found for each category and an alphabetical list of studies in classifications A, B, and C are provided in Table 1.

There were 35 studies in classification D, 17 in E, 23 in F, 13 in G, 18 in H, and 19 in I. Note that some studies appear in multiple classifications and that studies for which only abstracts were available (usually conference presentations) were not classified. The majority of studies explored the extra-enteric behavior of the traditional fecal indicator bacteria. This is not surprising, given that the current AWQC are based on levels of those fecal indicator bacteria (USEPA 1986), sampling for them is required in many settings, and data on the traditional fecal indicator bacteria are readily available. Some studies explored the extra-enteric fate of alternative indicator organisms (e.g., *Bacteroides* spp., bacteriophages, *Clostridium perfringens*; see Section 5 for further information). The majority of this section is devoted to specifically address the fate of fecal indicator bacteria. Given the nature of peer-reviewed publication, it is difficult to categorize across many types of studies from different areas, because of the fact that researchers are often trying to demonstrate advancement of the field. Therefore, overlap in study objectives is often difficult to assess simply based upon methodological descriptions. Nevertheless, a synthesis of available studies reveals something about what is known, what has been suggested, and helps to identify the data gaps and future work that is relevant to all types of fecal indicators and markers, including newly developed alternative indicators.

Table 1. Classification of studies reviewed for assessing behavior of indicator organisms

Study Classification	Number of Studies	Citations
A	29	Alkan et al. 1995, Alm et al. 2006, Bordalo et al. 2002, Byappanahalli and Fujioka 1998, Byappanahalli et al. 2003, Craig et al. 2004, Davies and Evison 1991, Davies et al. 1995, Davis et al. 2005, Desmarais et al. 2002, Fiksdal et al. 1985, Fujioka et al. 1981, Gerba and McLeod 1976, Haller et al. 2009, Hartel et al. 2005, Hartz et al. 2008, Kapuscinski and Mitchell 1983, Lee et al. 2006, Lo et al. 1976, McCambridge and McMeekin 1981,Noble et al. 2004 Parker and Mee 1982, Sinton et al. 1994, Sinton et al. 1999, Sinton et al. 2002, Wait and Sobsey 2001, Wang and Doyle 1998, Wright 1989, Yamahara et al. 2009
B	19	Alm et al. 2006, Anderson et al. 2006, Carillo et al. 1985, Craig et al. 2004, Davies at al. 2005, Harwood 2004, LaLiberte and Grimes, 1982, Lo et al. 1976, Lopez-Torres et al. 1987, Noble et al. 2004, Pérez-Rosas and Hazen 1988, Pérez-Rosas and Hazen 1989, Rhodes and Kator 1988, Van Donsel et al. 1967, Vigness et al. 2006, Wait and Sobsey 2001, Walters et al. 2009, Whitman et al. 2004, Wommack et al. 1996
C	74	Ackerman and Weisberg 2003, Alm et al. 2003, An et al. 2002, Ashbolt et al. 1997, Ashbolt and Roser 2002, Ashbolt and Bruno 2003, Aulicino et al. 2001, Balazs et al. 1993, Bernhard et al. 2003; Boehm et al. 2002; Boehm 2007; Bonilla et al. 2006, Bonilla et al. 2007, Borrego et al. 1990, Brion et al. 2002, Byamukama et al. 2005, Byappanahalli et al. 2006, Byappanahalli et al. 2007, Carillo et al. 1985, Characklis et al. 2005, Craig et al. 2002, Davis et al. 2005, Desmarais et al. 2002, Dwight et al. 2002, Evanson and Ambrose 2006, Ferguson et al. 1996, Ferguson et al. 2005, Ghinsberg et al. 1994, Grant et al. 2005, Grant et al. 2007, Haller et al. 2009, Hardina and Fujioka, 1991, Hartel et al. 2005, Hartz et al. 2008, He and He 2008, He et al. 2007, Ishii et al. 2006, Ishii et al. 2007, Isobe et al. 2004, Jeng et al. 2005, Jeong et al. 2005, Ki et al. 2007, Kinzelman et al. 2003, Kinzelman et al. 2008, Kistemann et al. 2002, Krometis et al. 2007, Leecaster and Weisberg 2001, Lee et al. 2006, LeFevre and Lewis 2003, Lipp et al. 2001, Luna et al. 2010, Mallin et al. 2001, Mill et al. 2006, Noble et al. 2000, Obiri-Danso and Jones 1999, Oshiro and Fujioka 1995, Paul et al. 1995, Rivera et al. 1988, Roll and Fujioka 1997, Rosenfeld et al. 2006, Santoro and Boehme 2007, Schiff et al. 2003, SEPA 2001, Seurnick et al. 2006, Shiaris et al. 1987, Shibata et al. 2004, Solo-Gabriele et al. 2000, Surbeck et al. 2009, Tunnicliff and Brickler 1984, Whitman and Nevers 2003, Whitman et al. 2004, Whitman et al. 2006, Yamahara et al. 2007

Although laboratory studies provide the most direct means for evaluating the importance of environmental factors on indicator organisms survival, summaries of many of those studies are provided in Table 13 (Appendix C) and the ranges of decay (or growth) rates observed in these studies are provided in Table 14 (Appendix C). However, *in situ* studies are important as they are able to take into account the myriad of factors relevant to degradation of *E. coli* and enterococci persistence and growth. Typical laboratory studies control for a single factor at a time, while studies conducted in ambient conditions permit assessment of compound factors of degradation, persistence, and growth. It is not possible in this document to summarize all of the studies that have been conducted, so this listing is not exhaustive, but should be considered representative of much of the work to date.

4.4 Classification of Environments

Sites at which measurements were made and from which samples were taken were classified according to their climate zone and the expected level of impact of fecal pollution in the vicinity of the site. Using the definitions of climate zones presented in Text Box 1, the most significant difference between tropical and subtropical climate zones is the amount and temporal distribution of precipitation. Based on those definitions, the following classifications were made:

- South Florida is considered to have a tropical climate. This designation is consistent with that given to the region by participants in the Tropical Water Quality Indicators Workshop (Fujioka and Byappanahalli 2003).
- Southern California is considered to have a semi-tropical climate.
- Sites on the southeastern and southern coasts of Australia are considered to have a temperate climate.

The classification of other locations referred to in this draft report was unambiguous (e.g., Puerto Rico has a tropical climate; the Great Lakes region has a temperate climate).

It has been hypothesized that the occurrence and persistence of fecal indicator bacteria at particular sites is related to the relative level of impact of human sources at that site (the "continuum of contamination," as described by Whitman et al. 2006 that may result in the introduction of fecal contamination (both human and animal sources). As described in Section 4.7.2.1, studies (e.g., Mallin et al. 2001, Ramirez et al. 2000) have shown significant correlations between level of anthropogenic influence as measured by land use and impervious surface coverage estimates and concentration/occurrence of fecal indicator bacteria. To assess the relationship between level of human impact and fecal indicator bacteria concentration, or the extent to which data are available to assess this relationship, study areas were classified according to their anticipated or known level of impact by human sources of fecal pollution. Human sources were considered to be (treated and/or untreated) human sewage and fecal indicator bacteria stemming from animal operations. Sites were given the following impact designations:

- expected or observed significant impacts from human sewage (HSI);
- expected or observed impacts from human agriculture/animal operations (HAI); and
- expected or observed limited impacts from human sources (LI).

Where possible, the impact designation was drawn from the studies themselves. Otherwise, the designation was made based on descriptions of the study area provided by the authors or based on other information regarding the study area that was drawn from other sources. In general, receiving waters in large urbanized areas were classified as HSI. However, sites were not classified in the absence of sufficient information in the primary literature and when the context did not suggest an obvious choice.

4.5 Factors Influencing the Occurrence, Persistence, and Growth of Fecal Indicator Organisms

Factors influencing the occurrence, persistence, and growth of fecal indicators may be grouped as general and context-specific. General factors pertain to the life cycle of the organisms and their influences are often assessed through the use of controlled laboratory experiments. These experiments generally comprise the inoculation of a water sample, sediment sample, or soil sample drawn from a particular source with a population of indicator organisms; control of physical, chemical, and biological parameters; and direct measurement of the growth or decay of the indicator organisms. General factors are often interrelated. For example, inactivation of fecal coliforms in sunlight is much more rapid in marine waters than in fresh waters (Anderson et al. 2005, Bordalo et al. 2002, Harwood et al. 2004, He et al. 2007, Jeong et al. 2005, Lipp et al. 2001, Šolić and Krstulović 1992). As listed in Table 9, Table 10, and

Table 11 in Appendix C, the general physical, chemical, and biological (respectively) features that are believed to influence extra-enteric survival of indicator organism include temperature, light, suspended solids, turbidity, salinity, pH, alkalinity, nutrients, organics, and the biological community within the water column.

Location-specific factors are those related to the setting in which the indicator organism concentration is measured and used to assess water quality. An illustration of the interconnected, site-specific processes that determine the amount of culturable *E. coli* in Lake Michigan beach waters was provided by Whitman et al. (2006) and is shown in Figure 1. As can be seen, numerous features determining the net *E. coli* concentrations in the lake are specific to the site. These include human and nonhuman inputs of fecal pollution, mixing intensity at the site, characteristics of streams that transport *E. coli* to the receiving water, storage capacity of soils and sediments, and morphology of the region near the beach. The authors also review the potential for naturally occurring *E. coli* across sand environments, and discuss the potential for an autochthonous source of such bacteria because of the consistent nature of measurements observed across the study sites.

Deducing the impact a particular factor has on indicator organism occurrence, persistence, or growth is often difficult, given the many and complex processes occurring in a particular environment. For example, several studies noted the rapid rebound of indicator organism concentration in the surf zone after sunset following sunny days (Boehme et al. 2003, Rosenfeld et al. 2006, Whitman et al. 2004) but none have definitively identified the cause for the rebound. Context-specific factors playing potential roles in the observed rebound were related to mixing (large or small spatial scale), resuspension, loading of "fresh" indicator organisms from sources outside the study area, repair of damaged organisms, or growth rate in the surf zone outpacing death rate in the absence of insolation. Location-specific features that have been determined to play a role in the extra-enteric occurrence and survival of fecal indicator bacteria include rainfall and runoff, mixing, and land use in the vicinity of study areas (e.g., Coulliette et al. 2008, Seurinck et al. 2006, Surbeck et al. 2010).

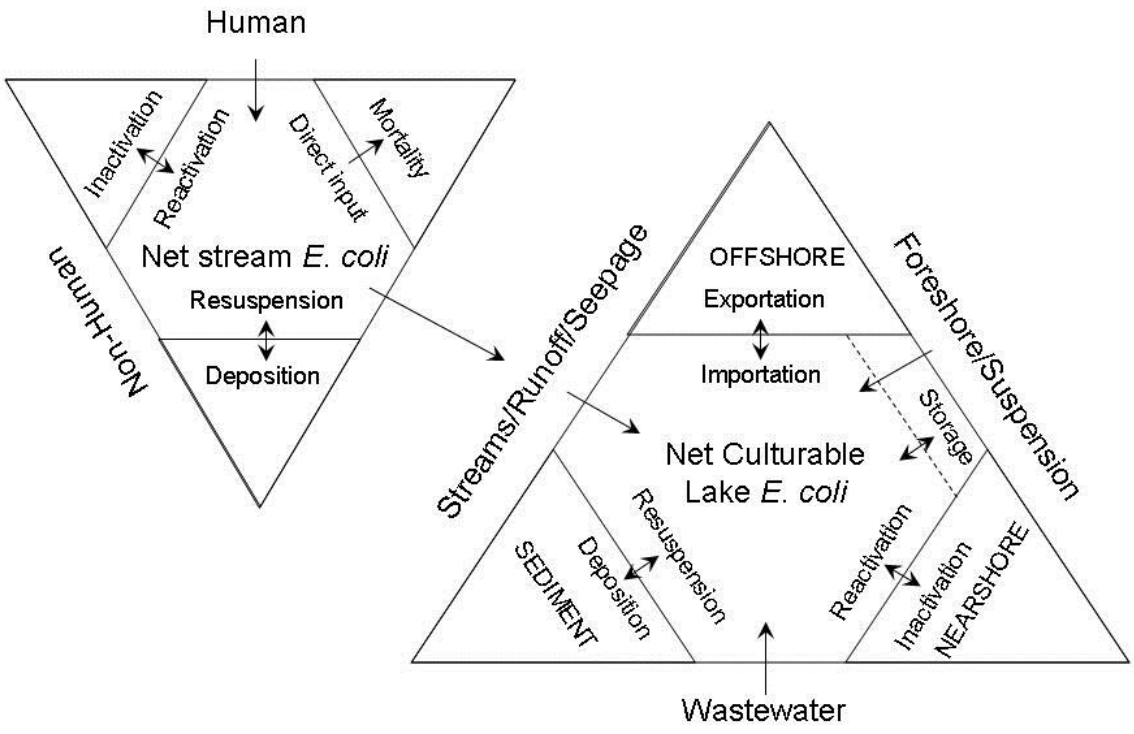

Figure 1. Illustration of the processes determining the incidence of culturable *E. coli* in Lake Michigan beach waters (SOURCE: Adapted from Whitman et al. 2006)

4.6 Evidence of Extra-Enteric Growth

In the literature, extra-enteric growth of fecal indicator bacteria is typically either measured directly (increase in counts of fecal indicator bacteria) or inferred for the climate-water-medium combinations shown in Table 2 (see also Table 12 and Table 13 in Appendix C). Again, the absence of confirmation of growth in a particular climate-water-medium combination does not confirm that growth cannot occur for that combination; rather, it indicates that in specific studies, this trend was not observed.

Section 4.6.1 presents evidence of direct growth that has been observed in documented studies. In most cases, direct observations of indicator organism growth are drawn from microcosm and mesocosm experiments in which the growth of organisms. Section 4.6.2 describes and analyzes studies in which authors attributed increased or slowly decreasing fecal indicator bacteria concentrations to growth. In these studies, growth is inferred from the net change in fecal indicator bacteria concentrations. Section 4.6.3 states the conditions under which growth is most likely and identifies data gaps regarding extra-enteric

Table 2. Conditions under which fecal indicator organism growth has been either measured or inferred in ambient conditions

Medium	Water Type	Climate		
		Tropical	**Subtropical**	**Temperate**
Water column	Marine			
	Estuarine	*E. coli*, enterococci		Total coliforms, *E. coli*, *Salmonella* spp.
	Fresh	*E. coli*, fecal coliforms		Total coliforms, *E. coli*, enterococci
Sediments	Marine		*E. coli*, enterococci, fecal coliforms	Fecal coliforms
	Estuarine			
	Fresh			Fecal coliforms
Soils	Marine			
	Fresh	*E. coli*, enterococci		*E. coli*, enterococci, fecal coliforms

4.6.1 Direct Observation of Growth

A range of peer reviewed studies documented growth of fecal indicator bacteria in sediments, soils, and in the water column (for a survey of studies, refer to Appendix C, Table 12). Growth was documented or inferred in fresh water, soils, and sediments associated with fresh water, and soils and sediments associated with marine or estuarine waters. Growth was not documented in the marine water column, and salinity drastically increases rates of loss of members of the fecal coliforms (including *E. coli*). The lack of observations of growth specifically in marine water columns should be treated as a data gap and not proof that growth cannot occur. Rhodes and Kator (1988) suggest that failure to observe growth of fecal indicator bacteria in some laboratory experiments may be a result of the harsh processes employed in preparing inocula, such as centrifugation and washing at cold temperatures.

The majority of growth observations have been made in soils, where fecal indicator bacteria likely benefit from protection from harsh environmental conditions and high organic matter contents (e.g., Alm et al. 2003, Anderson et al. 2005, Bonilla et al. 2007). Growth in soils has the potential to influence fecal indicator bacteria concentrations in the overlying water column and in sediments, permitting the benthos to serve as a reservoir of fecal indicator bacteria. If growth in soils by fecal indicator bacteria is mirrored by growth of pathogenic bacteria, there could be public health risks associated with contact with the soils during recreational activity. This has been demonstrated in the context of sand by Heaney et al. (2009), but has not been well documented for other soil types.

Most of the studies that documented fecal indicator bacteria growth were conducted in laboratory microcosms or *in situ* in diffusion chambers. Although numerous researchers have noted shortcomings associated with such microcosm experiments (e.g., slow response of mesocosms to changes in environmental conditions, difficulty in designing microcosms that simulate the complex array of physical, chemical, and biological factors present in environmental waters; Anderson et al. 2005), there is no clear alternative procedure for quantifying growth and decay in the presence of competing processes. A few researchers have noted the importance of grazing processes in experiments to assess growth, persistence and removal of fecal indicator bacteria

(e.g., Boehm et al. 2006). However, in mesocosms, grazing processes may largely be reduced through water sample manipulation. Some researchers even prefilter the ambient water used for mesocosm studies, thereby eliminating clearance of fecal indicator bacteria by grazers. Grazers are likely to be an important factor in removal of fecal indicator bacteria that is not well understood. Alm et al. (2006) found that growth (more than 3 logs) of *E. coli* occurred in diffusion chambers filled with moist autoclaved soil whereas *E. coli* concentrations decreased in sand adjacent to the mesocosms during the experiment. This finding indicates that conditions (nutrients, carbon sources, temperature, and others) in the soil are favorable for growth and that processes such as predation and soil washing by runoff reduce soil fecal indicator bacteria concentratins and may thus mask (or inhibit) growth. Davies et al. (1995) were able to demonstrate growth of fecal indicator bacteria in marine sediments when potential predators were inhibited with cycloheximide. Further, the authors inferred from deviation of decay kinetics from first-order that the die-off of the fecal coliforms reflected processes more complex than simple inactivation. Rhodes and Kator (1988) observed markedly different growth and decay rates in filtered and unfiltered estuarine water and attributed those differences to competition and predation.

While the extra-enteric growth of *E. coli*, enterococci, fecal coliforms, and total coliforms have been documented, the majority of growth observations have been made for *E. coli*. This is likely due to the ease of using and growing cultured *E. coli* in the laboratory. Also, *E. coli* is a single species, making quantification of the target cell easier than assessments of enterococci. In a small number of studies, the growth, persistence, or decay of other fecal indicator bacteria (other than *E. coli*, enterococci, and fecal coliforms) were reported. These studies present some interesting findings.

- growth of *Clostridium perfringens* was not observed under conditions in which *E. coli* was documented to grow (Desmarais 2002), though *C. perfringens* was persistent under those conditions; and
- *Bifidobacterium adolescentis* did not grow in a tropical stream under conditions for which *E. coli* growth was observed (Carillo et al. 1985).

In general, the lack of systematic study of alternative fecal indicator bacteria in controlled laboratory or *in situ* studies is a major data gap that may need to be filled before the efficacy of alternative indicators may be assessed.

Growth in sediments was observed at a site with a temperate climate and in marine and fresh waters. In most cases, growth was observed directly; in other cases, growth was inferred from deviation of indicator organism decay rate from first-order. Non-first-order decay indicates that decreases in indicator numbers may be a net result of growth and predation or other processes rather than inactivation alone (Davies et al. 1995).

Six studies documented growth in the water column (Carillo et al. 1985, Desmarais et al. 2002, Hardina and Fujioka 1991, Isobe et al. 2004, Okabe and Shimazu 2007, Rhodes and Kator 1988). Carillo and colleagues measured *E. coli* and *Bifidobacterium adolescentis* concentrations in diffusion chambers that were suspended in a tropical stream at two locations—one known to be impacted by sewage and a second at an elevation above suspected sources of fecal

contamination. *E. coli* was observed to increase in number over time at both sites while *B. adolescentis* did not. The observed growth of *E. coli* at a site believed to be relatively free of human sewage indicates that nutrient levels associated with relatively clean, unimpacted waters were sufficient to sustain an *E. coli* population. Growth of total coliforms in the water column was observed under temperate climate conditions typical of Tokyo, Japan (Okabe and Shimazu 2007). Growth was achieved in controlled laboratory investigations using unfiltered river water and seawater. Conditions at which growth was observed were a water temperature of 10°C and salinity at or below 10 ppt. Growth was not observed at 4°C and no salinity and at 20°C or above. Although fecal coliform growth was not observed at no salinity, 10°C, the decay rate was very low (0.02 d^{-1}), indicating the potential for a balance between growth and other processes such as predation or intoxication. In contrast to the effect of temperature on growth observed by Okabe and Shimazu, Rhodes and Kator (1988) observed that in *in situ* diffusion chamber experiments, growth (large negative values of decay constant) of both *E. coli* and *Salmonella* spp. was observed in the initial 2 to 3 days when temperature was greater than 18°C.

Studies of growth in soils report the ability of fecal indicator bacteria to survive adverse environmental conditions that have previously been thought to limit their ability to persist for long periods outside animal hosts. Ishii et al. (2007) determined that *E. coli* overwintered in frozen soils and subsequently grew in soils in a Lake Superior watershed. In the course of the study, the *E. coli* underwent several freeze/thaw cycles. Confirmation that *E. coli* regrew after winter was made based on knowledge of loadings in the soil plots where growth was observed and based on the genotype of *E. coli* observed before and after winter. Ishii et al. (2007) and others (Bonilla et al. 2007, Craig et al. 2004, Shiaris et al. 1987) have noted that the soil properties most conducive to growth or persistence are small particle size and sufficient organic content.

It is important to note that optimal growth temperature may differ in the water column and in soils. Data from systematic experiments indicate that water temperature above around 10° C are negatively correlated with fecal coliform, *E. coli*, and enterococci concentrations (e.g., Craig et al. 2004, Lipp et al. 2001). On the other hand, several authors (e.g., Hardina and Fujioka 1991, Ishii et al. 2007) suggest that high concentrations of fecal indicator bacteria in soils compared with those in the water column may be related to higher, more consistent temperatures encountered in soils. Clearly, there is empirical evidence of a myriad of complex processes relevant to bacterial growth and decay.

Other studies (Desmarais et al. 2002, Vigness et al. 2006) have demonstrated the ability of fecal indicator bacteria to regrow in soils after dessication. Vigness and colleagues demonstrated tolerance of dessication in sand plot experiments conducted near fresh waters. Specifically, they demonstrated that *E. coli* could tolerate low sand humidity conditions (<5 percent) and can resume active growth after rehydration. Desmarais and colleagues simulated drying-wetting conditions such as those experienced in tidal cycles. In that study, sediments from a stream with brackish water were dried then rehydrated in a simulation of tidal cycles. After rehydration, significant regrowth (1.5- to 2-logs) was observed for *E. coli* and enterococci in both the sediments and in the water column.

4.6.2 Indirect Evidence of Growth

Numerous studies have used indirect evidence to assert that growth of indicator organisms occurs in various climates and in various media. In some cases, persistence and the presence of conditions conducive to growth were taken as evidence that growth was likely or possible. For example, Byappanahalli et al. (2006) consistently found *E. coli* in soils in a protected natural area in a temperate climate (along the shores of Lake Michigan. In that study, numbers and population genetics of *E. coli* in soils at six separate study locations were observed between the months of March and October. The *E. coli* were persistent and able to regrow after soil desiccation and re-hydration. The authors took these observations to be evidence of the potential for growth and suggested that additional studies be performed at other temperate climate locations, focusing on the effect of soil chemical and physical characteristics and ecological characteristics (e.g., survival strategies, growth requirements, microbial interactions, population genetics).

Other studies have demonstrated that observations of indicator organism decline indicate a balance between ecological processes, including indicator organism growth, predation, the production of substances toxic to the indicator organisms, and other processes. For example, using *in situ* measurements (diffusion chamber) of fecal coliform numbers in microcosms, Davies et al. (1995) showed a significantly longer persistence of fecal coliforms in the absence of predators than when they were present. This finding indicates the potential for growth in suitable ecological conditions. In the same study the authors determined that marine sediments provide a favorable, non-starvation environment for *E. coli*. Both of these findings indicate the potential for extra-enteric growth if ecological conditions are favorable.

The rapid rebound of bacteria populations after daylight hours has been advanced by researchers as providing evidence of potential indicator organism growth. Boehm et al. (2002) suggested that growth outpacing die-off and predation is one of several possible reasons for rapid rebound of enterococci and other indicator bacteria after dark. Other potential explanations include resuscitation of damaged bacteria, resupply of bacteria, or resuspension of nearshore and foreshore bacteria by wave action (Rosenfeld et al. 2006).

Other studies (described in Section 4.7.1) have been conducted to evaluate the survival of indicator organisms in various conditions (*in situ*, in light and dark, under various light sources, etc). In those studies, decay rate constants for fecal indicator organisms kept in dark conditions were universally very low compared with those calculated for light conditions. Under dark conditions, it is possible that predation or other competing processes can mask the incidence of indicator organism growth. This uncertainty over whether or not in-water-column growth occurs over the range of environmental conditions encountered during recreational use seasons represents a data gap that could be filled through applied research so that growth (or lack thereof) may be separated from the other processes determining the net rate of change of indicator organisms.

4.6.3 Assessment of Conditions Supporting Growth and Identification of Data Gaps

From the preceding observations of growth, the following conclusions may be drawn:

- Growth of *E. coli* has been reported in all but one climatic condition. Although growth has not yet been observed in the subtropical climate, there does not appear to be any growth-related feature associated with the subtropical climate that is dissimilar enough from those in tropical or temperate climates to preclude growth.
- Growth of *E. coli* has been observed in all media-water combinations except in the marine water column.
- Predation by indigenous species may result in net decline of indicator organism population despite growth.
- The nutrient and carbon requirements for growth of *E. coli* in soils are modest (Hardina and Fujioka 1991, Whitman et al. 2006) and growth has been observed in soils and in the water column in areas in the absence of human sewage.
- Soils with higher silt and clay content are more conducive to indicator growth than those with high sand content.
- *E. coli* and possibly other indicator bacteria can survive harsh environmental conditions encountered outside intestines. These surviving organisms can then regrow in soils.
- Measurement of growth *in situ* in fresh waters is difficult because predation, introduction of indicator organisms from other external sources, and other processes mask the occurrence of growth.

Four data gaps preclude a full understanding of the incidence of extra-enteric growth and the conditions under which it occurs. First and foremost, data on the ability of alternative indicator organisms to grow in all climate zones and in all water types is lacking. The majority of studies reviewed in this draft report examined the occurrence and growth of traditional fecal indicator organisms, including *E. coli*, enterococci, and coliforms. Systematic study of the growth of candidate alternative fecal indicator organisms through research similar to that described above is essential in determining the efficacy of those organisms as indicators of fecal pollution.

Second, the ability of enterococci to survive in marine environments under favorable environmental conditions (low or no light, low or limited predation, favorable temperatures) could be determined through focused research. Improved knowledge of enterococci growth rates are essential for development of phenomenological models of indicator transport and fate for marine beaches and could help explain the observed rapid rebound of enterococci numbers at marine beaches after dark.

Third, research could be conducted to assess whether marine and relatively unimpacted fresh waters can support indicator organism growth. Among the water column studies described above, none documented growth in those two environments. *In situ* studies could be conducted in areas such as protected stream headwaters, marine waters near protected areas, and areas located far from known sewage discharges. Such studies could help establish whether indicator organism growth requirements are met in relatively unimpacted waters.

Fourth, potential intra-region spatial variation of fecal indicator bacteria growth needs to be quantified. Some studies have documented differences in growth among soils in various settings and distances from waters. Other studies might be conducted to assess the potential for growth as a function of depth in the water column, as a function of depth in the soil or sediment layer, or

from site-to-site within a given study area. Factors expected to vary spatially within study areas are salinity, soil type, nutrient availability, temperature, and others.

4.7 Evaluation of Factors Contributing to Occurrence, Persistence, or Growth of Indicator Organisms

The factors that influence the extra-enteric growth, persistence, and occurrence of fecal indicator organisms can be classified as general and context-specific. General factors are growth conditions such as temperature, salinity, solar flux, nutrient level, to name a few, which may be measured in a sample or at a particular location. General properties are intensive properties whose values are independent of the mass or extent of the system in which they are measured (Wark 1983). Regardless of climate zone or other features related to a site, indicator organism response (growth, death, persistence) to general properties will be essentially the same, regardless of site. Extensive properties are those that are specific to a site. Context-specific properties may influence site indicator organism concentration via loading or via their role in establishing the intensive properties at the site. The influence of context-specific properties is expected to vary significantly from site-to-site. For example, sewage outfalls influence indicator organism concentration via direct discharge of indicator organisms into receiving waters and sediments, influences on the nutrient level or substrate availability in receiving waters, changes in the concentration of suspended particles, or because they may give rise to density currents and increase mixing, etc. (e.g., Ashbolt and Bruno 2003, Krometis et al. 2007). The magnitude of the effects of sewer outfalls varies from site-to-site because outfalls discharge different volumes of water with different water qualities and because the orientation of outfalls varies from site-to-site.

Results of research on the influence of intensive and extensive properties on indicator organism occurrence, persistence, and possibly growth are summarized below. First, the influences of intensive properties are reviewed. The intensive conditions most favorable to growth and persistence in soils, sediments, and the water column are assessed and data gaps related to the influence of intensive properties are identified. Second, the influence of extensive features on occurrence, persistence, and growth are reviewed and assessed and data gaps related to extensive features are identified.

4.7.1 General Parameters

The current understanding of the influence of the general (intensive) parameters found in Table 9, Table 10, and

Table 11 in Appendix C and combinations of these parameters on fecal indicator bacteria fate is described below. For each parameter, the data gaps in current understanding of the effect of the parameter in various environments are listed. Summaries of findings of studies related to each of these parameters are presented in tabular form in Appendix C (Tables 15 to 19).

Sections for each parameter begin with a table showing the conditions under which the effect of the particular parameter has been studied. The conditions shown include the climate zone in which the study was conducted, the media in which studies were made, the water type in the study (marine, estuarine, or fresh), and the degree to which the region in which the study was conducted was impacted by human sources of fecal pollution. The impact designations (i.e., HSI, HAI, and LI) were described in Section 4.3.

4.7.1.1 Temperature

The conditions under which temperature influences on indicator organisms were assessed are presented in Table 3. Summaries of findings of studies on the effect of temperature are found in Appendix C, Table 15. As discussed below, temperature influences bacteria fate directly via dependence of microbial kinetics (growth and inactivation rates) on temperature and indirectly through mechanisms such as fluid motion via density currents.

Table 3. Settings for which temperature effects on extra-enteric fate were studied

Medium	Water Type	Impacts	Climate		
			Tropical	Subtropical	Temperate
Water column	Marine	HSI[a]	X		X
		HAI[b]			
		LI[c]	X		X
	Estuarine	HSI		X	X
		HAI			
		LI			
	Fresh	HSI	X		X
		HAI			
		LI	X	X	
Sediments	Marine	HSI			X
		HAI			
		LI			
	Estuarine	HSI			
		HAI			
		LI			
	Fresh	HSI			X
		HAI			
		LI			
Soils	Marine	HSI			
		HAI			
		LI			
	Estuarine	HSI			
		HAI			
		LI			
	Fresh	HSI			X
		HAI			
		LI			

[a] Expected or observed significant impacts from human sewage
[b] Expected or observed impacts from human agriculture/animal operations
[c] Expected or observed limited impacts from human sources

Some exceptions to the trend of increasing decay rate with increasing temperature were previously noted in the studies reviewed in this draft report. Carillo et al. (1985) found the densities of fecal coliforms and *E. coli* were positively correlated with water temperature for studies in a tropical freshwater stream in the temperature range 21.1 to 27.1° C. He et al. (2007) found that the influence of temperature on indicator survival in subtropical estuarine waters was different in ponded waters than in waters that were flowing; in ponded waters, fecal coliform, total coliform, and enterococci concentrations were positively correlated with temperature. Whitman and Nevers (2003) determined that temperature was positively correlated with *E. coli* counts in the water column at sites on Lake Michigan.

Fewer studies have been conducted on the effect of temperature in soils and sediments than the effects in the water column. Craig et al. (2004) observed that declines in *E. coli* populations in marine and river sediments were more rapid at higher temperatures within the temperature range 10 to 30° C. Whitman and Nevers (2003) found that water temperature was significantly correlated with *E. coli* counts in submerged sands (correlation coefficient [*r*] = 0.396). In studies of temperate soils, Whitman and Nevers found that foreshore sand *E. coli* counts were correlated with temperature and water temperature. Ishii et al. (2007) observed growth of *E. coli* in non-sterile soils for soil temperature above 30°C.

Findings (reported in the preceding paragraph) on the influence of temperature on indicator organisms in the water column versus the effect in soils and sediments yields a seeming paradox—low temperature (around 10°C) appears to favor persistence and growth of *E. coli* and other indicator organisms in the water column whereas higher temperatures appear to favor growth in soils and is sometimes associated with growth or slower decay in the water column. A possible explanation for this phenomenon is the difference in microbial ecology in sands and sediments versus that in the water column. This paradox indicates a research gap.

Several authors have noted synergy between temperature effects and effects of other general properties on indicator organism survival. For example, in studies in marine and fresh waters, Craig et al. (2004) determined that increased temperatures led to more rapid declines in *E. coli* numbers at a given light intensity. Bordalo et al. (2002) noted that daily variations in temperature may influence the microbial population which, in turn, influences the observed effect of sunlight on indicator organism decay rate.

In summary, optimal survival or growth conditions for fecal coliforms, *E. coli*, and enterococci in the water column appear to be at a temperature around 10°C, though several contradictory observations were found in the literature. Growth and survival in soils appears to favor higher temperatures (> 30°C), though limited data were available to make this determination. The most significant data gaps relate to the effect of temperature on fate in soils and sediments, particularly tropical and subtropical soils and sediments. Other data gaps are for soils and sediments in relatively unimpacted areas and for all media in subtropical regions. The lack of data on the temperature conditions in which growth may occur in soil is especially important, given the

likelihood that soils serve as indicator organism reservoirs and can be sources of the organisms during rain events.

4.7.1.2 Salinity

The conditions under which researchers have studied the influence of salinity on fecal indicator bacteria persistence are presented in Table 4. Summaries of findings of studies on the effect of salinity are found in Appendix C, Table 16. Salinity influences fecal indicator bacteria survival directly via contributions to inactivation of the organism, changes in osmotic potential, stress to membrane structures, and indirectly through microbial competition.

The studies reviewed for this draft report generally found salinity at levels observed in seawater to contribute to declines in fecal indicator bacteria populations. However, the specific effects of salinity differed among fecal indicator bacteria types and with other water quality parameters (temperature and others). In studies of the water column, Anderson et al. (2005) determined fecal coliform decay in salt water to be approximately 15-times that observed for fresh water under similar conditions (with the same inoculum and general properties). Bordalo et al. (2002) made similar observations for indicators in estuarine waters; Harwood et al. (2004) for waters from tropical sources; Lipp et al. (2001) and Jeong et al. (2005) for estuarine waters; He et al. (2007) for ponded and flowing waters; and by Šolić and Krstulović (1992) for polluted marine waters.

Salinity in sediments also appears to cause loss of fecal indicator bacteria populations, though decay rates in saline sediments appear to be less than those observed in the water column (Harwood 2004). Studies that examined the effect of salinity on survival in sediments include Anderson et al. (2005), Davies et al. (1995), Evanson and Ambrose (2006), Harwood (2004), and Lipp et al. (2001).

Synergy between sunlight inactivation and salinity was noted by several authors (e.g., Bordalo et al. 2002; Šolić and Krstulović 1992). Although a mixed effect has also been absent in a range of previous studies, the synergy is important to identify. Those researchers that did observe synergy between sunlight inactivation and salinity found that the bactericidal effects of sunlight were enhanced by even small increases in salinity. For example, Šolić and Krstulović found that the greatest impacts of salinity on survival were observed in the range 10 to 15‰; increasing salinity above 15‰ produced small changes in survival time compared with changes observed in the lower salinity range.

In summary, salinity has a negative impact on the survival or growth of fecal coliforms, *E. coli*, and enterococci in the water column and in sediments. The greatest effects appear to be in the low salinity range; changes in salinity above 15‰ were observed to produce only small changes in indicator organism survival. Data gaps in knowledge of the impact salinity has on indicator organism survival are the influence of salinity on survival in the low salinity range, the influence of salinity on survival or growth in all media in environments with limited fecal pollution impacts, and the influence of soil salinity on indicator organism growth and the make-up of the soil microbial population.

Table 4. Settings for which salinity effects on extra-enteric fate were studied

Medium	Impacts	Climate		
		Tropical	Subtropical	Temperate
Water column	HSI[a]	X	X	X
	HAI[b]			
	LI[c]			
Sediments	HIS	X	X	X
	HAI			
	LI			
Soils	HIS			
	HAI			
	LI			

[a] Expected or observed significant impacts from human sewage.
[b] Expected or observed impacts from human agriculture/animal operations.
[c] Expected or observed limited impacts from human sources.

4.7.1.3 Sunlight

The conditions under which researchers have studied the influence of light and sunlight on fecal indicator bacteria survival are presented in Table 5. Summaries of findings of studies on the effect of sunlight are found in Appendix C, Table 17. Sunlight influences indicator organism survival directly through inactivation and direct damage to DNA and RNA in the cells, and indirectly through changes in the microbial population. In particular, it is difficult to summarize sunlight-based studies, because not all researchers include data in their documentation relevant to "dose" of sunlight, i.e. measurements of PAR or other measures of solar irradiance for direct comparison.

Table 5. Settings for which light and sunlight effects on extra-enteric fate were studied

Medium	Water Type	Impacts	Climate		
			Tropical	Subtropical	Temperate
Water column	Marine	HSI[a]	X	X	X
		HAI[b]			
		LI[c]		X	X
	Estuarine	HSI	X		
		HAI			
		LI			
	Fresh	HSI			X
		HAI			
		LI	X		

[a] Expected or observed significant impacts from human sewage.
[b] Expected or observed impacts from human agriculture/animal operations.
[c] Expected or observed limited impacts from human sources.

In all studies, sunlight was seen to dramatically decrease fecal indicator bacteria concentrations as a function of applied dose. Significant decreases in survival were noted for fecal coliforms (Bordalo et al. 2002, Fujioka et al. 1981, Sinton et al. 1999, Šolić and Krstulović 1992), enterococci (Boehm et al. 2002, Noble et al. 2004, Rosenfeld et al. 2006), and *E. coli* (Alkan et al. 1995, Davies and Evison 1991, McCambridge and McMeekin 1981, Noble et al. 2004,

Rosenfeld et al. 2006, Whitman et al. 2004). In a single study of the impact of sunlight on bacteriophages (Wommack et al. 1996), (1) decay rates were much higher for light conditions than for dark conditions, and (2) decay rates for microcosms at the water surface were twice as high as those for microcosms suspended 1 m below the water surface in an estuary. In general, survival curves for indicator organisms exposed to sunlight exhibited "shoulder" behavior (e.g., Kapuscinki and Mitchell 1983, Sinton 2007) followed by first-order decay.

Several studies have noted temporal variations in fecal indicator bacteria concentrations related to daily sunlight cycles. Boehm et al. (2002) used spectral analysis to distinguish between fluctuations in enterococci concentration resulting from sunlight cycles and other causes. They found that significant sunlight-related fluctuations occurred daily, with the lowest concentrations of fecal indicator bacteria (enterococci) occurring in the mid-afternoon and the highest concentration occurring after dark. An interesting finding made in that study and another study of temporal fluctuations of indicator organisms (Rosenfeld et al. 2006) was that fecal indicator bacteria concentrations rebounded very quickly after daylight hours. Possible explanations for the rapid rebound are repair of damaged cells, resupply, growth, or entrainment of organisms in mixing processes.

Researchers have examined the relationship between fecal indicator bacteria populations and associated particles. Many studies have been conducted in soils and those related to potential inactivation processes in aquatic systems (Fujioka et al. 1999). In source fecal contamination, and throughout the wastewater treatment process, fecal indicator bacteria are frequently associated with particles. The bacteria are in effect protected from UV disinfection due to this particle association (Emerick et al. 2000). Research has been conducted to determine particle sizes associated with shielding UV light and quantify number of particles with embedded coliform bacteria (Emerick et al. 1999), to quantify bacteria and loss in the presence of wastewater solids (Loge et al. 1999), and to assess the decay rates of fecal indicator bacteria in wastewater treatment plant material (Loge et al. 2001). While there are several studies that have examined the protection of fecal indicator bacteria from UV irradiation and other environmental factors due to particle association, most have been conducted in either drinking water or in the wastewater stream, and have not been conducted in ambient waters. Fries et al. (2005) did not study decay per se, but did describe the portion of fecal indicator bacteria in an estuarine setting associated with particles. The findings indicated that surprisingly, upwards of 40% of the fecal indicator bacteria in the ambient waters were attached to particles, and that their settling velocities were therefore altered due to this association. It will be important in the future to study particle-association and the complex factors of nutrient status, and organic matter concentrations and quality to fully understand processes related to fecal indicator bacteria dynamics in ambient conditions.

Other important findings of studies of sunlight on fecal indicator bacteria survival include the following:

- significantly different inactivation rates have been observed between studies conducted with artificial light sources and those conducted in natural sunlight;

- inactivation rates reduce with water depth because the energy spectrum of light changes with penetration depth and because of reflection and absorption of solar energy in turbid waters; and
- there is apparent synergy between (1) solar radiation and salinity (the effect of solar radiation is enhanced in saline waters), (2) solar radiation and temperature (the effect of solar radiation is enhanced at higher temperatures), and (3) solar radiation and the effects of competition and predation (McCambridge and McMeekin 1981).

Data gaps in the understanding of the effects of sunlight on extra-enteric survival of indicator organisms include the following:

- the capacity of fecal indicator bacteria to repair from sunlight damage; and
- the impact of sunlight on fecal indicator bacteria across a range of different climate types.

4.7.1.4 Turbidity and Suspended Solids

The conditions under which various researchers have studied the influence of suspended solids and turbidity on indicator organism survival are presented in Table 6. Summaries of studies on the effect of temperature are found in Appendix C, Table 18. Turbidity and suspended solids influence indicator organism survival through reflection of sunlight (Davies et al. 1995), adsorption and sheltering of indicator organisms (particle-associated bacteria), and possibly other mechanisms (Characklis et al. 2005).

Studies of turbidity and total suspended solids (TSS) have either examined the extent to which the two parameters were correlated with the concentration of indicator organisms or sought to quantify the indicator organism load associated with settleable and non-settleable particulate matter. Indicator organism concentrations have correlated poorly with turbidity and suspended solids concentrations in most cases. In a study of south Florida beaches, Shibata et al. (2004) found no correlation between turbidity and *E. coli* or enterococci (though *Clostridium perfringens* and total coliforms were correlated with turbidity). Similarly, Jeong et al. (2005) found no consistent correlations between fecal coliforms, *E. coli*, or enterococci and turbidity for samples taken in an estuary with known human sewage impacts. However, Mallin (2001) found significant correlation between turbidity and fecal coliforms across multiple sites in a tidally-influenced creek while Tunnicliff and Brickler (1984) noted that in the Colorado River, fecal coliforms were positively correlated with turbidity during storm conditions.

Several authors studied the relationship between TSS and the proportion of indicator bacteria associated with particles. Jeng et al. (2005) determined that for samples taken during 2 storms, 9.8 to 27.5% of fecal coliforms, 21.8 to 30.4% of *E. coli*, and 8.3 to 11.5% of enterococci in the water column were associated with particles. Enterococci tended to associate with small particles and were less likely to be removed from the water column through settling. This differential partitioning of enterococci to small particles was also noted by Krometis (2007), who similar results for *Clostridium perfringens*. Although TSS has been shown to be important for association with fecal indicator bacteria, there is little direct evidence showing that TSS serves as a direct predictor of survival or growth of fecal indicator bacteria. Given the array of particles that can qualify as "total suspended solids" it is likely that the chemical makeup of the solids,

and the quality of the organic matter within (labile vs. refractory) is more important than the total quantity.

Important data gaps related to turbidity and suspended solids include the following:

- relationship of different types of particles as a portion of the TSS signal;
- carbon to nitrogen rations in a range of different suspended solids, and TSS from a range of different environments, and the relationship to fecal indicator bacteria concentration, survival, and growth;
- their impacts in settings with limited fecal impacts in waters and sediments and in all climate zones;
- the relationship between the recession of stream hydrographs, the recession of turbidity and TSS from peak values after storms, and the recession of fecal indicator organism concentrations from peak values after storms for watershed of all sizes, physical characteristics, and land uses; and
- the relationship between turbidity in deep waters and net reduction in indicator counts due to solar radiation attenuation at a particular site or in a particular water body.

Table 6. Settings in which suspended solids and turbidity effects on extra-enteric fate were studied

Medium	Water type	Impacts	Climate		
			Tropical	Subtropical	Temperate
Water column	Marine	HSI[a]	X		
		HAI[b]			
		LI[c]			X
	Estuarine	HSI	X	X	X
		HAI			X
		LI			
	Fresh	HSI			
		HAI			
		LI			X

[a] Expected or observed significant impacts from human sewage.
[b] Expected or observed impacts from human agriculture/animal operations.
[c] Expected or observed limited impacts from human sources.

4.7.1.5 Nutrients

Because comparatively few studies have systematically investigated the influence of nutrients on indicator organism survival, those studies are reviewed individually below.

Alm et al. (2006) concluded that ample nutrients are present in Lake Huron beach sands to sustain growth of *E. coli*. In controlled laboratory microcosm studies using autoclaved beach sand inoculated with *E. coli* strains previously isolated from ambient beach sand, *E. coli* densities increased from 2 CFU/g to more than 2×10^5 CFU/g sand after 2 days of incubation at 19°C, and remained above 2×10^5 CFU/g for at least 35 days. In field studies using similarly inoculated microcosms filled with sterilized beach sands, growth was observed in microcosms but not adjacent sands. The lack of growth in adjacent sands was taken as evidence of predation or washing of indicators from the sands.

The presence of sufficient nutrients for growth in unimpacted tropical soils was demonstrated by Byappanahalli and Fujioka (1998). This study found that (1) *E. coli* grew on 10% soil extract agar, (2) populations of fecal coliforms and *E. coli* from sewage were shown to immediately increase by about 3-logs when simple nutrients (glucose and salts) were added to natural soil, and (3) fecal coliforms and *E. coli* increased by 2-logs within 24 hours when a minimal amount of sewage was added to cobalt-irradiated soil.

In studies in fresh waters in Lake Michigan, Byappanahalli (2003) determined that *Cladophora* (macro-alga) provides a suitable environment for indicator bacteria to persist for extended periods and to grow under natural conditions. Observed growth of *E. coli* and enterococci was directly related to the concentration of algal leachate.

Carillo et al. (1985) studied growth of indicator organisms in fresh tropical waters. Correlations between bacterial densities, nitrates, phosphates, and total phosphorus indicated that all viable counts were related to nutrient levels, regardless of the site sampled. Sites sampled in that study ranged from highly impacted from sewage discharge to limited expected impacts. *In situ* studies in unimpacted waters indicated that nutrient levels in the water column were sufficient to sustain significant indicator organism growth.

In studies of soils in the swash zone of marine waters, Genthner et al. (2005) determined that entrapment may partially account for increased bacteria densities; however, biological factors (e.g., nutrients, protection from predation) and physical factors (e.g., particulate matter, periodic wetting and drying, protection from solar irradiation) may not only allow the enhanced survival of bacteria but may actually provide a growth-promoting environmental niche on the beach.

In a study by Lopez-Torres et al. (1987), statistical analyses indicated a positive correlation between concentrations of fecal coliforms and increasing concentrations of phosphates, total phosphorus, and nitrates. This finding implies that fecal coliforms follow a familiar pattern of association with productive environments and high nutrients—conditions that are conducive to growth.

The following two general conclusions can be drawn from these findings:

- Nutrient requirements for growth of indicators in soils and the water column are modest and have been shown to be present in sufficient quantities for growth in relatively unimpacted waters and soils.
- High nutrient levels are associated with relatively high counts of fecal coliforms in soils.

4.7.1.6 Organic Matter

Because comparatively few studies systematically investigated the influence of organic matter on fecal indicator bacteria survival, those studies are reviewed individually below.

Seawater samples along an area of the Tyrrhenian coast near the Tiber River mouth were examined for coliforms, fecal streptococci (enterococci), enteroviruses, *Salmonella* spp.,

coliphages, *Bacteroides fragilis* phages, *Pseudomonas*, alophilic *Vibrios*, *Aeromonas*, and yeasts (Aulicino et al. 2001). Their results showed that the area studied was characterized by the presence of organic matter originating from land that can support the presence of opportunistic pathogens and other microbial flora.

Evanson and Ambrose (2006) observed spatial differences in enterococci counts at two sites in a tidal wetland. One site tended to undergo flowing waters whereas stagnant conditions were generally present at the other site. Differences between sites were attributed to sediment differences, such as organic content and finer grain size and/or discrete sources of fecal indicator bacteria.

Ferguson et al. (2005) examined water and sediment samples for a range of fecal indicator bacteria from six sites in an urban estuary located in Sydney, Australia. In that study, the presence of total organic matter was associated with a significant increase in the density of both fecal coliforms and fecal streptococci.

In a study on the effects of estuarine sediments on the survival of *E. coli* in marine waters in Texas, Gerba and McLeod (1976) observed longer survival of *E. coli* in sediments than in the water column and attributed the increased survival to the greater content of organic matter present in the sediment compared with that in seawater.

Lee et al. (2006) showed that fecal indicator bacteria levels in overlying water at two beaches were related to sediment organic content.

These studies collectively show that organic matter is associated with the occurrence and growth of fecal indicator bacteria. Research is necessary to derive further information about the roles of specific types of organic matter and their relationships to fecal indicator bacteria growth and persistence. Specifically, the quality of the organic matter, including molecular weight, and carbon to nitrogen ratios, is probably important to ascertain. In receiving waters, it is also important to understand whether organic matter is serving as a substrate for growth, or as a protective shield from irradiation and predation, or both.

4.7.1.7 pH and Alkalinity

Only two studies were identified that systematically investigated the influence of pH on indicator organism survival and only one investigated alkalinity effects; these are reviewed individually below.

Carillo et al. (1985) observed negative correlations between bacterial densities (*Bifidobacterium* spp., fecal coliforms, *E. coli*) and pH in the range $6.2 < pH < 7.0$. Studies were conducted at multiple sites on a tropical freshwater stream. In Croatian marine waters, Šolić and Krstulović (1992) found that the optimum pH for fecal coliform survival was between pH 6 and 7 with rapid decline occurring both above and below this range. Carillo et al. (1985) also found that fecal coliform and *E. coli* counts in the water column were correlated positively with alkalinity.

Systematic research could be conducted on the effect of pH and alkalinity on fecal indicator bacteria survival in fresh and marine waters and on the synergy of pH effects with salinity, sunlight, and temperature effects. The results from such studies could be used in developing predictive models for net indicator organism occurrence in recreational waters.

4.7.2 Context-Related Parameters

4.7.2.1 Rainfall and Runoff

The conditions under which rainfall and runoff influence indicator organisms are shown below in Table 7. Summaries of findings of studies on the effect of rainfall and runoff are found in Appendix C, Table 19.

Table 7. Settings for which rainfall and runoff effects on extra-enteric fate were studied

Medium	Water Type	Impacts	Climate		
			Tropical	Subtropical	Temperate
Water column	Marine	HSI[a]	X	X	
		HAI[b]			
		LI[c]			
	Estuarine	HSI		X	
		HAI			
		LI			
	Fresh	HSI			
		HAI			
		LI			
Sediments	Marine	HSI	X	X	X
		HAI			
		LI			
	Estuarine	HSI		X	
		HAI			X
		LI			
	Fresh	HSI			
		HAI			
		LI			X
Soil	Marine	HSI	X		
		HAI			
		LI			
	Estuarine	HSI			
		HAI			
		LI			
	Fresh	HSI			
		HAI			
		LI			

[a] Expected or observed significant impacts from human sewage.
[b] Expected or observed impacts from human agriculture/animal operations.
[c] Expected or observed limited impacts from human sources.

Rainfall potentially influences the occurrence of fecal indicators through the following mechanisms:

- initiation of sewer overflows;
- advection of fecal indicator organisms into stormwater conveyances and directly into receiving water, soils, and sediments;
- increase in streamflow and storm sewer flow rates; and
- washing of indicator organisms from soils and sediments to down-slope soils and sediments or into receiving waters.

Other, less-direct, influences that rainfall may have on indicator organism occurrence, survival, decay, and growth include the following:

- changes in sunlight penetration related to changes in turbidity associated with high runoff rates and entrainment of sediments into the water column;
- changes in particle concentrations with attendant change in proportion of particle-associated bacteria;
- changes in water temperature; and
- advection of nutrients and carbon sources to soils, sediments, and the water column.

Although runoff, rainfall patterns, and land use are of great interest in predictions of fecal indicator bacteria input to aquatic systems, a review of that literature is not possible within this document. This section is merely to summarize some of the recent work in the area and to give the reader some examples to follow for further reading. There are many mechanisms by which rainfall and runoff may influence indicator organism occurrence and survival. As noted by Ackerman and Weisberg (2003) although rain-based water quality warnings are attractive due to their simplicity and the speed (relative to microbial enumeration) with which they may be issued, the underlying relationships between rainfall and beach concentrations remains poorly understood. Characklis et al. (2005) assert that "there is no such thing as a 'typical' storm." This assertion is based on the variability of indicator organism counts (fecal coliforms, *E. coli*, enterococci, *Clostridium perfringens*, total coliphages, and particle concentration) among storms that occurred after at least 3 consecutive days without appreciable rainfall and that caused stream discharge to increase by at least a factor of 4.

Several studies have sought to identify threshold rainfall amounts at which fecal-indicator events such as exceedances of AWQC are likely to occur. In analyses of rainfall and fecal indicator organism concentrations observed at beaches in southern California, Ackerman and Weisberg (2003) determined that storms with rainfall less than 2.5 mm produced no change in indicator organism concentration above background levels and that all storms with rainfall amounts greater than 25 mm resulted in an increase in the number of beaches failing state water quality standards.

Other studies attempted to relate the timing of rainfall to peaks in concentrations of fecal indicator bacteria . Ashbolt and Bruno (2003) determined that rainfall on the day of sampling (as opposed to 24 hours prior to sampling) was the strongest predictor of enterococci counts in the water column in marine waters near a highly-urbanized area. In contrast, Mallin et al. (2001) and Seurnick et al. (2006) found rainfall in the 24 hours prior to sampling to have a significant correlation with fecal coliform concentration in the water column. Ackerman and Weisberg (2003) found that indicator organism counts in the water column peaked two days after small

storms and one day after large storms. Craig et al. (2002) determined that rainfall in the two days prior to sampling was correlated to fecal coliform counts in marine sediments and LeFevre and Lewis (2003) found that increased enterococci concentrations in sediments were observed approximately 1 day after a rain event.

The duration of high indicator organism counts after rainfall is highly variable among sites and from storm-to-storm. Ackerman and Weisberg (2003) found that at beaches in the City of Los Angeles, average fecal coliform concentrations fell to background levels within 5 days of rain events. Krometis et al. (2007) found that in a freshwater stream in North Carolina where institutional and low density residential land use dominate the drainage, fecal coliform and *E. coli* concentrations rose much more rapidly at the onset of a storm and receded much faster than did enterococci and *Clostridium perfringens*. One possible explanation for the longer occurrence of enterococci after storms is its tendency to associate with smaller, slower-settling particles (Characklis et al. 2005; Krometis et al. 2007). In eastern North Carolina, Coulliette et al. have demonstrated the importance of stormwater as a conduit of fecal indicator bacteria to estuarine systems.

Data gaps related to rainfall and runoff include the influence of rainfall and runoff from drainages with significant confined animal operation land use, the impact of rainfall and runoff on soils indicator organism concentrations for all water types and climate zones, and systematic studies on the influence of rainfall and runoff on indicator organism concentrations in the water column and sediments of fresh waters. Studies of indicator organism dynamics could also be conducted, since rainfall has been widely suggested as an important element of beach water quality forecasting. However, as rainfall patterns can be highly regional this work typically needs to be conducted on a small scale.

4.7.2.2 Mixing and Circulation

The conditions under which researchers have studied the influence of mixing and circulation on indicator organism survival are presented in Table 8 and the studies in the literature pertaining to rainfall and runoff are summarized in Appendix C, Table 20.

Mixing and circulation influence fecal indicator organism survival and occurrence directly through transport of the organisms, dilution, and resuspension. Indirect influences on indicator organism populations are the transport of nutrients and substrate to indicator organisms in all media and through changes in physical and chemical properties related to indicator organism survival and growth. Mixing occurs at many time and length scales in the natural environment. Mixing may be related to tidal cycles, wind effects, wave action (LeFevre and Lewis 2003), groundwater circulation, stratification or turnover of lakes, turbulence, density currents, and numerous other causes. Boehm et al. (2002) demonstrated the dependence of indicator organism concentration on mixing and fluid transport processes that occur on monthly, daily, hourly, and 10-minute time scales. Measured indicator organism concentrations at a given time are a result of the net effect of these processes with different time scales.

Table 8. Settings for which mixing and circulation effects on extra-enteric fate were studied

Medium	Water Type	Impacts	Climate		
			Tropical	Subtropical	Temperate
Water column	Marine	HSI[a]	X	X	
		HAI[b]			
		LI[c]			
	Estuarine	HSI			
		HAI			
		LI			
	Fresh	HSI			X
		HAI			
		LI			
Sediments	Marine	HSI			X
		HAI			
		LI			X
	Estuarine	HSI		X	
		HAI			
		LI			
	Fresh	HSI			
		HAI			
		LI			
Soils	Marine	HSI			
		HAI			
		LI			
	Estuarine	HS			
		HAI			
		LI			
	Fresh	HSI			X
		HAI			
		LI			

[a] Expected or observed significant impacts from human sewage.
[b] Expected or observed impacts from human agriculture/animal operations.
[c] Expected or observed limited impacts from human sources.

Several studies related changes in indicator organism concentrations to tidal processes. Boehm et al. (2002) noted that the following tidal processes have the potential for influencing indicator organism concentrations: tidal flushing of estuaries and channels, tidally-modulated near-shore circulation patterns, exfiltration of bacteria-contaminated groundwater via tidal pumping, and the movement of offshore wastewater fields by internal tides. Shibata et al. (2004) observed that enterococci and *Clostridium perfringens* concentrations were elevated at the shoreline of a tropical ocean beach during high tides while Solo-Gabriele et al. (2000) observed that the highest *E. coli* measurements made during their study during periods between rain events were at high tides. Jeong et al. (2005) found increased indicator counts at ebb tides at a beach in southern California and attributed this finding to reduction in salinity attendant to ebb tides. Rosenfeld et al. (2006) determined that contamination events at southern California beaches occurred more frequently with larger tidal range.

Mixing and turbulence may result in the washing of indicator organisms from soils or sediments into the water column or the resuspension of individual bacteria or particle-associated bacteria into the water column. An et al. (2002) related the purchase of gasoline at a marina (an indicator

of power boat use) to levels of indicator organisms during periods with no storms. The authors concluded that turbulence generated by boats that resuspend sediments that are laden with fecal indicator bacteria, resulting in elevated counts in the water column. The action of waves on beach sands may also entrain indicator organisms into the water column. Oshiro and Fujioka (1995) associated wave action with low concentrations in near-shore sand indicator organism concentrations. Waves were hypothesized to wash indicator organisms from soils, resulting in low indicator organism concentration in soils near the shore than in soils further upslope.

Mixing processes and circulation processes are complex and can influence indicator organism concentrations in myriad ways. The current understanding of the role mixing plays in the occurrence and persistence of indicator organisms is limited and probably insufficient for *a priori* prediction of the influence of mixing at sites where no data have been taken. Relatively few studies were found in the literature dealing with mixing and circulation directly. Data gaps related to mixing and circulation are data for all media and impact classifications for tropical climates, data for all media and water types in areas classified as LI and HAI, and data related to impacts in temperate climate water column and marine soils.

4.7.2.3 Land Use

Several studies were identified that directly related indicator organism occurrence and survival to land use. Other studies that related watershed characteristics to indicator organism dynamics in a less direct or circumstantial way are not reviewed here. In general, relating indicator organism stocks and flows to watershed characteristics is difficult given the resolution of data usually available. As noted by Kistemann et al. (2002), "for every situation in a watercourse, an individual analysis has to be carried out, taking into account geographical conditions in catchment areas as well as variability in precipitation and runoff."

Mallin et al. (2001) analyzed several data sets within coastal North Carolina (estuaries and fresh water) with the goal of assessing the impact of demographic, landscape, and meteorological factors on aquatic fecal coliform bacteria pollution. On a watershed scale, an analysis of several tidal creeks found strong correlations between mean estuarine fecal coliform bacterial counts and watershed population, percent developed area, and especially with percent impervious surface coverage. An analysis of rural watersheds in the coastal plain found that stream fecal coliform counts and turbidity were both strongly correlated with rainfall in the previous 24 hours in watersheds containing extensive industrial swine and poultry operations, as well as in watersheds containing more traditional agriculture and cattle husbandry. In contrast to these findings, in watersheds rich in swamp wetlands these relationships were not significant—even in watersheds containing extensive animal production.

Ramirez et al. (2000) sampled at several marine water stations near La Parguera (southwest Puerto Rico) because of the incidence of onshore development and stormwater runoff in their drainages and the littoral and coastal systems at these locations. The parameters that were measured suggest that there are effects from onshore development, with stations down-current and those influenced by new development generally with higher concentrations of chemical and physical constituents and higher enterococci counts. Subsequent water quality analysis showed that areas adjacent to new development are significantly impacted.

Traister and Anisfeld (2007) conducted a study in a river basin and showed a strong relationship to land use characteristics for fecal indicator bacteria, while also identifying patterns in fecal indicator bacteria attributable to base flow versus storm flow.

Finally, Yamahara et al. (2007) found the presence of a putative bacterial source (i.e., river discharges), the degree of wave shelter, and surrounding land use explained variation in both enterococci and *E. coli* densities in beach sands along the California coast. Other parameters that were found to influence sand *E. coli* density were moisture and organic carbon content.

4.8 Modeling

A range of researchers have begun to incorporate the array of literature values for fecal indicator bacteria decay into context specific models at beaches to improve predictions of fecal indicator bacteria concentrations given environmental conditions. An example of this approach is by Liu et al (2006), where an assessment of processes relevant to fecal indicator bacteria fate and transport were studied. The researchers incorporated inactivation rates factoring in sunlight, sedimentation, and temperature, and loading from tributaries to describe dynamics of fecal indicator bacteria. In another study, Boehm et al. 2005 constructed a modeling of enterococci concentration in the surf zone at a marine beach incorporating transport via alongshore currents, inactivation, and grazing. For this study, they relied on inactivation parameters from the literature for relevant environments. The researchers also conducted specific laboratory experiments to parameterize grazing rates. The researchers showed that dilution was the main cause of loss of enterococci along the beach, while grazing and inactivation were less important. Other recently available computer models for predicting surface water quality include HSPF (Hydrologic Simulation Program-Fortran), WASP (Water quality Analysis Simulation Program), Qual2K (river and stream water quality model) and EFDC (Environmental Fluid Dynamics Code). These models simulate natural aquatic systems by including parameterization for physical processes as dispersion, dilution, mixing. The models also often rely on first order decay parameters derived from the literature, of particular high incorporation of first order decay rates of fecal coliforms. Given the recent shift away from the fecal coliform group, and the observation in recent years that first-order decay is rarely observed in ambient waters, it will be necessary to conduct additional research to more fully understand the variability in the behavior of fecal indicator bacteria, and also to incorporate more complex behavior into future computer models. A recent paper by Liu et al. 2006, demonstrates an effort to model transport and inactivation of *E. coli* and enterococci in Lake Michigan. These researchers successfully incorporated loading data from tributaries, and inactivation as driven by sunlight, temperature and sedimentation in a hydrodynamic modeling effort. There are a range of studies that have been conducted additionally to try and develop more simplistic predictive and probabilistic models (as opposed to mechanistic models of environmental processes, fate and transport). A summary of those exercises is beyond the scope of this document. For example, Wong et al. 2009 conducted a study of Lake Michigan using a range of markers and fecal indicator bacteria types to try and develop a relational model of viruses and indicators. These researchers and a range of others have identified the lack of correlation between fecal indicator bacteria and viral pathogens. This is an area of research that continues to confound our ability to measure pathogens directly as a means for protection of public health risk.

4.9 Summary and Conclusions

Findings of studies assessing the impact of physical, chemical, and biological factors on the extra-enteric extended occurrence, survival, fate, and growth of fecal indicator bacteria were reviewed systematically in an attempt to (1) ascertain the factors most important in determining indicator organism survival under ambient conditions; and (2) contrast the behavior of fecal indicator bacteria in different waters (marine, estuarine, and fresh), climate zones (tropical, subtropical, and temperate), and areas characterized by different levels of fecal impact (expected or observed significant impacts from human sewage, expected or observed impacts from human agriculture/animal operations, and expected or observed limited impacts from human sources).

The preceding literature review supports the following conclusions:

- Fecal indicator organism die-off and decay in laboratory controlled experiments has been generally well studied. However, it is clear that examination of single factors in factorially designed, laboratory-based experiments is not the optimal way to assess the behavior of fecal indicator bacteria under natural conditions. These controlled experiments might be more relevant to wastewater and drinking water studies.
- Given favorable environmental conditions, fecal indicator bacteria growth has been shown to occur widely, except in the marine water column. This finding highlights the importance of the appropriate selection of fecal indicator bacteria for specific environments (i.e. the widespread use of enterococci in marine environments), and also highlights the importance of local knowledge in interpretation of fecal indicator bacteria data that could possibly be a direct result of reservoir populations.
- Occurrence and persistence of fecal indicator bacteria in soils and sediments are consistently greater than those observed in the water column, regardless of media, climate, or water type. Important factors that enhanced survival of indicator organisms in soils and sediments include the availability of nutrients and organic material, shelter from sunlight, and the presence of conditions more favorable to the survival of the indicator organisms than their predators. Enhanced persistence of fecal indicator bacteria in soils, sand, and sediments is significant because fecal indicator bacteria stored or growing in soils may be mobilized via washing of soils or resuspension. Thus, mobilized indicator organisms represent a potentially important source of fecal indicator bacteria that is not specific to a recent fecal contamination event, and may indicate decoupling of the fecal indicator bacteria population from pathogens of concern.
- Among intensive factors influencing the extra-enteric survival of fecal indicator bacteria, the most important appear to be solar radiation, salinity, and temperature. Based on findings in studies in which all three of these parameters were varied systematically, their order of importance for fecal indicator bacteria in the water column appears to be (1) solar radiation, (2) salinity, and (3) temperature. Synergistic effects of these three parameters have been observed, with both temperature and salinity enhancing the effect of sunlight. Among studies assessing the role of nutrient and organic material availability, and particle-association of fecal indicator bacteria, the findings have been much more variable and difficult to generalize.
- Among extensive (context-specific) factors influencing the extra-enteric fate of fecal indicator bacteria, the most important appear to be rainfall, antecedent rainfall, runoff,

and land use. Insufficient data are available to make general characterizations on the influence of particular categories of land use on the fate of fecal indicator bacteria, except to state that impervious surface causes input of fecal indicator bacteria into receiving waters much more rapidly that non-impervious surface, reducing the potential for fecal indicator bacteria degradation in the process. The impacts of both rainfall/runoff and mixing on fecal indicator bacteria concentrations vary widely spatially and temporally and impacts are realized at multiple temporal and spatial scales. There was no consistency among studies on the time period over which wet weather contributes to elevated water column indicator organism concentrations or the period or amount of rainfall that was the best predictor of water column concentrations or water quality standard exceedances. Therefore, it is vital for water quality agencies to conduct their own assessments of the relationships between fecal indicator bacteria concentration and rainfall amount, duration, and intensity.

- Newly developed models that take into account more complex parameters are necessary o capture the great range of variability observed in aquatic systems. Notably, models that can incorporate a range of processes related to degradation/loss/survival/growth of fecal indicator bacteria are likely to be superior to those that only incorporate first-order decay.

Data from the literature present several opportunities for further analysis, though data gaps must be filled for the analyses to be conducted. Three potential topics for further research are discussed below.

First, studies in which microbial kinetics (growth or decay) were explored under controlled conditions could be used to compare dynamics in different climate zone-fecal impact combinations. Appropriate statistical techniques for this analysis are analysis of covariance (ANCOVA) and possibly nonlinear multiple regression. A potential impact of this study would be testing of the hypothesis that a single set of environmental conditions at which survival or growth of indicator organisms is optimal may be defined in terms of general (site-independent) parameters, irrespective of climate zone and level or source of fecal pollution. Obstacles to this analysis are lack of complete data on factors controlling microbial dynamics and lack of data for many climate-zone-fecal impact combinations. As described above, observed decay is not well-described by first-order models due to predation and competition, differences in inactivation rate between individual and particle-associated organisms, and likely other factors. Data available in the studies are not sufficient to allow quantification of these factors. Also, controlled experiments have not been performed for many media-climate zone-fecal impact combinations, particularly for waters in areas impacted by agriculture and in soils and sediments. An example of a study similar to that proposed (but with many fewer factors explored) is presented by Wuenschel et al. (2005).

Second, the relationship between rainfall and temporal variations in fecal indicator bacteria concentrations could be quantified. The objective of such an analysis would be to relate timing and magnitude of peak indicator organism occurrence to watershed characteristics. Current research in the combined fields of hydrology and fecal indicator bacteria fate and transport will improve our abilities to quantify loading throughout the extent of the hydrograph, and to relate fecal indicator bacteria dynamics to antecedent rainfall, rainfall duration, and rainfall intensity. Results from this analysis could be used to develop input to predictive models for forecasting

and "nowcasting" periods of exceedances of water quality standards (e.g., Frick et al. 2008). Challenges to performing this analysis are the extreme spatial and temporal variability in observed fecal indicator bacteria concentrations (Boehm et al. 2007), the small number of studies available for analysis, and the general lack of complete data sets [for indicator organism counts] for most sites (Frick et al. 2008). Examples of analyses that might be performed relating watershed characteristics to indicator organism occurrence are refinements of regression analyses, such as those advocated by Frick et al. (2008), or ANCOVA, as demonstrated by Bishop et al. (2005) in their assessment of the impacts of agricultural best management practices on stream water quality. Coulliette et al. (2009) used a space/time model of the Newport Estuary as a means to integrate estuarine characteristics, tidal flushing, antecedent rainfall, and rainfall duration and intensity. It may be possible in the future to combine space/time (geospatial) models of fecal indicator bacteria concentration in large scale systems, with hydrodynamic models.

Third, there is the potential for exploring the role that storage and growth in sands and sediments and subsequent resuspension play in occurrence of indicator organisms in the water column. As with indicator organisms in the water column, indicator concentrations in sediments and near-shore soils can exhibit high variability. Among studies surveyed, the ratio of indicator organism concentration in sediments or soils to those observed in the water column ranged from 0.076 (Brownell et al. 2007; fecal coliforms in marine environment during dry weather) to 23 (Bonilla et al. 2007; enterococci in wet sands in the tidal zone in a marine environment) to 460 (Bonilla et al. dry sands above the tidal zone in a marine environment). Study of these data could potentially be used in predictive models relating contributions of indicator organisms from sediments and soils to water column indicator concentrations under various rainfall and mixing conditions.

Given new interest in molecular methods for quantification of fecal indicator bacteria and pathogens it will be vital to understand the persistence of DNA/RNA signals in relation to existing culture-based methods. This work has been initiated by a range of researchers and as QPCR-based methods become more commonly implemented in water quality agencies, these data will be important for interpretation of potential public health risk.

In summary, a systematic determination, through a formal meta-analysis, of the role, if any, that climate zone, water types, and relative level of impact by fecal pollution play in the extra-enteric fate of fecal indicator bacteria in recreational waters was hampered by the lack of data for many climate-water type-impact level combinations. In general, the literature on this subject are characterized by a high density of published studies conducted in specific regions (southern California, the beaches of southern Lake Michigan and Lake Huron, beaches in the vicinity of Sydney, Australia and in south Florida, and sands, soils, and surf-zone waters located near large urban settings in Hawaii). There are relatively few studies in areas with limited fecal pollution impacts, regions with high fecal pollution impacts from sources associated with confined animal feeding operations, and river, stream or other inland water (particularly flowing water) settings. In addition to data gaps related to regional and water quality differences, there are data gaps related to the variability in fecal indicator bacteria at particular sites.

5. Alternative Indicators for Tropical and Subtropical Regions

Culturable *E. coli* and enterococci have several widely documented limitations as indicators of waterborne pathogens. These traditional fecal indicator bacteria are often found in high concentrations in animal feces, and the public health risk associated with contact with animal feces is poorly understood. These traditional fecal indicator bacteria also survive and grow in sand and other beach sediment (Solo-Gabriele et al. 2000, Desmarais et al. 2002, Byappanahalli and Fujioka 2004), particularly in tropical climates. This survival and growth decouples the fecal indicator bacteria from the fecal pollution they are intended to represent.

In response, a variety of alternative indicators and indicator approaches have been proposed and assessed in recreational waters. Many of these are reviewed by Griffin et al. (2001), Fujioka (2001), Savichtcheva and Okabe (2006), and more recently in Chapter 2 of EPA's *Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria* (USEPA 2007a). Such alternative approaches can be divided broadly into those that are microbiologically-based and those that involve the use of chemical markers. Much of the research into alternative indicators of water quality—especially for the use of chemicals—is tied closely to microbial (fecal) source tracking efforts (see Field and Samadpour 2007). Not surprisingly, whether an approach is chemically- or microbiologically-based, these alternative methods are often used in conjunction with traditional fecal indicator bacteria to assess their validity and utility. Table 21 in Appendix C includes a representative survey of studies (in ascending chronological order by alternative indicator used) that have employed one or more of the alternative microbiological and chemical indicators to assess microbial water quality in tropical, subtropical, and temperate regions reviewed in this section.

Until the last decade or so, most alternative microbiological indicators that have been used to assess recreational water quality criteria have been culturable bacteria. With the growing development and use of molecular detection techniques (e.g., PCR), a broader variety of bacteria and now viruses (especially viruses of bacteria or phages) have been identified as potential alternatives to traditional fecal indicator bacteria. Notably, these techniques have also given rise to the ability to identify and track the sources of microbial contamination using both traditional indicator bacteria as well as many of the novel bacteria and viruses that are discussed subsequently in this section. Given these technological advances, some researchers have advocated approaches to detect so-called indicator bacteria (e.g., *Aeromonas*, *Staphylococcus aureus*, *Vibrio vulnificus*) and viruses (e.g., enteroviruses) that are actually or potentially pathogenic, especially to immunocompromised persons (Fujioka 2001, Griffin et al. 2001). However, a discussion of the direct detection of frank and opportunistic pathogens to assess microbial water quality is beyond the scope of this draft report.

5.1 Alternative Indicators of Fecal Contamination: Microbiological

5.1.1 Clostridium perfringens

Clostridium perfringens is a bacterial indicator that has been used to assess water quality for decades (NRC 2004), but it is still considered widely to be an alternative indicator. *C.*

perfringens is a Gram-positive, anaerobic, rod-shaped bacterium. Oxygen tolerance of *C. perfringens* has been poorly characterized in the environment: some studies show survival in atmospheric oxygen for at least 72 hours (Rolfe et al. 1977), while others have observed cell death after an hour in an aerobic environment (Trinh et al. 2000). However, *C. perfringens* forms highly resistant endospores which limit inactivation due to physical stresses such as light, heat, desiccation, and water and wastewater treatment processes.

Research demonstrating that *C. perfringens* is consistently present in sewage (Fujioka and Shimuzura 1985) and that its density in river water is significantly correlated with the presence of enteric pathogens (Payment and Franco 1993, Brookes et al. 2005) recommend it as an indicator of recreational water quality. In fact, *C. perfringens* may be a better indicator than *E. coli* and enterococci for tropical and subtropical waters because (1) it is more stable in environmental waters than some waterborne pathogens (Medema et al. 1997); (2) its concentration in tropical soil is orders of magnitude lower than fecal coliforms and enterococci (Roll and Fujioka 1997); (3) it is found less often in wildlife and ruminant domestic animal feces (Cox et al. 2005); (4) it has been used successfully to monitor sewage contaminated streams and ocean water, particularly in Hawaii (Fujioka 2001, Fujioka and Shizumura 1985); and (5) it is less susceptible to inactivation and degradation than fecal coliforms and enterococci, meaning that it is offers a conservative indication of sewage (Davies et al. 1995).

Unfortunately, this extended persistence may simultaneously limit the usefulness of *C. perfringens* as an alternative indicator. Because spores can survive for years (Hill et al. 1996), their detection in environmental media can be difficult to interpret in the absence of a known source of fecal contamination. The presence of spores could indicate normal microflora in an environment, represent a "historical" (nonrecent) fecal contamination event, or reflect recent fecal contamination that is relevant to public health (Mueller-Spitz et al. 2010). Persistence may be limited in the tropics relative to temperate latitudes because rates of *C. perfringens* degradation correlate significantly with exposure to radiant energy (Burkhardt et al. 2000). However, this correlation is very weak, and the use of *C. perfringens* as a fecal indicator organism remains equivocal in light of uncertainties associated with determining the age of spores (NRC 2004). However, researchers have reported the successful use of *C. perfringens* to assess microbial water quality. For example, Ferguson et al. (1996) reported *C. perfringens* to be the most useful indicator of fecal pollution in water and sediment in an Australian estuarine system. Fujioka (2001) concluded that *C. perfringens* is a conservative indicator that may be used to determine whether recreational water is contaminated by fecal pollution. That is, "If monitoring results show no or low levels of *C. perfringens*, one can be assured that the quality of that recreational water is high (Fujioka, 2001)."

5.1.2 Bacteroides

Bacteroides, Gram-negative anaerobes, have also long been suggested as alternative indicators to *E. coli* and enterococci (Fiksdal et al. 1985). In sewage samples, *Bacteroides* and fecal coliform concentrations correlate strongly (Dick and Field 2004), and because *Bacteroides* are present at more than 1,000-fold-higher densities than fecal coliforms (Fiksdal et al. 1985), *Bacteroides* could serve as more sensitive indicators of human fecal contamination than fecal coliform bacteria from a purely numerical standpoint. In fact, in a wide range of surface water samples,

Bacteroides concentrations were more predictive of the presence of bacterial pathogens than traditional indicators (Savichtcheva et al. 2007).

Bacteroides have also been touted as good alternative indicators because they are strict anaerobes and are not expected to survive for extended periods of time under aerobic conditions (Rolfe *et al.* 1977). Indeed, *Bacteroides* have been shown to lose viability in less than a day after exposure to environmental water (Rolfe et al. 1977, Kreader 1998). There has been some concern that the DNA markers used to measure *Bacteroides* may persist long after cell death, but at tropical water temperatures the DNA signal rapidly decays within 1 to 2 days (Okabe and Shimazu 2007, Walters et al. 2009). This persistence is similar to that of infective enterovirus (Walters et al. 2009).

Recent studies have demonstrated host specificity in certain 16S rRNA genes (Bernhard and Field 2000); therefore, *Bacteroides* gene markers may be useful in distinguishing human from animal fecal pollution. Universal, bovine-, swine-, dog-, and human-specific *Bacteroides* assays have been developed (Carson et al. 2005, Seurinck et al. 2005, Layton et al. 2006, Kildare et al. 2007, Okabe et al. 2007, Stricker et al. 2008, Yampara-Iquise et al. 2008), but some cross-reactivity between sources has been observed for nearly all of these assays. The bovine assays appear to be the most specific, probably because bovine *Bacteroides* cluster much more tightly than those from other hosts (Layton et al. 2006). Nevertheless, the source specificity of *Bacteroides* assays is often greater than 90% (Shanks et al. 2010), and human-specific assays have been used successfully in tropical environments to distinguish between human and non-human fecal pollution (Betancourt and Fujioka 2006, Bonkosky et al. 2009).

5.1.3 Bifidobacteria

For many of the same reasons as *Bacteroides*, *Bifidobacteria* have been recommended as alternative indicators. *Bifidobacteria* are also strict anaerobes and comprise a significant fraction of human-intestinal bacteria (Resnick and Levin 1981). Like *Bacteroides, Bifidobacteria* persistence is limited in environmental waters particularly when temperatures are warm (Resnick and Levin 1981, Rhodes and Kator 1999, Bonjoch et al. 2009, Ottoson 2009). Also similar to *Bacteroides,* many "source-specific" *Bifidobacteria* assays have been developed (Bernhard and Field 2000, Nebra et al. 2003, Bonjoch et al. 2004, King et al. 2007), though many so-called source-specific species have actually been shown to be quite cosmopolitan (Lamendella et al. 2008).

Due to early difficulties in accurate detection and enumeration by both culture and molecular methods (Carrillo et al. 1985, Wang *et al.* 1996, Bernhard and Field 2000), *Bifidobacteria* have received much less attention than *Bacteroides* in the literature. Nevertheless, *Bifidobacteria* have been used in tropical streams and temperate estuaries to distinguish human from animal fecal pollution and recent from residual pollution (Mara and Oragui1985, Carrillo et al. 1985, Morrison *et al.* 2008, Mushi et al. 2010).

5.1.4 Coliphages

Bacteriophages (or simply phages) are nonpathogenic viruses that infect bacteria, and bacteriophages that infect *E. coli* or other closely related coliforms are called coliphages. There is a relatively extensive history of research documenting the possible uses of phages as indicators of fecal contamination—especially indicators of waterborne viruses (e.g. Hilton and Stotzky 1973, Kott et al. 1974, Stetler 1984, Havelaar et al. 1993). Taxonomically, coliphages are very diverse and include a total of six virus families: three families of double-stranded DNA viruses, two families of single-stranded DNA viruses, and one family of single-stranded RNA viruses (USEPA 2007a). Coliphages that infect via the host cell wall of *E. coli* are commonly called somatic coliphages. Male-specific coliphages (also called F+ coliphages) infect *E. coli* by attaching to the F-pili, hair-like appendages protruding from the host. Although somatic phages have been explored as alternative fecal indicators (Palmateer et al. 1991; Brion et al. 2002), little is known about the specificity of their occurrence in human or animal feces (USEPA 2007a). Furthermore, a recent nonpoint source epidemiological study in California found that somatic coliphages were not predictive of human health risks from bathing in marine recreational water (Colford et al. 2007). As a result most research using coliphages as alternative indicators has focused on male-specific coliphages.

Like anaerobic fecal bacteria, male-specific coliphages have been recommended as alternative indicators of fecal pollution because of their prevalence and ready detection in sewage (Calci et al. 1998) and unlikely replication outside the enteric environment even in tropical waters (Hernandez-Delgado and Toranzos 1995, Jofre 2009). Male-specific coliphages are expected to be particularly good indicators of pathogenic enteric viruses because of their similar size, shape, and stability in environmental- and waste-water (Stetler 1984, Havelaar et al. 1993). Indeed, concentrations of male-specific coliphage are significantly correlated with concentrations of enteric viruses in surface water at both temperate and tropical latitudes (Havelaar et al. 1993, Ogorzaly et al. 2009, Espinosa et al. 2009) and health risk associated with bathing in recreational water (Colford et al. 2007). In persistence studies coliphage has had similar or longer survival than many enteroviruses and noroviruses (Nasser and Oman 1999, Allwood et al. 2003, Love et al. 2010). Coliphage persistence may be slightly shortened at tropical latitudes because accumulated light energy speeds inactivation (Burkhardt *et al.* 2000), but because the mechanisms of sunlight inactivation for coliphages and enteric viruses are similar (Fujioka and Yoneyama 2002), this shortened persistence should not affect the relationship between indicator and pathogens. Little is known about relative rates of inactivation of enteric viruses and coliphage in turbid water or sediments, where the viruses are relatively protected from sunlight. Skraber et al. (2009) demonstrated that coliphage can persist for longer than one month in freshwater sediments. As a result, coliphages can be susceptible to the same weakness as *C. perfringens*—where there is resuspension of sediment there can be difficulty in distinguishing between recent and older contamination.

Nevertheless, male-specific coliphage may be useful in distinguishing between sewage and animal waste. Of the four coliphage serogroups, two are usually found in animal waste, while the other two are primarily found in human feces (Hsu et al. 1995, Griffin et al. 2000, Cole et al. 2003, Blanch et al. 2006). These serogroups have been used to confirm putative animal and human waste in both tropical and temperate environmental waters (Griffin et al. 2000, Brion et al. 2002, Luther and Fujioka 2004, Stewart-Pullaro et al. 2006, Griffith et al. 2009). However, the relationship between subgroups is complicated by their differential persistence, and some

researchers discourage the sole use of male-specific coliphage genotyping for source tracking (Muniesa *et al.* 2009).

5.1.5 Bacteroides *Phages*

Bacteroides phages, or viruses that specifically infect *Bacteroides* spp., have been used as indicators of fecal contamination of water and sediment in Spain (Lucena et al. 1996, Tartera et al. 1989), the United States (Chung and Sobsey 1993, McLaughlin and Rose 2006) and more recently in an inland river in the United Kingdom (Ebdon et al. 2007). Bacteroides phages are proposed as alternative indicators for many of the same reasons as coliphages: (1) similar size, shape, and transport as enteric viruses; (2) apparent source specificity (Tartera and Jofre 1987, Ebdon et al. 2007); (3) higher concentrations than enteric viruses in sewage (Tartera and Jofre 1987); (4) similar or longer survival than enteric viruses and sediment and seawater (Chung and Sobsey 1993, Lucena et al. 2003, Mocè-Llivina et al. 2005); and 5) inability to replicate outside the intestinal tract (Tartera et al. 1989). However, the utility of these phages as indicators is currently limited because the diversity of phages, including their specificity for human host strains, remains poorly characterized over a range of locations and quantification methodologies are not fully developed (USEPA 2007).

5.2 Alternative Indicators of Fecal Contamination: Chemical Biomarkers

There are a large number of organic compounds that are specific primarily to human fecal contamination, including chemical indicators that can be either natural products found in human feces (e.g., fecal steroids) or synthetic chemicals found in products that are specific to household and community waste streams (NRC 2004). However, the cost and expertise required to analyze chemical biomarkers may limit their utility for routine water quality monitoring, and more research is needed gain a better understanding of the transport, transformations, and persistence of these compounds under various environmental conditions (NRC 2004). Additionally, most chemical biomarkers have only been used for source-tracking. Few have been associated with traditional bacterial indicators, let alone pathogens. Epidemiology studies relating chemical biomarker concentrations with health risk will be required before their application as indicators.

5.2.1 Fecal Steroids

Fecal steroids (i.e., sterols, stanols, stanones) have been used somewhat frequently for differentiating between human and nonhuman sources of fecal contamination in the aquatic environment (e.g., Nichols et al. 1993, Noblet et al. 2004, Tyagi et al. 2007, Wang et al. 2010). The steroid used most frequently in such studies is coprostanol, which is produced by catabolism of cholesterol in the intestinal tract of higher vertebrates and is the most abundant (~60% of the total sterols) in human feces (Leeming et al. 1996). However, differences in dietary sterol intake, metabolic production, and gut microbiota yield differences in sterol profiles between humans and animals (Leeming et al. 1996), and as a result, most recent studies have used suites of steroids to distinguish between fecal sources. Additionally, the ratio of epicocoprostanol and coprostanol has been successfully used to determine whether or not sewage has been treated before it reaches receiving waters (Froehner et al. 2009).

Steroid concentrations are significantly correlated with *E. coli* concentrations in temperate and tropical Asian marine water, though this correlation appears to be seasonal and dependent upon climatic conditions such as rainfall (Isobe et al. 2004). Although fecal sterols should be microbially degraded in the aerobic water column in 1 to 2 weeks, they may persist on the scale of months when deposited in the tropical and sub-tropical sediments (Bartlett 1987, Pratt et al. 2008). Indeed, bacterial indicator degradation is rapid when compared with steroid degradation in the sediment (Pratt et al. 2008). In situations with likely resuspension of sediment, fecal steroids may overestimate the freshness of fecal contamination. For this reason, many researchers have not embraced the measurement of steroids alone as indicators for fecal contamination, preferring to use this in conjunction with microbial indicators (USEPA 2007). However, the combined use of fecal steroids and *E. coli* has allowed fecal source identification that was not possible with the use of fecal indicator bacteria alone (Noblet et al. 2004).

5.2.2 Optical Brighteners

Optical brighteners may be inexpensive and potentially sensitive alternative chemical indicators, which are detected by fluorometry. In the United States, optical brighteners are almost always added to laundry detergents to help compensate for undesirable yellowing in clothes (Poiger et al. 1998). Because household plumbing systems typically mix effluent from washing machines with wastewater from toilets, the detection of optical brighteners is associated with human sewage (Poiger et al. 1998, Boving et al. 2004).

In order to use optical brighteners as an alternative indicator of human fecal contamination, they must be combined with use of fecal indicator bacteria counts or other microbial source tracking methods (McDonald et al. 2006). Although researchers have documented instances of strong fluorescent signal and high numbers of fecal enterococci (McDonald et al. 2006), cases of no correlation between fluorometry and fecal bacteria counts have also been reported (Wolfe et al. 1995). Two key confounding factors have been interference due to the fluorescence of organic matter (Boving et al. 2004) and photodegradation of optical brighteners (Kramer et al. 1996). Though steps can be taken to account for the presence of fluorescing organic matter (Hartel et al. 2007, Cao et al. 2009), rapid photodegradation in sunlight may limit the utility of optical brighteners in tropical settings, where high UV indexes may decrease optical brightener detection.

5.2.3 Pharmaceuticals and Personal Care Products

More than 6 million pharmaceutical and personal care products (PPCPs) are commercially available worldwide, and many have been detected in streams across the United States (Koplin et al. 2002, Daughton 2004). Though their environmental release during manufacture, processing, and distribution is neglible, many are released in sewage effluent (Daughton and Ternes 1999, Ellis 2006), and their presence in surface waters is taken to be indicative of sewage contamination (Yu and Chu 2009). As a result, PPCPs have garnered a great deal of attention recently as potential indicators of human-specific fecal contamination, including diclofenac, urobiline, caffeine, and triclosan. Because PPCPs represent a broad spectrum of organic contaminants with varying susceptibilities to biodegradation, photodegradation, and wastewater

treatment, it is difficult to make generalized statements about their potential persistence in surface waters (Buser et al. 1998, Daughton and Ternes 1999, Andreozzi et al. 2003, Zhang et al. 2008, Kim et al. 2009, Benotti and Brownawell 2009, Musolff et al. 2009). Furthermore, few compounds have been correlated with traditional indicators (Peeler et al. 2006, Young et al. 2008, Haack et al. 2009, Knee et al. 2010). Much more work is needed on persistence of specific compounds and their relationships with traditional indicators and pathogens before a PPCP will be useful as an indicator.

5.3 Summary

Researchers have been documenting issues and limitations associated with the use of traditional indicator bacteria for assessing the presence of fecal contamination in recreational waters for decades, and many researchers have questioned the validity of continued reliance on the periodic measurement of fecal indicator bacteria in the water column as the sole means of determining the microbial quality of ambient waters.

To help address and potentially resolve these limitations, researchers have evaluated and reported on the use of a wide variety of alternative (and increasingly sophisticated) microbiological and chemical indicators of fecal contamination in ambient waters in a variety of regions. In many cases, these alternative indicators and indicator approaches have focused on microbial source tracking and related research. As a result, many researchers suggest a tiered assessment of water quality, in which traditional fecal indicator bacteria are initially used to identify potential contamination and alternative indicators are used in subsequent steps to determine the source and age of the contamination. Some general approaches have been described in the literature that rely on a set or toolbox of indicators—including alternative indicator and indicator approaches (e.g., NRC, 2004)—to assess the microbial quality of ambient waters. Confidence in the use of alternative indicators to supplement current indicators could increase if EPA and others conduct health studies that demonstrate a statistically valid correlation between the presence of one or more alternative indicators with an increased incidence of illness in exposed persons, thus ensuring that the use of a new indicator is based on health risk. Also, although molecular-based methods show promise for identifying human-specific indicators and pathogens, the methods currently show wide variability both within a single laboratory and between laboratories. Continued work is needed to validate and standardize these methods.

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Appendix A

Summary of 2001 Tropical Indicators Workshop

In recognition of the growing body of literature and research on potential limitations of the continued reliance on fecal indicator bacteria for assessing the microbial quality of recreational waters in tropical and subtropical regions, on March 1-2, 2001, a group of 18 national and international experts convened the "Tropical Water Quality Indicator Workshop" in Honolulu, Hawaii (Fujioka and Byappanahalli 2003). Primary funding for the workshop was provided by the EPA Office of Water; matching funds were provided by the Department of Health of the State of Hawaii and by the Water Resources Research Center of the University of Hawaii. Given the importance and relevance of this workshop to the sections that follow, details of objectives and goals and outcomes are summarized in detail below. The overall goal of the workshop was to address issues identified under the "Tropical Indicators" section of the EPA Action Plan for Beaches and Recreational Waters (USEPA, 1998), which states the following:

> Currently recommended fecal indicators may not be suitable for assessing human health risks in the tropics. Studies have suggested that at tropical locales such as Puerto Rico, Hawaii, and Guam, *E. coli* and enterococci can be detected in waters where there is no apparent warm-blooded animal source of contamination.
>
> Whether or not current indicator bacteria proliferate naturally in soil and water under tropical conditions must be determined. If so, the range of conditions (such as nutrients, temperature, pH, and salinity) under which the bacteria proliferate will be characterized and their geographical boundaries defined. If the phenomenon is widespread under tropical conditions, additional research will be conducted to modify approaches for monitoring, or to develop new tropics-specific indicators. Further evaluation of *Clostridium perfringens* and other microbial indicators (including coliphages) that do not flourish naturally in the tropics will be conducted to determine their usefulness as alternative indicators.

To meet this goal, the workshop had several objectives, including (1) to critically evaluate published information related to sources, persistence, and multiplication of EPA-approved fecal indicators (i.e., fecal coliforms, *E. coli*, and enterococci) in tropical locations and the impact of such findings on the suitability of existing water quality criteria for these locations and (2) to critically evaluate the use of alternative water quality indicators in tropical locations. The invited experts were sent a guidance document, which is included as Appendix D to the workshop proceedings and report (Fujioka and Byappanahalli 2003). The guidance document included five questions to help focus the deliberations of the workshop participants. These questions, reprinted below, are followed in bold text by appropriate consensus-based (i.e., acceptable to at least 75% of experts) statements from the workshop participants, which are considered to be the most important products of this workshop:

> 1. Are there sufficient experimental and monitoring data to conclude that the assumption used in interpreting water quality standards ([i.e.,] there are no significant environmental sources of fecal coliforms, *E. coli*, and enterococci) is not applicable in tropical areas

(Hawaii, Guam, Puerto Rico, south Florida) because these bacteria can be recovered in high concentrations from ambient environments (water, soil, plants) in these areas?

Soil, sediments, water, and plants may be significant indigenous sources of indicator bacteria in tropical waters.

2. Are there sufficient experimental and monitoring data to conclude that the EPA criteria (*E. coli*, enterococci) used to assess the quality of environmental waters are not reliable in tropical locales (Hawaii, Guam, Puerto Rico, south Florida) because the selected fecal bacteria persist in these ambient environments and represent non-fecal contamination?

No consensus-based statement was developed or reported.

3. Are there sufficient experimental and monitoring data to conclude that the EPA recommended recreational water quality standards are not suitable to assess the hygienic quality of environmental waters in Hawaii, Guam, Puerto Rico, and south Florida?

The inherent environmental characteristics of the tropics affect the relationships between indicators of fecal contamination (*E. coli*, fecal coliforms, enterococci) and health effects observed in bathers, which may compromise the efficacy of EPA guidelines.

4. Are there sufficient experimental and monitoring data to conclude that fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) can multiply in tropical environments and that bacteria from these sources are indicative of lower health risk than those from fecal sources?

Fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) can multiply and persist in soil, sediment, and water in some tropical/subtropical environments (Hawaii, Guam, Puerto Rico, south Florida).

5. Are there sufficient experimental and monitoring data to conclude that the proposed alternative criteria and recreational water quality standards for Hawaii and Puerto Rico are more useful than the current EPA criteria and standards?

Recreational water quality guidelines for the tropics/subtropics should be supplemented with additional alternative indicators (*C. perfringens*, coliphages) for watershed assessment (or sanitary survey).

Although five of eighteen experts accepted the above statement, they preferred the following alternate statement: **In the absence of a predominant point source pollution, recreational water quality guidelines for the tropics/subtropics should be supplemented with additional alternative indicators (*C. perfringens*, coliphages) for watershed assessment (or sanitary survey).**

Lastly, it is important to emphasize that the focus of the workshop was to evaluate the problems associated with appropriate water quality standards in tropical locations, as described and reported by experts/scientists from Hawaii, Guam, Puerto Rico, and south Florida. Collectively, the consensus statements concur with the previous reports by these experts (e.g., Byappanahalli and Fujioka, 1998) that due to environmental sources of fecal indicator bacteria in their

respective tropical locations, reliable interpretations of the current recreational water quality standards in tropical locations may be compromised. Thus, the consensus statements represent agreements in understanding how environmental factors can control the fate of indicator microorganisms and how these factors can affect the development and use of water quality standards in tropical environments. The workshop participants also identified several overall recommendations and research needs.

Appendix B

Literature Search Strategy and Results—Review of Fecal Indicator Organism Behavior in Ambient Waters and Alternative Indicators for Tropical Regions

The literature search strategy conducted in late 2007 consisted of a number of combined approaches. Search terms and a synopsis of information needed were given to a professional librarian to search the online DIALOG databases. To supplement the DIALOG searches, individual authors used free search engines on the internet to find articles pertaining to specific information needed. Experts that participated in EPA's Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria[2] were contacted by email and requested to contribute literature they felt was important. The titles of literature cited in specific reports, books, review articles, and conference proceedings were evaluated for relevance.

B.1 Initial Literature Search Strategy Conducted by Professional Librarian

Selection of DIALOG data base files used for this search:

```
File 155:MEDLINE(R) 1950-2007/Nov 30
       (c) format only 2007 Dialog
File 266:FEDRIP 2007/Sep
       Comp & dist by NTIS, Intl Copyright All Rights Res
File 144:Pascal 1973-2007/Nov W3
       (c) 2007 INIST/CNRS
File 110:WasteInfo 1974-2002/Jul
       (c)  2002 AEA Techn Env.
File 245:WATERNET(TM) 1971-2007Jul
       (c) 2007 American Water Works Association
File 117:Water Resources Abstracts 1966-2007/Aug
       (c) 2007 CSA.
File   5:Biosis Previews(R) 1926-2007/Nov W4
       (c) 2007 The Thomson Corporation
File  40:Enviroline(R) 1975-2007/Oct
       (c) 2007 Congressional Information Service
File 143:Biol. & Agric. Index 1983-2007/Oct
       (c) 2007 The HW Wilson Co
File   6:NTIS 1964-2007/Dec W3
       (c) 2007 NTIS, Intl Cpyrght All Rights Res
File  72:EMBASE 1993-2007/Dec 05
       (c) 2007 Elsevier B.V.
```

Search strategies used for this search:

#1: **ANY OF THE SEARCH TERMS COMBINED WITH ANY OF THE AUTHORS: 1985-PRESENT**

[2] Report from this workshop: http://www.epa.gov/waterscience/criteria/recreation/

```
S1       2045   (FECAL OR FAECAL)()INDICATOR? ?
S2     334552   TROPICAL OR SUBTROPICAL OR RESUSPENSION OR REGROWTH OR
                INDIGENOUS
S3         50   S1 (5N) S2
S4   19961011   PY=1920:1984
S5         47   S3 NOT S4
S6         59   (DIFFERENCE? ? OR COMPAR? OR CONTRAST?) AND TEMPERATE
(S) -
                TROPICAL AND WATER()QUALITY
S7       2619   BACTERIA? AND INDICATOR? ? AND (SAND OR SANDS OR
SEDIMENT? OR
                BEACH OR SHORE)
S8      21488   ENVIRONMENTAL? AND (ENTEROCOCCI OR (E OR
ESCHERICHIA)()COLI)
S9      26837   AU=(FUJIOKA? OR BYAPPANAHALLI? OR ISHII S? OR ISOBE K?
OR -
                ROSE J? OR SOLO-GABRIELE H? OR TORANZOS G? OR TIEDJE J?
OR
                HAZEN T?)
S10      4572   AU=(CALDERON R? OR SADOWSKY M? OR GENTHNER F? OR
AKAZAWA E?
                OR GERBA C? OR SLIFKO T? OR YATES M? OR MOE C? OR JAY
J?)
S11   5776949   DISPERSAL OR PH OR ALKALINITY OR TEMPERATURE OR
RAINFALL OR
                SALINITY OR RUNOFF OR TURBIDITY OR NUTRIENTS
S12    422744   ORGANICS OR ORGANIC()(FOAM OR FOAMS) OR
SUSPENDED()(SOLID -
                OR SOLIDS) OR WATER()QUALITY OR LIGHT (S) (INTENSITY OR
                DURATION)
S13    623176   DEPTH OR STRATIFICATION OR AQUATIC()PLANTS OR BIOFILM?
? OR
                PREDATION OR BIOLOGICAL()COMMUNIT? (S) (WATER()COLUMN
OR
                PLANTS OR EPIPHYTIC)
S14     29926   (TOTAL OR FECAL OR FAECAL)()COLIFORMS OR
CLOSTRIDIUM()PERF-
                RINGENS OR (SULFITE OR SULPHITE)()REDUCING()CLOSTRIDIA
OR
                BIFIDOBATERI?
S15     52396   RHODOCOCCUS()COPROPHILUS OR BACTEROID? OR
HUMAN()ENTERIC()-
                VIRUS? OR (SOMATIC OR F()SPECIFIC)()COLIPHAGE? ? OR
                PLASTICS()GREASE
S16    115764   (FECAL OR FAECAL)()(STEROID? ? OR STEROL? ? OR STANONE?
?)
                OR CAFFEINE OR DETERGENTS
S17     21427   S6 OR S7 OR S8) NOT S4
S19       422   S5 OR S17) AND (S9 OR S10)
S20       253   RD S19  (unique items; deduped)
```

#2: **(Fecal set within 5 words of Tropical Set) OR Difference set: 1985-present**

```
S1       2045   (FECAL OR FAECAL)()INDICATOR? ?
S2     334552   TROPICAL OR SUBTROPICAL OR RESUSPENSION OR REGROWTH OR
```

```
                INDIGENOUS
S3          50  S1 (5N) S2
S4    19961011  PY=1920:1984
S5          47  S3 NOT S4
S6          59  (DIFFERENCE? ? OR COMPAR? OR CONTRAST?) AND TEMPERATE
(S) -
                TROPICAL AND WATER()QUALITY
S27         56  S6 NOT S4
S28         89  (S27 OR S5) NOT (S9 OR S10)
S29         51  RD S28  (unique items, deduped)
```

#3: **Bacteria set OR (Fecal set in same field as any term in Sets 11, 12, 13, 14, 15 OR 16): 2002-present**

```
S7        2619  BACTERIA? AND INDICATOR? ? AND (SAND OR SANDS OR
SEDIMENT? OR
                BEACH OR SHORE)
S1        2045  (FECAL OR FAECAL)()INDICATOR? ?
S11    5776949  DISPERSAL OR PH OR ALKALINITY OR TEMPERATURE OR
RAINFALL OR
                SALINITY OR RUNOFF OR TURBIDITY OR NUTRIENTS
S12     422744  ORGANICS OR ORGANIC()(FOAM OR FOAMS) OR
SUSPENDED()(SOLID -
                OR SOLIDS) OR WATER()QUALITY OR LIGHT (S) (INTENSITY OR
                DURATION)
S13     623176  DEPTH OR STRATIFICATION OR AQUATIC()PLANTS OR BIOFILM?
? OR
                PREDATION OR BIOLOGICAL()COMMUNIT? (S) (WATER()COLUMN
OR PLA-
                NTS OR EPIPHYTIC)
S14      29926  (TOTAL OR FECAL OR FAECAL)()COLIFORMS OR
CLOSTRIDIUM()PERF-
                RINGENS OR (SULFITE OR SULPHITE)()REDUCING()CLOSTRIDIA
OR
                BIFIDOBATERI?
S15      52396  RHODOCOCCUS()COPROPHILUS OR BACTEROID? OR
HUMAN()ENTERIC()-
                VIRUS? OR (SOMATIC OR F()SPECIFIC)()COLIPHAGE? ? OR
                PLASTICS()GREASE
S16     115764  (FECAL OR FAECAL)()(STEROID? ? OR STEROL? ? OR STANONE?
?)
                OR CAFFEINE OR DETERGENTS
S22       1205  S1 (S) (S11 OR S12 OR S13 OR S14 OR S15 OR S16)
S33       2814  (S7 OR S22) NOT (S4 OR S19 OR S23 OR S9 OR S10 OR S6 OR
S5)
S34       1593  RD S33  (unique items, deduped)
S39        584  S34/2002:2007
```

Dates: 1985-present [2002-present is more crucial]
Language: No restrictions

Descriptions of these databases are available at http://library.dialog.com/bluesheets/.

Retrieve: Titles and year

Format: MS Word
Additional information: Interested in international and domestic journals and government reports.

Search terms:
Tropical fecal indicator*
Sub[-]tropical indicator*
Difference* temperate tropical water quality
Bacteria* indicator* sand
Bacteria* indicator* sediment*
Environmental E. coli
Environmental enterococci
Fecal indicator* AND

- Dispersal
- pH
- Alkalinity
- Temperature
- Light [duration, intensity]
- Rainfall
- Salinity
- Runoff
- Suspended Solids
- Turbidity
- Nutrients
- Organics
- Organic foams
- Water Quality
- Biological community in water column
- Water Depth
- Stratification
- Depth
- Mixing (wind, waves, etc.)
- Resuspension from sediments and sand
- Regrowth
- Presence of aquatic plants – epiphytic bacterial communities on plants
- biofilms
- Predation

fecal indicator* AND

- Total coliforms
- Fecal coliforms
- *Clostridium perfringens*
- sulfite-reducing Clostridia
- *Bifidobacterium*
- *Rhodococcus coprophilus*
- *Bacteroid**

- Human enteric viruses
- Somatic coliphages
- F-specific coliphages
- *Bacteroide** phage
- Sanitary plastics grease
- Fecal steroids (sterols, stanols, stanones)
- Caffeine
- Detergents
- Rainfall
- Turbidity

Fecal indicator* resuspension
Fecal indicator* regrowth
Fecal indicator indigenous

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Specific literature requested by EPA:

1) Yamahara, K.M., Layton, B.A., Santoro, A.E., Boehm, A.B. 2007. Beach sands along the California Coast are diffuse sources of fecal bacteria to coastal waters. Environ. Sci. Technol. 41: 4515-4521. Supporting information for beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. Environ. Sci. Technol. Supplemental to 41: 4515-4521, 17 pp.
2) National Research Council (NRC). 2004. Indicators for waterborne pathogens. National Academies Press. Washington, DC. 315 pp.
3) USEPA. 2007. Report of the experts scientific workshop on critical research needs for the development of new or revised recreational water quality criteria. EPA 823-R-07-006. Available online at http://www.epa.gov/waterscience/criteria/recreation.

Partial list of specific issues and questions issues sought to be addressed from this literature search:

- What does the available data say in regards to indicator behavior in ambient waters?
- What differences exist in currently used fecal indicators when they are applied in tropical (and possibly sub-tropical) locations versus temperate locations?
- discuss and evaluate the extent to which indicator organisms and other accompanying organisms derived from fecal sources are impacted by temperature regime (e.g., temperate vs. tropical).
- potential alternative indicators that may provide more meaningful results in tropical environments if the current indicators are determined to be limited in application in a tropical environment.
- Examine indicator behavior in ambient water, sand, and sediments associated with water bodies, and other aquatic micro environments, such as plant life and biofilms [cover indicator regrowth as well as indigenous populations]
    - Separate out fecal origin and established indigenous populations

1. How do potential regrowth and establishment (or extra-enteric sources) of indicator organisms in the environment affect assessment of risk to Human health? Are the documented cases where this has happened?
2. How does that phenomenon effect compliance? (TMDLs and compliance—could be spending billions on TMDLs that are not required)
3. What are the differences in performance of currently used indicators in tropical waters? Compare data for currently used versus the ones that are proposed (e.g., enterococcus versus *Clostridium perfrignens*)
4. What factors can affect both the organism and its recovery and enumeration?
5. Do data support a more appropriate indicator in these situations (potential for regrowth and establishment) than the indicators currently recommended by EPA?
6. What differences exist in fecal indicators when applied in tropical and subtropical areas?

Based on the review of the initial results of the literature search, the co-lead writers reviewed a 68-page document of titles (approximately 700 titles, including many duplicates) for immediate relevance or irrelevance (i.e., article would not be ordered). Those found to be immediately relevant (about 150 titles) were compared to ICF's and CEC's in-house library of documents and those already ordered during the literature search and retrieval processes for the associated white papers "Review of Published Studies to Characterize Relative Risks from Different Sources of Fecal Contamination in Recreational Water" (USEPA, 2009a) and "Review of Zoonotic Pathogens in Ambient Waters" (USEPA, 2009b). For those that were not immediately identified as relevant or irrelevant, an abstract was requested (if available). This resulted in the review of about 30 abstracts, of which 7 articles were assessed to determine whether they were already obtained or ordered elsewhere. The results of this literature review were then combined into a library with the relevant citations from a reverse literature citation search on

several of the key studies (e.g., National Research Council's 2004 report *Indicators for Waterborne Pathogens report*; see more below). Lastly, as the white paper authors began to review the literature, several successive literature requests, resulting in about 40 additional articles, were sent to be checked against the library and ordered if not already possessed or ordered elsewhere.

B.2 Summary of Literature Search Results

This process resulted in a total order of 371 documents (primarily peer reviewed scientific articles), of which a total of 285 (77 percent) were received during the expedited writing process, not all of which could be reviewed. There are many more papers in the peer-reviewed literature, and this by no means represents all of them. Of the articles reviewed, 174 citations were included in the white paper.

B.3 Supplemental Literature Search Strategy

In addition to the literature search conducted by the ICF professional librarian, several other resources were consulted. The flowing experts in the field were contacted directly by email and asked to suggest references:

Nicholas Ashbolt, USEPA
Thomas Atherholt, New Jersey Department of Environmental Protection
Michael Beach, Centers for Disease Control and Prevention
Bart Bibler, Florida Department of Health
Alexandria Boehm, Stanford University, California
Rebecca Calderon, USEPA
Jennifer Clancy, Clancy Environmental Consultants
Jack Colford, University of California, Berkeley
Elizabeth Doyle, USEPA
Alfred Dufour, USEPA
Lee Dunbar, Connecticut Department of Environmental Protection
Lora Fleming, University of Miami School of Medicine and Rosenstiel School of Marine and Atmospheric Sciences, Florida
Charles Hagedorn, Virginia Tech
Joel Hansel, USEPA
Lawrence Honeybourne, Orange County Health Care Agency, Santa Ana, California
Donna Francy, U.S. Geological Survey
Roger Fujioka, University of Hawaii, Manoa
Toni Glymph, Wisconsin Department of Natural Resources
Mark Gold, Heal the Bay, California
Paul Hunter, University of East Anglia, U.K.
Dennis Juranek, Centers for Disease Control and Prevention (retired)
David Kay, University of Wales, U.K.
Sharon Kluender, Wisconsin State Laboratory of Hygiene
Erin Lipp, University of Georgia
Graham McBride, National Institute of Water and Atmospheric Research, New Zealand

Charles McGee, Orange County Sanitation District, California
Harvey Minnigh, RCAP Solutions Inc.
Samuel Myoda, Delaware Department of Natural Resources
Charles Noss, USEPA
Robin Oshiro, USEPA
James Pendergast, USEPA
Linwood Pendleton, University of California, Los Angeles
Mark Pfister, Lake County Health Department, Illinois
John Ravenscroft, USEPA
Graciella Ramirez-Toro, CECIA-IAUPR
Stephen Schaub, USEPA
Mark Sobsey, University of North Carolina, Chapel Hill
Jeffrey Soller, Soller Environmental, California
Michael Tate, Kansas Department of Health and Environment
Peter Teunis, RIVM (National Institute of Public Health and the Environment), Netherlands
Gary Toranzos, University of Puerto Rico, Rio Piedras
Timothy Wade, USEPA
John Wathen, USEPA
Stephen Weisberg, Southern California Coastal Water Research Project
David Whiting, Florida Department of Environmental Protection
Richard Zepp, USEPA

In addition to contacting experts in the field, specific reports were obtained and the titles of the references cited in the reports were reviewed for relevance and ordered if not already obtain or ordered elsewhere.

- National Research Council (NRC). 2004. Indicators for waterborne pathogens. National Academies Press. Washington, DC. 315 pp.
- USEPA. 2007. Report of the experts scientific workshop on critical research needs for the development of new or revised recreational water quality criteria. EPA 823-R-07-006. Available online at http://www.epa.gov/waterscience/criteria/recreation
- Fujioka, R.S, and Byappanahalli, M.N. (eds.) 2003. Proceedings and report: Tropical Water Quality Indicator Workshop. Special Report SR-2004-01. University of Hawaii at Manoa, Water Resources Research Center. Available online at http://www.wrrc.hawaii.edu/tropindworkshop.html
- References cited by the Natural Resources Defense Council reviewers of the EPA Critical Path Science Plan
- Boehm et al. 2008. A sea change ahead for recreational water quality criteria (peer review in progress).

In addition, Clancy Environmental Consultants, Inc., ICF International, Soller Environmental, WaltJay Consulting, and EPA's Health and Ecological Criteria Division all maintain extensive literature databases and reference lists from previously completed projects. All of those in house resources were also sources of literature.

Appendix C

Summary Tables of Findings on the Influence Individual Parameters have on Extra-Enteric Occurrence and Survival of Fecal Indicator Organisms

Table 9. Summary of potential physical parameters that may affect the behavior of fecal indicators in different geographic regions

Physical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
Temperature	Alkan et al. 1995	United Kingdom	*E. coli*, enterococci
	Anderson et al. 2005	Tropical	*E. coli*, enterococci, fecal coliforms
	Byappanahalli et al. 2006	Great Lakes	*E. coli*
	Carillo et al. 1985	Puerto Rico	*E. coli*, *Bifidobacterium*
	Craig et al. 2004	Australia	*E. coli*
	He et al. 2007	Southern California	*E. coli*, enterococci, total coliforms, fecal coliforms
	Ishii et al. 2007	Lake Superior, Minnesota	*E. coli*
	Isobe et al. 2004	Malaysia and Vietnam	Enterococci, fecal coliforms
	Lipp et al. 2001	Southern California	Enterococci, fecal coliforms, *Clostridium perfringens*
	Noble et al. 2004	Southern California	*E. coli*, enterococci, total coliforms, F+ coliphage
	Okabe and Shimazu 2007	Japan	*Bacteroides-Prevotella*, total coliforms, fecal coliforms
	Rhodes and Kator 1988	Chesapeake Bay	*E. coli*, *Salmonella* spp.
	Seurinck et al. 2006	Belgium	*E. coli,* fecal streptococci, *Bacteroides*
	Shibata et al. 2004	Florida	*E. coli*, enterococci, fecal coliforms, total coliforms, *C. perfringens*
	Sinton et al. 2007	New Zeeland	*E. coli*, *Campylobacter jejuni, S. enterica*
	Šolić and Krstuvolic 1992	Croatia	Fecal coliforms
	Wait and Sobsey 2001	Coastal North Carolina	*E. coli*, *Salmonella typhi*, *Shigella sonnei*, poliovirus type 1, parvovirus
	Wang and Doyle 1998	Georgia	*E. coli* O157:H7
	Whitman and Nevers 2003	Great Lakes	*E. coli*
	Whitman et al. 2004	Great Lakes	*E. coli*

Physical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
Rainfall	Ackerman and Weisberg 2003	Southern California	Fecal coliforms
	Ashbolt and Bruno 2003	Australia	Thermotolerant coliforms, enterococci
	Boehm et al. 2002	Southern California	Total coliforms
	Bonilla et al. 2007	South Florida	Somatic coliphages, *enterococci*, *E. coli*, fecal coliforms
	Brion et al. 2002	Kentucky	F-specific RNA coliphage
	Characklis et al. 2005	North Carolina	*E. coli, C. perfringens*, fecal coliforms, enterococci, total coliphages
	Craig et al. 2002	Adelaide, Australia	Fecal coliforms
	Curriero et al. 2001	United States	*E. coli*, *Giardia*, *Cryptosporidium*
	Dwight et al. 2002	Southern California	Total coliforms
	Evanson and Ambrose 2006	Southern California	*E. coli*, enterococci, total coliforms
	Ferguson et al. 1996	Australia	Fecal coliforms, enterococci, *C. perfringens*
	Jeng et al. 2005	Lake Pontchartrain, Louisiana	*E. coli*, fecal coliforms
	Kistemann et al. 2002	Germany	*E. coli*, coliforms, enterococci, *C. perfringens*
	Krometis et al. 2007	North Carolina	*E. coli*, fecal coliforms, enterococci, *C. perfringens*
	LeFevre and Lewis 2003	New Zeeland	Enterococci
	Lipp et al. 2001	Southern California	Enterococci, fecal coliforms, *C. perfringens*
	Mallin et al. 2001	North Carolina	Fecal coliforms
	Paul et al. 1997	Hawaii	Coliphages
	SEPA 2001	Scotland	Total and fecal coliforms
	Shibata et al. 2004	Florida	*E. coli*, enterococci, fecal coliforms, total coliforms, *C. perfringens*
	Seurinck et al. 2006	Belgium	*E. coli,* fecal streptococci, *Bacteroides*
	Tunnicliff and Brickler 1984	Grand Canyon	Fecal coliforms
Light (duration,	Alkan et al. 1995	United Kingdom	*E. coli*, enterococci

Physical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
Intensity)	Ashbolt and Bruno 2003	Australia	Thermotolerant coliforms, enterococci
	Boehm et al. 2002	Southern California	Total coliforms
	Davies and Evison 1991	United Kingdom	Fecal coliforms, fecal streptococci, *E. coli, C. perfringens*
	Fujioka et al. 1981	Hawaii	Fecal coliforms, enterococci
	Ki et al. 2007	Southern California	Fecal coliforms, total coliforms, enterococci
	McCambridge and McMeekin 1981	Tasmania	*E. coli, Enterobacter aerogenes*, *Salmonella typhimurium* M48, *Klebsiella pneumoniae*, *Streptococcus faecium*, *Erwina herbicola* 851
	Noble et al. 2004	Southern California	*E. coli*, enterococci, total coliforms, F+ coliphage
	Rosenfeld et al. 2006	Southern California	Total coliforms, fecal coliforms, enterococci
	Shibata et al. 2004	Florida	*E. coli*, enterococci, fecal coliforms, total coliforms, *C. perfringens*
	Sinton et al. 1999	New Zealand	Fecal coliforms, coliphages
	Sinton et al. 2002	New Zealand	*E. coli*, enterococci, fecal coliforms
	Šolić and Krstuvolic 1992	Croatia	Fecal coliforms
	Sukias and Nguyen 2003	New Zealand	*E. coli*
	Whitman et al. 2004	Great Lakes	*E. coli*
	Wommack et al. 1996	England	Bacteriophage
Runoff	Boehm et al. 2002	Southern California	Total coliforms
	Coulliette et al. 2008	North Carolina	*E. coli,* enterococci
	Currier et al. 2001	United States	*E. coli*, *Giardia*, *Cryptosporidium*
	Isobe et al. 2004	Malaysia and Vietnam	Enterococci, fecal coliforms
	Kistemann et al. 2002	Germany	*E. coli*, coliforms, enterococci, *C. perfringens*
	Oshiro and Fujioka, 1995	Hawaii	*E. coli*, enterococci, fecal coliforms

Physical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
Suspended solids	Characklis et al. 2005	North Carolina	*E. coli*, enterococci, fecal coliforms, *C. perfringens*
	Jeng et al. 2005	Lake Pontchartrain, LA	*E. coli*, fecal coliforms
	Krometis et al. 2007	North Carolina	*E. coli*, *C. perfringens*
	Noble et al. 2004	Southern California	*E. coli*, enterococci, total coliforms, F+ coliphage
	Ramirez 2000	Puerto Rico	*E. coli*, enterococci, total coliforms, fecal coliforms
Turbidity	Alkan et al. 1995	United Kingdom	*E. coli*, enterococci
	He et al. 2007	Southern California	*E. coli*, enterococci, total coliforms, fecal coliforms
	Jeong et al. 2005	Southern California	*E. coli*, fecal coliforms, enterococci
	Kistemann 2002	Germany	*E. coli*, coliforms, enterococci, *C. perfringens*
	Mallin et al. 2000	North Carolina	*E. coli*, fecal coliforms
	Shibata et al. 2004	Florida	*E. coli*, enterococci, fecal coliforms, total coliforms, *C. perfringens*
	Tunnicliff and Brickler 1984	Grand Canyon	Fecal coliforms
Water depth	Kay et al. 1994	United Kingdom	Enterococci
	Paul et al. 1995	Florida	Fecal coliforms, enterococci, *C. perfringens*
	Whitman and Nevers 2003	Lake Michigan, Illinois	*E. coli*
Stratification	Craig et al. 2003	Australia	*E. coli*, enterococci, coliphage, *Salmonella*
Mixing (wind, waves, tides)	Alkan et al. 1995	United Kingdom	*E. coli*, enterococci
	An et al. 2002	Oklahoma	*E. coli*
	Ashbolt and Bruno 2003	Australia	Thermotolerant coliforms, enterococci
	Boehm et al. 2002	Southern California	Total coliforms
	Grant et al. 2005	Southern California	*E. coli*, enterococci, total coliforms
	Jeong et al. 2005	Southern California	Total coliforms, *E. coli*, enterococci
	Ki et al. 2007	Southern California	*E. coli*, enterococci, fecal coliforms, total coliforms
	LeFevre and Lewis 2003	New Zeeland	Enterococci
	Mill et al. 2006	Australia	*E. coli*, enterococci, total coliforms

Physical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
	Oshiro and Fujioka 1995	Hawaii	*E. coli*, fecal coliforms, enterococci
	Rosenfeld et al. 2006	Southern California	*E. coli*, enterococci, fecal coliforms
	Santoro and Boehm 2007	Southern California	Total coliforms, fecal coliforms, *Bacteroides*
	Shiaris 1987	Massachusetts	Fecal coliforms, enterococci, *Vibrio*
	Shibata et al. 2004	Florida	*E. coli*, enterococci, fecal coliforms, total coliforms, *C. perfringens*
	Solo-Gabriele et al. 2000	Florida	*E. coli*
	Whitman and Nevers 2003	Great Lakes	*E. coli*
	Yamahara et al. 2007	California coast	Enterococci

Table 10. Summary of potential chemical parameters that may affect the behavior of fecal indicators in different geographic regions

Chemical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
Resuspension from sediments and sands	LaLiberte and Grimes 1982	Minnesota	*E. coli*
	LeFevre and Lewis 2003	New Zealand	Enterococci
	Lipp et al. 2001	Florida	Fecal coliforms, enterococci, *C. perfringens*, coliphages
	Oshiro and Fujioka 1995	Hawaii	*E. coli*, enterococci, fecal coliforms
	Shiaris et al. 1987	Massachusetts	Fecal coliforms, enterococci, *Vibrio*
pH	Carillo et al. 1985	Puerto Rico	*E. coli*, *Bifidobacterium*
	He et al. 2007	Southern California	*E. coli*, enterococci, total coliforms, fecal coliforms
	Kistemann 2002	Germany	*E. coli*, coliforms, enterococci, *C. perfringens*
	Parker and Mee 1982	Australia	Fecal coliforms
	Šolić and Krstulović 1992	Croatia	Fecal coliforms
Alkalinity	Carillo et al. 1985	Puerto Rico	*E. coli*, *Bifidobacterium*
Salinity	Anderson et al. 2005	Tropical	*E. coli*, enterococci, fecal coliforms
	Bernhard et al. 2003	Oregon	16s ribosomal DNA markers
	Bordalo et al. 2002	Thailand	Enterococci, fecal coliforms
	Davies et al. 1995	Sydney, Australia	Fecal coliforms, fecal streptococci, *C. perfringens*
	Evanson and Ambrose 2006	Southern California	*E. coli*, total coliforms, enterococci
	Fujioka 1981	Hawaii	Fecal coliforms, enterococci
	Harwood 2004	Florida	Fecal coliforms, enterococci
	He et al. 2007	Southern California	*E. coli*, enterococci, total coliforms, fecal coliforms
	Lipp et al. 2001	Southern California	Enterococci, fecal coliforms, *C. perfringens*
	Jeong et al. 2005	Southern California	*E. coli*, enterococci, fecal coliforms
	Mallin et al. 2000	North Carolina	*E. coli*, fecal coliforms
	Okabe and Shimazu 2007	Japan	*Bacteroides-Prevotella*, total coliforms, fecal coliforms
	Sinton et al. 2002	New Zealand	*E. coli*, enterococci, fecal coliforms
	Šolić and Krstulović 1992	Croatia	Fecal coliforms
Nutrients	Alm et al. 2006	Great Lakes	*E. coli*

Chemical Parameters	Study/Year	Geographic Region	Indicator and Pathogenic Microorganism(s)
	Byappanahalli and Fujioka 1998	Hawaii	*E. coli*, fecal coliforms
	Byappanahalli et al. 2003	Great Lakes	*E. coli*, enterococci
	Carillo et al. 1985	Puerto Rico	*E. coli*, *Bifidobacterium*
	Genthner et al. 2005	Florida	Enterococci
	He et al. 2007	Southern California	*E. coli*, enterococci, total coliforms, fecal coliforms
	Kistemann 2002	Germany	*E. coli*, coliforms, enterococci, *C. perfringens*
	Lopez-Torres et al. 1987	Puerto Rico	*Klebsiella pneumoniae*, fecal coliforms
	Noble et al. 2004	Southern California	*E. coli*, enterococci, total coliforms, F+ coliphage
	Ramirez 2000	Puerto Rico	*E. coli*, enterococci, total coliforms, fecal coliforms
	Shiaris et al. 1987	Massachusetts	Fecal coliforms, enterococci, *Vibrio*
	Shibata et al. 2004	Florida	*E. coli*, enterococci, fecal coliforms, total coliforms, *C. perfringens*
Organics	Aulicino et al. 2001	Tyrrhenian coast	Coliforms, streptococci, enteroviruses, *Salmonella* spp., coliphages, *Bacteroides fragilis* phages
	Craig et al. 2004	Australia	*E. coli*
	Desmarais et al. 2002	Florida	*E. coli*, enterococci, *C. perfringens*
	Evanson and Ambrose 2006	Southern California	*E. coli*, total coliforms, enterococci
	Ferguson et al. 1996	Australia	Fecal coliforms, enterococci, *C. perfringens*
	Gerba and McLeod 1976	Gulf Coast	*E. coli*, fecal coliforms
	Lee et al. 2006	Southern California	*E. coli*, enterococci
	Shiaris et al. 1987	Massachusetts	Fecal coliforms, enterococci, *Vibrio*
	Sukias and Nguyen 2003	New Zealand	Fecal coliforms, enterococci, *C. perfringens*
	Yamahara et al. 2007	California	*E. coli*, enterococci

Table 11. Summary of potential biological parameters that may affect the behavior of fecal indicators in different geographic regions

Biological Parameters	Study/Year	Geographic Region	Indicator Microorganism(s)
Biological community in the water column	Shiaris et al. 1987	Massachusetts	Fecal coliforms, enterococci, *Vibrio*
	Wymer et al. 2005	Maryland, Massachusetts, Lake Michigan, Michigan, California	*E. coli*, enterococci
Regrowth	Desmarais et al. 2002	Florida	*E. coli*, enterococci, *C. perfringens*
	Hartel et al. 2005	Georgia, New Hampshire, Puerto Rico	Enterococci
	Harwood and Rose 2004	Florida	Fecal coliforms, enterococci
	Vigness et al. 2006	Wisconsin	*E. coli*
Epiphytic bacterial communities on plants	Hernandez-Delgado et al. 1991	Puerto Rico	Fecal coliforms, coliphage
	Rivera et al. 1988	Puerto Rico	*E. coli*
Other support matrices for biofilms	Decho 2000	Temperate and (sub)tropical	Biofilms
	Gerba and McLeod 1976	Texas	*E. coli*
	Harwood et al. 1999	Florida	*E. coli*, fecal coliforms
Predation	Davies et al. 1995	Australia	Fecal coliforms, enterococci, *C. perfringens*

Table 12. Summary of studies in growth of fecal indicator bacteria (FIB) was measured or inferred

Study	Climate	Water Type	Media	Growth Conditions	Observation
Alm et al. 2006	Temperate	Fresh	Swash zone sands collected from bathing beach	Laboratory microcosms of autoclaved Lake Huron beach sands, incubated at 19°C in the dark; *in situ* measurements at Lake Huron beach sites in diffusion chambers in the swash zone. Daily air temperature ranged from 17.7° C–26.7° C.	5-logs of *E. coli* growth were observed within 2 days and persistence at high numbers was observed for 35 days in laboratory (incubated) microcosm High initial growth (to > 10^7 CFU/100g sand) and sustained growth of *E. coli* observed *in situ* in the absence of predation and competition
Byappanahalli and Fujioka1998	Tropical	Fresh	Soils collected from a research station; proximity of soil to water at time of collection unknown	Hawaiian soil samples (irradiated and natural) inoculated with sewage; soils were maintained at 60% water holding capacity and inoculated with indicator organisms derived from sewage	>2 logs of growth observed for fecal coliforms and *E. coli* in both sterile and natural soils; minimal inputs of nutrients and carbon were provided during incubation for natural (non-sterilized) soils Growth depends on competition from indigenous (extra-enteric) organisms
Byappanahalli et al. 2006	Temperate	Fresh	Soils in a temperate, protected region believed to be relatively free of sewage contamination	*In situ* experiments were conducted in soils in the vicinity (20m or less) of streams or wetlands; soils were desiccated and rehydrated	The likelihood of growth was inferred from persistence of *E. coli* in soils, the lack of known external inputs of *E. coli* to the soils and from the presence of conditions conducive to growth
Carillo et al. 1985	Tropical	Fresh	Water column in a stream (*in situ*) via diffusion chambers	Water temperature between 21– 22 °C for two sites at which *in situ* studies were conducted; nitrite + nitrate concentration were 0.43 and 0.53 mg/L at the two sites, respectively; total phosphorous concentration 3.48 and 3.04 μg/L for the two sites at which *in situ* studies were conducted	*E. coli* density increased with time in *in situ* experiments at all locations along a river reach; some study sites were characterized as relatively unimpacted by sewage and others were directly impacted by sewage *B. adolescentis* (an alternative indicator organism evaluated by the study authors) did not increase over time for *in situ* growth experiments

Study	Climate	Water Type	Media	Growth Conditions	Observation
Davies et al. 1995	Temperate	Marine	Sediment drawn from the pool below a stormwater outfall and collected 0.4 km offshore and used in laboratory microcosm studies	Experiments were performed in the laboratory with two source waters; growth was evaluated in the presence and absence of predators	Fecal coliforms may grow in fresh water and marine water sediments in the absence of predators; net growth was not observed in marine sediments but was inferred based on persistence of fecal coliforms In the presence of predators, growth in sediments is still likely, but is outpaced by predation Because calculated fecal coliform decay rates did not exhibit first-order kinetics, the authors infer that observed decay rates are the result of a balance between growth and predation
Davies et al. 1995	Temperate	Fresh	Sediment drawn from a stream known to be subject to flow of raw sewage and deployed in diffusion chambers for *in situ* growth observation	*In situ* experiments performed in a freshwater stream	Because calculated fecal coliform decay rates did not exhibit first-order kinetics, the authors inferred that observed decay rates are the result of a balance between growth and predation For fecal streptococci a first-order decay model adequately described observed counts
Desmarais et al. 2002	Tropical	Estuarine	Waters from a brackish creek mixed with sterile sediments	Laboratory experiments conducted with waters from a brackish creek	Within 30 hours of addition of small amounts of sterile soils to water samples, significant growth of *E. coli* and enterococci was observed; no growth or decline of *Clostridium perfringens* was observed under the same conditions
Ferguson et al. 2005	Subtropical	Marine	Sediments drawn from the intertidal zone of marine waters away from bird droppings	Counts of enterococci, fecal coliforms, and *E. coli* made in samples collected from intertidal and marine sites	High indicator levels [of all indicators]…of sediments indicate long-term survival and regrowth of indicator bacteria
Gerba and McLeod 1976	Tropical	Estuarine	Sediments and water column from areas known to be receiving domestic sewage pollution	Experiments were performed with and without sterilization of the media, with and without sediments mixed with waters and with and without nutrients eluted from sediments	Growth and sustained high levels of *E. coli* occurred in waters dosed with nutrients eluted from sediments; growth of approximately 5 logs was realized within 3 days

Study	Climate	Water Type	Media	Growth Conditions	Observation
Hardina and Fujioka 1991	Tropical	Fresh	Water from a tropical stream with known impacts of human sewage; observations were performed *in situ* and in laboratory experiments	Experiments performed in the field in a tropical stream with known sewage impacts and with a temperature range of 18 – 26°C; water from the stream was allowed to diffuse to water in the test apparatus Experiments performed in the laboratory with water drawn from a tropical stream and with incubation temperature in the range 23 – 25°C	Under laboratory conditions, fecal coliforms concentration increased by more than 2 logs within two days and maintained high concentration through a third day *E. coli* was observed to grow in both laboratory and field conditions during the first 24 hours of incubation; growth was greater under field conditions Enterococci did not experience growth under laboratory or filed conditions Although growth was observed in water, multiplication in streams was deemed unlikely to be the cause of high levels of *E. coli* and enterococci in streams because the residence time in streams is low and because growth conditions are less than ideal due to suboptimal temperatures, nutrients and competition with other bacteria
Hardina and Fujioka 1991	Tropical	Fresh	(1) Soil from the banks and 10 m away from two streams in Hawaii in a forested area and (2) soils from a grassy area approximately 200 m away from and 5 m above the stream elevation.	Soils and sediment samples were drawn at depths of 0, 16, and 36 cm; temperature and soil descriptions not provided	Circumstantial evidence led the authors to believe there is growth of *E. coli* and enterococci in soils and that high levels of the *E. coli* and enterococci in soils is the cause for high levels of FIB in streams Evidence includes high levels of both indicators at all locations; indicator bacteria concentration increases with decreasing elevation (downstream); soils maintain a more constant warmer temperature than their environment; and soils concentrate nutrients from the water phase
Hartel et al. 2005	Temperate and tropical	Marine	Sediments collected at two temperate and one tropical location; sediments had very different sand, silt, and clay contents	Laboratory experiments in which sediments were dried for 2, 30, or 60 days	A fraction of enterococci survived desiccations of 60 days in all soils; survival was poorest in Puerto Rico soils that had high sand content Regrowth was observed in Georgia soil samples that were dry for 60 days

Study	Climate	Water Type	Media	Growth Conditions	Observation
Ishii et al. 2006	Temperate	Fresh	Soils from (1) a partial wetland, (2) the overbanks of a river (sandy soils, low organic content), and (3) the overbank of a river on a site of a former wastewater treatment facility	*In situ* measurements and laboratory experiments with sediments from three sites (one from each study area)	*E. coli* strains appear to have naturalized in the soils studied *E. coli* was observed routinely in soil samples *E. coli* survived winter months, including multiple freeze-thaw cycles Growth in laboratory studies was observed in unamended, unsterilized soils when soil temperatures were at or above 30°C
Isobe et al. 2004	Temperate	Fresh	Water column in a river; samples were drawn from a reach believed to be free of inputs of sewage and classified as "control sites" by the authors	*In situ* measurements made during one calendar year; the study area (Tokyo) has four seasons and water temperature ranged from below 10°C to above 25°C	*Total coliforms*, *E. coli*, and fecal streptococci (enterococci) were detected at control sites irrespective of season Coprostanol concentrations measured at control sites concurrent with FIB concentrations were below the detection limit, indicating that *E. coli* and fecal streptococci were naturally present in soils or feces of animal origin
Lee et al. 2006	Subtropical	Marine	Water from the water column with or without sediment present in the microcosm	Marine water overlaying approximately 10 g of autoclaved sediment and inoculated with *E. coli* or *Enterococci*	Significant growth of both *E. coli* and *Enterococci* occurred when microcosm waters were on top of a small quantity of sediments Sediments may contribute organic content required for growth
Noble et al. 2004	Temperate	Marine and Freshwater	Mesocosm seeded with range of inocula and nutrients and incubated at different temperatures	Nutrients, total suspended solids, temperature, and bacteria inocula varied factorially	Die off of E. coli and enterococci specifically related to temperature, Decay more rapid at higher temperatures.
Okabe and Shimazu, 2007	Temperate	Fresh and low salinity waters	Unfiltered river water and unfiltered marine water diluted with unfiltered river water	Laboratory observations made of growth/decay of 6 fecal indicators. Fresh and seawater samples were mixed to achieve a desired salinity, inoculated with human, pig, and cow feces suspensions and maintained at target temperatures.	Total coliform growth was observed at T = 10°C and salinities of 0 ppt (k = 0.11 d^{-1}) and 10 ppt (k = 0.07 d^{-1}) Net decay of total coliforms was observed at salinities greater than 10 ppt Net decay of fecal coliforms was observed at all salinities and temperatures, though decay rate in fresh water at water temperature of 10°C was very low (0.02 d^{-1})

Study	Climate	Water Type	Media	Growth Conditions	Observation
Parker and Mee 1982	Temperate	Soils near septic systems	Coarse sands from dunes inoculated with *Salmonella adelaide* and fecal coliforms and septic tank effluent	Moisture and nutrient conditions similar to those near septic systems were maintained in sands	For most of the simulations performed, die-off of fecal coliforms and *S. adelaide* were minimal Fecal coliform growth in the first 2 – 3 days was observed in all but two simulations
Rhodes and Kator 1988	Temperate	Estuarine	Filtered and unfiltered estuarine water inoculated with suspensions of *E. coli* and *Salmonella* spp.	*In situ* experiments in diffusion chambers. Growth and decay were monitored during all 4 seasons and with and without predators and competition.	*E. coli* and *Salmonella* spp. grew in filtered and unfiltered water for about 2 days after inoculation Decay rate after the growth period was much higher in unfiltered water than in filtered water
Whitman and Nevers 2003	Temperate	Fresh	Foreshore beach sand at a Lake Michigan beach in Chicago, IL	Sustained and increasing concentrations of *E. coli* were observed in Lake Michigan foreshore beach sands. Features considered to contribute to potential growth were the moist environment and supply of nutrients with gentle groundwater circulation, protection from sunlight, and high surface area for biofilms.	*E. coli* concentration in sands responded (increased) to rain events and wind events, but relatively quickly fell to pre-event levels where they were maintained *E. coli* concentrations in sand gradually increased during the course of a summer *E. coli* recolonized beach sands that were replaced, even though temperatures were relatively low and no rain events occurred during the observation period
Yan et al. 2010	Tropical	Marine	Sand inoculated with range of organisms/treaments		

Table 13. Summary of laboratory studies

Study	Media	Organisms	Temperature	Light	Salinity	Amendments
Alkan et al. 1995	Filtered sterilized seawater	*E. coli* and enterococci	10, 15, 20, 25, 30°C	100, 300, 500, 700, 900 W/m^2	34.5 to 35.0 ‰	Sewage
Alm et al. 2006	Autoclaved swash zone sands	*E. coli*	19°C	Darkness	Fresh water	None
Bordalo et al. 2002	Natural estuarine water	Fecal coliforms, enterococci	27.6 to 34.6°C	Natural sunlight and darkness	0.7 psu, 25.2 psu	Sewage
Byappanahalli and Fujioka 1998	Irradiated and natural soils, maintained at 60% water holding capacity	Fecal coliforms, *E. coli*	23 to 25°C	Darkness	Fresh water	Nutrients and glucose
Byappanahalli et al. 2003	Unfiltered lake water	*E. coli,* enterococci	25, 30, 35°C	Darkness	Fresh water	*Cladophora* leachate
Craig et al. 2004	Sediment cores mixed with estuarine water	*E. coli*	10, 20, 30 °C	Unknown	(TDS) 23,450 to 28,663	Sediment organic carbon and nutrients
Davies and Evison 1991	Seawater and freshwater	*E. coli, Salmonella typhimurium*	5, 15, 25°C	Natural sunlight, artificial light, darkness	Sea water and fresh water	None
Davies et al. 2003	Marine and fresh sediments, with and without predator inhibition; sterilized sea water	Fecal coliforms, fecal streptococci, *C. perfringens*	20°C	Darkness	Marine and fresh waters	Predator inhibitor
Davis et al. 2005	Unfiltered fresh lake water	Total coliforms, fecal coliforms, enterococci, *E. coli*	Unknown	Sunlight and darkness	Fresh water	None
Desmarais et al. 2002	Sterile and unsterilized sediments with river water, river water, sediments with sterilized river water	*E. coli,* enterococci, *C. perfringens*	28°C	Darkness	Fresh water	None
Fiksdal et al. 1985	Canal water	*Bacteroides fragilis, E. coli, Streptococcus faecalis*	12°C	Darkness	Unknown	None
Fujioka et al. 1980	Seawater with known fecal contaminant, uncontaminated seawater	Poliovirus type 1	24°C	Unknown	Seawater	None
Gerba and McLeod 1976	Estuary water and estuary water mixed with estuary sediments	*E. coli*	24°C	Darkness	4 to 38 g/kg	Sediments (and nutrients eluted from sediments)

Study	Media	Organisms	Temperature	Light	Salinity	Amendments
Hartel et al. 2005	Sediments from 3 locales, desiccated and rewetted	Enterococci	20 to 22°C	Darkness	Unknown	none
Kapuscinski and Mitchell 1983	Filtered seawater	*E. coli,* MS2 bacteriophage, ϕx-174 bateriophage, T7 bacteriophage	10, 15, 23°C	Natural sunlight and darkness	21 to 26 g/kg	None
Lee et al. 2006	Mixtures of sterilized beach sand and beach water, washed sand and beach water or sterilized beach water	*E. coli and E. faecalis*	Unknown	Unknown	Unknown	Unknown
Lo et al. 1976	Synthetic seawater	Poliomyelitus type-1, echovirus-6, coxsackievirus B-5	4, 15, 25, 37°C	Unknown	10, 20, 34 ppt	None
McCambridge and McMeekin 1981	Autoclaved estuarine water	*Klebsiella pneumoniae, E. coli, Salmonella typhimurium, Streptococcus faecium*	22°C	Direct sunlight, artificial light, darkness	Unknown	None
Noble et al. 2004	Freshwater and seawater	Total coliforms, *E. coli* and enterococci	14°C and 20°C	Direct sunlight typical of winter or summer day	Range	Total suspended solids, nutrients, sewage influent, sewage effluent
Parker and Mee 1982	Coarse sands dosed with septic system effluents	Fecal coliforms and *Salmonella adelaide*	15°C	Darkness	Fresh water	Septic system effluent
Stinton et al. 1994	Seawater	Fecal coliforms and enterococci	10 to 19.6°C	Natural sunlight, darkness	Seawater	WWTP effluent and meat processing plant effluent
Stinton et al. (1999)	Seawater	Fecal coliforms, enterococci, somatic coliphage, F-RNA phage, F-DNA phage, *B. fragilis* phage	9 to 19.0 °C	Natural sunlight and darkness	Seawater	Raw sewage and waste stabilization pond effluent

Study	Media	Organisms	Temperature	Light	Salinity	Amendments
Stinton et al. 2002	Fresh and seawaters	Fecal coliforms, enterococci, *E. coli,* somatic coliphages, F-RNA phages	14°C	Natural sunlight and darkness	Seawater	Waste stabilization pond effluent
Wait and Sobsey 2001	Seawater, collected once in each of 4 seasons	*E. coli, Salmonella typhi, Shigella sonnei*, poliovirus type 1, parovirus	12, 20, 28°C	Unknown	31.5 to 35 mg/L	None
Wang and Doyle 1998	Filtered, autoclaved tap water, fresh water from 3 reservoirs	*E. coli* O157:H7	8, 15, 25°C	Unknown	Conductivity (mS/m) 4.3, 11.0, 26.4, 34.1	None
Wright 1989	Well water, water from ephemeral stream, river water	Fecal coliforms, fecal streptococci, *Clostridium perfringens, Salmonella* spp.	24.8 to 31.2°C	Unknown	Conductivity (mS/m) 8 to 66	None
Yamahara et al. 2009	Beach sands	enterococci, *E. coli*	20°C	Dark	33 ‰ (seawater)	None

Salinity (‰) is defined as the weight in grams of dissolved inorganic matter in 1 kg of seawater after all BR^- and I^- have been replaced by the equivalent quantity of Cl^- and all HCO_3^- and CO_3^{2-} are converted to oxide.

Table 14. Range of decay and growth rates observed in laboratory studies

Study	Water Type	Media	Organism†	Decay Constant or Range (min^{-1})	Experimental Conditions
Alkan et al. 1995	Marine	Water	EC	k_{min}= 0.0066	Low light intensity, T = 20°C
				k_{max}= 0.0823	Medium light intensity, T = 20°C
			Ent	k_{min}= 0.0065	Low light intensity, T = 20°C
				k_{max}= 0.0830	Medium light intensity, T = 20°C
Alm et al. 2006	Fresh	Soil	EC	Growth	Dark, T = 19°C
Bordalo et al. 2002 ‡	Fresh	Water	FC	k_{min}= 0.00047	Dark, ambient temperature (Bangkok)
				k_{max}= 0.00103	Natural sunlight, ambient temperature
			Ent	k_{min}= 0.00039	Dark, ambient temperature (Bangkok)
				k_{max}= 0.00094	Natural sunlight, ambient temperature
	Marine	Water	FC	k_{min}= 0.00180	Dark, ambient temperature (Bangkok)
				k_{max}= 0.00265	Natural sunlight, ambient temperature
			Ent	k_{min}= 0.00121	Dark, ambient temperature (Bangkok)
				k_{max}= 0.00186	Natural sunlight, ambient temperature
Byappanahalli and Fujioka 1998	Fresh	Soil	FC	Growth	Sterilized and unsterilized soils
			EC	Growth	Sterilized and unsterilized soils
Byappanahalli et al. 2003	Fresh	Water	EC	Growth	Water augmented with algal leachate
Craig et al. 2004 ‡	Marine	Water	EC	k_{min}= 0.00034	Dark, T = 10°C
				k_{max}= 0.00340	Dark, T = 30°C
		Sedi-ments	EC	k_{min}= 0.00021	Dark, T = 10°C
				k_{max}= 0.00178	Dark, T = 30°C
Davies and Evison 1991	Marine	Water	EC	k_{min}= 0.00173	Artificial light, T = 25°C
				k_{max}= 0.00583	Natural sunlight, ambient temperature
	Fresh	Water	EC	Persistence	Artificial light source
				k_{min}= 0.00104	Natural light, ambient temperature
Davies et al. 1995	Marine	Water	FC	Non-1st-order decay	Predators absent and predators present, T = 20°C
Davis et al. 2005	Fresh	Water	FC	k = -0.00058	Growth (negative decay); dark, unknown T
			Ent	k > 0.00053	Dark, unknown temperature
Desmarais et al. 2002	Marine	Water	EC	Growth	Sediment present, , T = 28°C, dark
			Ent	Growth	Sediment present, , T = 28°C, dark
			EC	Decay	No sediment present, , T = 28°C, dark
			Ent	Decay	No sediment present, , T = 28°C, dark
Fiksdal et al. 1985	Marine	Water	EC	Growth	Growth then slow decay, T = 12°C, dark
Fujioka et al. 1981	Marine	Water	FC	k_{min}= 0.00080	Dark, T = 24°C
				k_{max}= 0.00183	Dark, T = 24°C
				k_{min}= 0.0256	Natural sunlight, T = 24°C
				k_{max}= 0.0770	Natural sunlight, T = 24°C
			FS	k_{min}= 0.00046	Dark, T = 24°C
				k_{max}= 0.00107	Dark, T = 24°C
				k_{min}= 0.01279	Natural sunlight, T = 24°C
				k_{max}= 0.03838	Natural sunlight, T = 24°C
Gerba and McLeod 1976	Estua-rine	Water	EC	Growth	Presence of sediments, T = 24°C, dark
				Decay	Absence of sediments, T = 24°C, dark
		Sedi-ments	EC	Growth	Presence of sediments, T = 24°C, dark
				Decay	Absence of sediments, T = 24°C, dark
Hartel et al. 2005	Marine	Sedi-ments	Ent	Growth	Dark, T = 25°C

Study	Water Type	Media	Organism†	Decay Constant or Range (min^{-1})	Experimental Conditions
Kapuscinski and Mitchell 1983	Marine	Water	EC	k_{min}= 0.02067	Natural sunlight, T = 15°C
				k_{max}= 0.04867	Natural sunlight, T = 23°C
				k_{min}= 0.0256	Dark, T = 15°C
				k_{max}= 0.0770	Dark, T = 23°C
Lee et al. 2006	Marine	Water	EC	Decay	Rapid decline reported, T unknown
				Growth	Marine water overlying sediments
Lo et al. 1976	Marine	Water	PMV	T_{90} ca. 20 wks	Low salinity, T = 15°C
			EV6	T_{90} ca. 8 wks	Low salinity, T = 15°C
			CB-5	T_{90} ca. 20 wks	Low salinity, T = 15°C
McCambridge and McMeekin 1981	Estua-rine	Water	EC	T_{90} ca. 2.5 days	Sunlight; non-1st-order decay observed
				T_{90} ca. 3 days	Dark; non-1st-order decay observed
Noble et al. 2004	Marine	Water	TC	T_{90} ca. 5.0 days	Seawater, T = 14°C
			EC	T_{90} ca. 4.6 days	Seawater, T = 14°C
			Ent	T_{90} ca. 7.4 days	Seawater, T = 14°C
Noble et al. 2004	Marine	Water	TC	T_{90} ca. 3.5 days	Seawater, T = 20°C
			EC	T_{90} ca. 3.3 days	Seawater, T = 20°C
			Ent	T_{90} ca. 4.8 days	Seawater, T = 20°C
			EC	T_{90} ca. 0.7 days	Seawater, High Solar Irradiation, T = 20°C
			Ent	T_{90} ca. 0.4 days	Seawater, High Solar Irradiation, T = 20°C
Parker and Mee 1982 ‡	Fresh	Soil	FC	k_{min} < 0.00002	Unsaturated soil, T = 15°C
				k_{max} = 0.00010	Saturated soil, T = 15°C
Sinton et al. 1994	Marine	Water	FC	k_{min} = 0.00033	Dark, cold, with sewage addition
				k_{max} = 0.00047	Dark, warm, with sewage addition
			Ent	k_{min} = 0.00008	Dark, cold, with sewage addition
				k_{max} = 0.00013	Dark, warm, with sewage addition
			FC*	k_s = 0.515 n_s = 1.45	Natural sunlight, sewage added
			Ent*	k_s = 0.310 n_s = 2.58	Natural sunlight, sewage added
Sinton et al. 1999	Marine	Water	FC	k_{min} = 0.00060	Dark, cold, with sewage addition
				k_{max} = 0.00078	Dark, warm, with sewage addition
			Ent	k_{max} = 0.00008	Dark, warm, with sewage addition
			FC*	k_s = 0.47 n_s = 1.23	Natural sunlight, sewage added
			Ent*	$k_{s,max}$ = 0.27 n_s = 9.47	Natural sunlight, sewage added
Sinton et al. 2002	Fresh	Water	FC	k_{min} = 0.00012	Dark, temperature unknown
				k_{max} = 0.00027	Dark, temperature unknown
			EC	k_{min} = 0.00029	Dark, temperature unknown
				k_{max} = 0.00038	Dark, temperature unknown
			Ent	k_{min} = 0.00020	Dark, temperature unknown
				k_{max} = 0.00028	Dark, temperature unknown
			FC*	$k_{s,min}$ = 0.084 n_s = 1.1	Warm, raw sewage inoculums
				$k_{s,max}$ = 0.275 n_s = 3.8	Cold, wastewater stabilization pond inoculum
			EC*	$k_{s,min}$ = 0.073 n_s = 1.0	Warm, raw sewage inoculums
				$k_{s,max}$ = 0.287 n_s = 4.8	Cold, wastewater stabilization pond inoculum
			Ent*	$k_{s,min}$ = 0.110 n_s = 1.0	Cold, wastewater stabilization pond inoculum
				$k_{s,max}$ = 0.276	Warm, wastewater stabilization pond

				n_s = 1.3	inoculum

Table 15. Summary of studies examining the influence of temperature on persistence and growth of indicator organisms

Climate	Media	Study	Setting	Indicator Organism Response	Temperature Range
Tropical	Water column	Carillo et al. 1985	A tropical stream in a **protected area** at high elevation, impacted by **agriculture and direct sewage releases** at mid-elevations and dominated by **small towns** at low elevations	• Densities of fecal coliforms and *E. coli* were positively correlated with water temperature • Densities of *Bifidobacterium* spp. were not correlated with temperature	21.1–27.1°C
		Shibata et al. 2004	**Marine water** from 2 southern Florida beaches. One beach had no known sewage impacts (although pets are allowed on the beach) but regularly has poor water quality; the second has no known sewage impacts and good water quality.	• No correlation found between temperature and indicator organisms for pooled data for all sample sites • Temperature negatively correlated with total coliforms and *E. coli* at two sample sites and positively correlated with *Clostridium perfringens* at one site	25.6–30.7°C
	Sediments				
	Soils				
Subtropical	Water column	He et al. 2007	**Ponded** and **flowing fresh** waters sampled in a region dominated by **undeveloped lands** and **recreational areas**	• Fecal coliform, total coliform, and enterococci concentrations positively correlated with temperature for ponded waters	
		Lipp et al. 2001	**Estuary** waters from 12 sites; drainages for 6 sites had high off-site disposal system density; drainages for rivers draining to estuary had low urban use	• Fecal coliform bacteria negatively correlated with temperature (correlation coefficient -0.318) • Enterococci concentration negatively correlated with temperature (correlation coefficient -0.383) • Water column *Clostridium perfringens* concentration negatively correlated with temperature (r = -0.224)	17.0–31.5°C
		Noble et al. 2004	**Marine water** collected from Santa Monica Bay near Malibu Beach, Malibu, CA (**assumed impacted by human sewage**) and **fresh water** from Malibu Creek State Park **(heavily forested, undeveloped site)**	• All bacterial indicators (total coliforms, *E. coli*, and enterococci) degraded more rapidly in sea water at 20 °C than at 14 °C under dark conditions	14 and 20°C

		Sinton et al. 1997	**Marine and fresh** water collected from marine and freshwater sources whose level of fecal impacts is not described. Mesocosms exposed to natural sunlight and atmospheric temperature or kept in the dark.	• Decay of *E. coli* was faster at 8–10°C than at 15–20°C • No significant difference in decay rate was observed as temperature was raised above 10°C	8–20°C
	Sediments				
	Soils				
Temperate	Water column	Alkan et al. 1995	**Marine water** collected from the North Sea Coast of the UK, **away from know sewage outfalls or sludge dumping grounds**; waters were sterilized and inoculated with settled sewage and test organisms	• Temperature was not found to be correlated with *E. coli* or enterococci die-off rates (decay constants)	10–30°C
		Craig et al. 2004	**Marine** and **river** sites in Adelaide, Australia; sites likely **impacted** by runoff from Adelaide and other sources	• Decline in *E. coli* populations was more rapid at increased temperature (indication of synergy between solar radiation and temperature)	10–30°C
		Isobe et al. 2004	**Fresh** water in 3 rivers in Tokyo, Japan; rivers received waters of waste water treatment plant effluent (secondary treatment) and septic system discharges. Control locations believed to be free of sewage loads were also sampled.	• *E. coli* concentration was 1–2 orders of magnitude less during winter months than in summer concentrations	Less than 10°C in the winter and more than 25°C in the winter
		Okabe and Shimazu 2007	**Fresh** water from the Atsubetsu River in Japan (no description of land use or water quality provided) and **marine water** from Ishikari Bay, Hokkaido, Japan	• In laboratory experiments conducted at 4, 10, 20, and 30°C, total coliforms were found to have their lowest decay rate or even to grow at 10°C. The highest decay rate for total coliforms was observed at 4°C. • In laboratory experiments conducted at 4, 10, 20 and 30°C, fecal coliforms were found to have their lowest decay rate (0.02 d^{-1}) at 10°C. The highest decay rate for fecal coliforms was observed at 4°C.	4, 10, 20, and 30°C

		Rhodes and Kator, 1988	**Estuarine** water at two sites in a subestuary of the Chesapeake Bay. The subestuary received 0.6×10^6 L/d (0.16 millions of gallons/day) secondary effluent from a wastewater treatment plant.	• *In situ* experiments were conducted with raw and filtered estuary water • At water temperatures >18°C, multiplication occurred during the initial exposure phase (2–3 days) in filtered and unfiltered water for *E. coli* and *Salmonella* spp. • After the initial exposure phase, *E. coli* mortality rates were inversely correlated with temperature • After the initial exposure phase, *Salmonella* spp. Mortality rates were not correlated with temperature	5.9–28.2°C
		Seurinck et al. 2006	**Estuarine** water (presumed to be estuarine based on sample site descriptions) at and near bathing beaches in Belgium. Sample sites **impacted** by wastewater treatment plant, combined sewer overflows, swimmer bacteria loads, and stormwater runoff.	• Water temperature below 10°C was significantly associated with detection of human-specific *Bacteroides* marker, fecal coliforms, and fecal *streptococci* • Water temperature below 10°C was not significantly associated with detection of *E. coli*	
		Šolić and Krstulović, 1992	**Marine** water (**moderately to highly polluted)** used in laboratory and field (mesocosm) experiments	• Temperature strongly effected the survival of fecal coliforms in seawater—survival time (as T_{90}) decreased "exponentially" with temperature (14°C–25°C) [exponential trend was proposed but not evaluated by the authors] • Significant interaction between solar radiation and temperature effects was observed	14–25°C
		Wait and Sobsey, 2001	**Marine water** drawn from a source with unknown level of impact from sewage	• In laboratory experiments, in the temperature range studied, inactivation was slowest at 6°C for *E. coli* and *Salmonella typhi* • Only minor differences in decay rate were observed in the temperature range 12°C–28°C	6–28°C

		Wang and Doyle, 1998	**Fresh** water drawn from one raw water reservoir, two recreational lakes and one treated water source in **Georgia**. No information provided on land use near source waters or suspected impacts by sewage.	• *E. coli* O157:H7 survived well (ca. 2 logs reduction over 13 weeks) for all four waters at 8°C. Survival was progressively worse at 15 and 25°C. • At higher temperatures there was more variation in survival among the four waters tested, with the highest survival observed for treated water. This variation indicates synergy between temperature effects and effects related to differences in water quality between source waters.	
		Whitman and Nevers, 2003	**Fresh** water, Lake Michigan in Chicago, IL. Water assumed to be **impacted by human sources** (runoff) and **impacted by animal sources** (bird feces).	• Air temperature was significantly correlated with *E. coli* counts in water (r = 0.327) • Water temperature was significantly correlated with *E. coli* counts in water (r = 0.333)	Air temperature: 1.35–23.11°C Water temperature: 4.3–22.0°C
		Whitman et al. 2004	**Fresh** water, Lake Michigan in Chicago, IL. Water assumed to be **impacted by human sources** (runoff) and **impacted by animal sources** (bird feces).	• For *in situ* mesocosm experiments conducted at 45 cm depth, temperature had an explained variance of 0.073 (significant) for mean *E. coli* counts.	Temperature range for mesocosm experiments not reported
	Sediments	Craig et al. 2004	**Marine** and **river** sites in Adelaide, Australia; sites likely **impacted** by runoff from Adelaide and other sources	• Decline in *E. coli* populations was more rapid at increased temperature	10–30°C
		Whitman and Nevers, 2003	**Fresh** water submerged sands at a beach on Lake Michigan in Chicago, IL. Sands assumed to be **impacted by human sources** (runoff) and **impacted by animal sources** (bird feces).	• Air temperature was significantly correlated with *E. coli* counts in submerged sands (r = 0.401) • Water temperature was significantly correlated with *E. coli* counts in submerged sands (r = 0.396)	Air temperature: 1.35–23.11°C Water temperature: 4.3–22.0°C
	Soils	Byappanahalli et al. 2006	**Soils** sampled from a **protected natural area**	• Occurrence of *E. coli* in soils was not found to be correlated with temperature, indicating no trend with seasons	
		Ishii et al. 2006	**Soils** from (1) a partial wetland (2) the overbanks of a river (sandy soils, low organic content) and (3) the overbank of a river on a site of a former wastewater treatment facility	• Growth of *E. coli* in non-sterile soils was observed for soil temperature >30°C	4–37°C

		Whitman and Nevers, 2003	**Fresh** water, foreshore sands, Lake Michigan in Chicago, IL. Water assumed to be **impacted by human sources** (runoff) and **impacted by animal sources** (bird feces).	• Air temperature was significantly correlated with *E. coli* counts in foreshore sands (r = 0.593) • Water temperature was significantly correlated with *E. coli* counts in foreshore sands (r = 0.592)	Air temperature: 1.35–23.11°C Water temperature: 4.3–22.0°C

Table 16. Summary of studies examining the influence of salinity on persistence and growth of indicator organisms

Climate	Media	Study	Setting	Indicator Organism Response	Salinity Range
Tropical	Water column	Anderson et al. 2005	Mesocosms of **Fresh water** drawn from the Hillsborough River (freshwater river in Florida) and **marine water** drawn from the Gulf of Mexico	• Fecal coliform decay rate in salt water inoculated with wastewater were 15-times those observed in fresh water (-4.2 $\log_{10}$ (CFU/100mL) d^{-1} vs. -0.27) • Enterococci decay rate in salt water inoculated with wastewater were 3.4 times those observed in fresh water (-0.31 $\log_{10}$ (CFU/100mL) d^{-1} vs. -1.05)	Fresh water to ~ 30 ppt
		Bordalo et al. 2002	**Estuarine waters** with adjusted salinity in **laboratory microcosms.** Waters drawn from an estuary receiving flows from areas of Thailand's largest urban areas and are considered **impacted by human sewage.**	• Salinity had an adverse effect on fecal coliform and enterococci survival • Fecal coliform T_{90} in low- and high-salinity water under light conditions was 37.1 and 14.5 hours, respectively • Enterococci T_{90} in low- and high-salinity water under light conditions was 40.9 and 20.6 hours, respectively	0.8–25.2 PSU
		Fujioka et al. 1981	**Marine waters** from a bathing beach at a depth of 1.2 m, and **fresh waters** from a stream near a forest preserve	• Under laboratory conditions, fecal streptococci and fecal coliforms remained stable for up to 3 days, whereas both the same populations were drastically reduced during the second and third days of incubation • Fecal streptococci (enterococci) and fecal coliforms were more resistant to the bactericidal effect of sunlight in fresh water than in marine water	Not reported
		Harwood, 2004	**Fresh waters** from the Hillsborough River, south Florida (assumed **impacted** by human sewage and agricultural sources) and **marine waters** from the Gulf of Mexico	• Decay rates of fecal coliforms and enterococci in salt water are much higher than those observed in fresh water	Not reported

	Sediments	Anderson et al. 2005	**Mesocosms of sediments** from the Hillsborough River (freshwater river in Florida) and from the Gulf of Mexico (marine)	• Fecal coliform decay rate in salt water sediments inoculated with wastewater were > 100 times those observed in fresh water (-3.1 $\log_{10}$ (CFU/100mL) d^{-1} vs. -0.03) • Enterococci decay rate in salt water sediments inoculated with wastewater were nearly the same as those for fresh water (-0.22 $\log_{10}$ (CFU/100mL) d^{-1} vs. -0.21)	Fresh water to ~30 ppt
		Harwood, 2004	**Fresh waters** from the Hillsborough River, south Florida (assumed **impacted** by human sewage and agricultural sources) and **marine waters** from the Gulf of Mexico	• Decay rates of fecal coliforms and enterococci in salt water are much higher than those observed in fresh water • In fresh water decay rate of fecal coliforms in the water column was much greater than that observed in sediments; in salt water the difference in the decay rate in sediments and water column was not as great	Not reported
	Soils				
Subtropical	Water column	Lipp et al. 2001	**Estuary** waters from 12 sites. Drainages for 6 sites had high off-site disposal system density. Drainages for rivers draining to estuary had low urban use.	• Water column fecal coliform concentration negatively correlated with salinity (correlation coefficient -0.601) • Water column enterococci concentration negatively correlated with salinity (correlation coefficient -0.671)	Monthly mean for entire study area 3.5–25.9 ‰
		Jeong et al. 2005	**Estuary water** samples taken at Marinas in Newport Beach, CA. Headwaters for the drainage to the study region are an ecological reserve. Other parts of the drainage included significant human inputs of pollutions (presumably some with human sewage).	• Water column fecal indicator organism (fecal coliforms, *E. coli*, and enterococci) counts were all strongly negatively correlated with salinity	Approx 27.0–33.4 PSU

		Evanson and Ambrose, 2006	**Brackish waters from tidally-influenced wetlands.** Wetlands known to be impacted by human sewage and are a suspected source of fecal indicator organisms in beach waters. Two sites were assessed—one receiving direct urban runoff and a second within the wetland.	• Within the wetland, salinity was the only environmental condition correlated to water column total coliform and enterococci concentrations. Precipitation, tidal range and wind were not correlated.	Approximately 10–35 ppt
		He et al. 2007	**Ponded** and **flowing fresh** waters sampled in a region dominated by **undeveloped lands** and **recreational areas**	• Fecal coliform, total coliform, and enterococci decreased with salinity for ponded waters • Fecal coliform, total coliform, and enterococci concentration showed significant correlation with temperature and conductivity combined for ponded waters	Approximately 1–9 mS/cm
	Sediments	Evanson and Ambrose, 2006	**Brackish sediments from tidally-influenced wetlands.** Wetlands are known to be impacted by human sewage and are a suspected source of fecal indicator organisms in beach waters. Two sites were assessed—one receiving direct urban runoff and a second within the wetland.	• Within the wetland, salinity was not correlated with total coliform, *E. coli*, and enterococci counts in the sediments	Approximately 10–35 ppt
	Sediments	Lipp et al. 2001	**Estuary** sediments from 12 sites. Drainages for 6 sites have high off-site disposal system density. Drainages for rivers draining to estuary have low urban use.	• Sediments fecal coliform concentration negatively correlated with salinity (correlation coefficient -0.536)	Monthly mean for entire study area 3.5–25.9‰
	Soils				
Temperate	Water column	Šolić and Krstulović , 1992	**Marine** water (**moderately to highly polluted)** used in laboratory and field (mesocosm) experiments	• Fecal coliform survival time (measured as T_{90}) reduced with increasing salinity • The effect of salinity in the range 15–40 ‰ was smaller than the observed effect in the range 10-15‰ • There is likely synergy between the effects of solar radiation and salinity on fecal coliform inactivation	10–40‰

		Bernhard et al. 2003	**Estuarine** and **fresh water**. Estuarine water is known to be **impacted by human sewage** and fresh waters are in a region whose primary land use is **agriculture**.	• Salinity appeared to influence the occurrence of bovine ribosomal DNA markers from fecal *Bacteroides* and *Prevotella.* In saline regions where dairy farming is known to contribute fecal indicators to receiving waters, the occurrence of bovine markers differed. This effect was attributed to either influences of salinity on the PCR process or differing effects of salinity on the survival of different fecal indicator organisms.	0–32 ppt
	Sediments	Davies et al. 1995	**Fresh water** sediments from a source with **known human sewage impacts** and **marine sediments** from a location adjacent to a deepwater sewage outfall	• In marine sediments the elimination of fecal coliform predators appeared to have minimal influence on fecal coliform persistence or growth (net decay was observed). In fresh water sediments fecal coliforms were observed to grow in the absence of predators.	1.6–35.4‰
	Soils				

Table 17. Summary of studies examining the influence of incident natural or artificial light on persistence and growth of indicator organisms

Climate	Media	Study	Setting	Indicator Organism Response	Solar Radiation Range
Tropical	Water column	Bordalo et al. 2002	**Estuarine waters** with adjusted salinity in **laboratory microcosms.** Waters were drawn from an estuary receiving flows from areas of Thailand's largest urban areas and are considered **impacted by human sewage.**	• Sunlight had an adverse effect on fecal coliform and enterococci survival • Fecal coliform T_{90} in dark and light conditions for low salinity waters was 82.0 and 37.1 hours, respectively • Enterococci T_{90} in dark and light conditions for low salinity waters was 97.5 and 40.9 hours, respectively • Fecal coliform T_{90} in dark and light conditions for high salinity waters was 21.3 and 14.5 hours, respectively • Enterococci T_{90} in dark and light conditions for high salinity waters was 31.6 and 20.6 hours, respectively	Not reported; natural sunlight was used in experiments
		Fujioka et al. 1981	**Marine waters** from a bathing beach at a depth of 1.2 m, and **fresh waters** from a stream near a forest preserve.	• Populations of sewage-borne fecal coliforms and fecal streptococci in seawater were inactivated rapidly (within a few hours) in the presence of sunlight and persisted significantly longer in the absence of sunlight • The decline of fecal coliforms and fecal streptococci in sunlight was faster in marine waters than in fresh waters • Analysis of inactivation at various water depths indicates that the visible light spectrum is primarily responsible for inactivation, not the UV light spectrum	0–0.012 W/cn^2
	Sediments				
	Soils				
Subtropical	Water column	Boehm et al. 2002	**Marine** waters at beaches near **highly urbanized** region in southern California	• Indicator organism concentration falls sharply with increasing solar intensity • Indicator organism concentrations rebound quickly with decreasing solar intensity	
		Ki et al. 2007	**Marine** waters at a beach and in a coastal marsh near **highly urbanized** region in southern California	• Sunlight dominates the concentration dynamics of fecal indicator bacteria • Sunlight does not dominate dynamics in a coastal marsh	

		Rosenfeld et al. 2006	**Marine** waters at ankle depth, sampled at 17 locations in a **highly urbanized** region and with potential for receiving sewage from an ocean **sewage outfall**	• Nighttime concentrations of total coliforms, *E. coli*, and enterococci were significantly higher than daytime concentrations • Enterococci exceedances of water quality standards (>104 CFU/100 mL) occurred overwhelmingly between sunset and sunrise • Fecal and total coliform exceedances did not follow sunrise/sunset cycles • Enterococci concentrations rebounded faster after sunset than coliform concentrations	
		Sinton et al. 1999	**Marine water** collected from a location selected based on low coliform counts	• All organisms inactivated more rapidly in sunlight than in the dark • Fecal coliform inactivation exhibited shoulder behavior • Influence of sunlight on fecal coliform decay greater than that for F+ RNA coliphages and somatic coliphages	Reported as net insolation (0–25 MJ/m^2); experiments performed in sunlight and in dark
		Sinton et al. 2002	**Fresh river** water collected from a stream with low indicator counts; **marine** water collected from a site with low indicator counts in Auckland, New Zealand	• In dark, fresh water conditions, decay constant of indicators in waste stabilization pond effluent were greater than those in raw sewage. Predation and inhibitory substances are suggested as mechanisms for dark inactivation. • Inactivation via solar radiation increases with increasing salinity for all indicators; salinity had the least effect on enterococci • Inactivation in sunlight is around 10-times that observed in the dark for all indicators	Reported as net insolation (MJ/m^2); experiments performed in natural sunlight and dark
		Noble et al. 2004	**Marine water** collected from Santa Monica Bay near Malibu Beach, Malibu, CA (**assumed impacted by human sewage**) and **Fresh water** from Malibu Creek State Park **(heavily forested, undeveloped site).**	• For all bacterial indicators (total coliforms, *E. coli*, and enterococci), decay rate in sunlight was greater than that in the dark by at least a factor of 5	
	Sediments				
	Soils				
Temperate	Water column	Alkan et al. 1995	**Marine water** collected from the North Sea Coast of the UK, **away from know sewage outfalls or sludge dumping**	• The effect of light (as irradiance in W/m^2) on decay rate of *E. coli* was linear for samples at the water surface of the mesocosm • There was no die-off observed in *E. coli*	100–900 W/m^2

			grounds. Waters were sterilized and inoculated with settled sewage and test organisms.	population at a depth of 1 m (the bottom of the laboratory apparatus) for light intensity up to 500 W/m^2	
		Ashbolt and Bruno, 2003	**Marine** waters at beaches near **highly urbanized** region in Sydney, Australia	• Sunlight hours on the day of sampling contributed to "significant decrease in counts"	No quantitative data provided
		Davies and Evison, 1991	**Marine water** collected 7 miles off the northeast coast of Britain, well away from sludge dumping grounds	• Declines in *E. coli* concentrations in seawater exposed to natural sunlight were faster than those observed in fresh water; authors suggest synergy between salinity and exposure to natural sunlight • Significant differences in decay rate were noted between experiments with artificial and natural light sources	Not reported; light sources were sunlight and light from an artificial source
		McCambridge and McMeekin, 1981	Natural **estuarine** water—no description provided of sample location	• In the dark and in the presence of predators, *E. coli* reduced from 5×10^8 to 600 organisms per mL after 10 days • When exposed to sunlight and in the presence of predators, *E. coli* reduced from 5×10^8 to 600 organisms per mL after 8 days • In the absence of predators, the number of indicator organism cells decreases linearly with cumulative incident radiation	Experiments were conducted in dark conditions, under artificial light and in natural sunlight
		Šolić and Krstulović, 1992	**Marine** water (**moderately to highly polluted)** used in laboratory and field (mesocosm) experiments	• Fecal coliform survival time (measured as T_{90}) was inversely proportional to solar radiation in the range (510 830 W/m^2) • There is apparent synergy between solar radiation and temperature in fecal coliform inactivation • There is apparent synergy between solar radiation and salinity in inactivation	510–830 W/m^2
		Whitman et al. 2004	**Fresh** water beach near a **highly urbanized** region. Chicago, IL; chief pollutant sources gulls and near-shore sands	• *E. coli* counts were higher for samples taken in the morning than for afternoon samples • *E. coli* counts rarely exceeded 235 CFU/100mL on sunny mornings but regularly exceeded 235 CFU/100 mL on cloudy mornings • *E. coli* counts decreased logarithmically with hour of the day on sunny days • *E. coli* populations rapidly rebounded at night. Attributed to entrainment of bacteria from soils in	

				the swash zone	
		Wommack et al. 1996	**Estuarine** water from the York River estuary; descriptions of land use in the study area catchment not described	• Decay rates for two bacteriophages were significantly different for microcosms exposed to natural sunlight and for microcosms kept dark • Decay rates based on direct viable counts were twice a high for microcosms held at the surface than for microcosms suspended at 1 m depth	
	Sediments				
	Soils				

Table 18. Summary of studies examining the influence of turbidity and suspended solids on the persistence and growth of indicator organisms

Climate	Media	Study	Setting	Indicator Organism Response	Turbidity or TSS Range
Tropical	Water column	Shibata et al. 2004	**Marine water** from two southern Florida beaches. One beach has no known sewage impacts (although pets are allowed on the beach) but regularly has poor water quality; the second has no known sewage impacts and good water quality.	• *Clostridium perfringens* and total coliforms correlated strongly with turbidity (r = 0.60 and r = 0.57) • *E. coli* and enterococci counts did not correlate strongly with turbidity	~0 to <25 NTU
	Sediments				
	Soils				
Subtropical	Water column	Jeong et al. 2005	**Estuary water** samples taken at Marinas in Newport Beach, CA. Headwaters for the drainage to the study region were an ecological reserve. Other parts of the drainage included significant human inputs of pollutions (presumably some with human sewage).	• No consistent relation was noted between turbidity and water column fecal indicator organism (fecal coliforms, *E. coli*, and enterococci) • Total coliforms were positively correlated with turbidity at more sites than *E. coli* or enterococci during one phase of the study	Approx 27.0–33.4 PSU
		Noble et al. 2004	**Marine water** collected from Santa Monica Bay near Malibu Beach, Malibu, CA (**assumed impacted by human sewage**) and f**resh water** from Malibu Creek State Park **(heavily forested, undeveloped site).**	• TSS levels were not significant factors in the inactivation rates of *E. coli*, enterococci, or F+ specific coliphage inactivation rate in fresh and marine waters	(TSS) 0–500 mg/L
	Sediments	Jeng et al. 2005	**Estuarine waters** from the outfall of a pumped stormwater canal on Lake Pontchartrain, LA and a nearby beach believed to be under the influence of waters from the outfall. Stormwater was presumed **highly impacted by**	• Measured fraction of fecal coliforms attached to particles was 9.8% and 27.5% for two storms sampled • Measured fraction of *E. coli* attached to particles was 21.8% and 30.4% for two storms sampled • Measured fraction of enterococci attached to particles was 8.3% and 11.5% for two storms	Mean TSS concentration during two rainfall events were 170 and 203 mg/L

			human sources such as sewer overflows and dense residential use.	sampled • Enterococci tended to associate with small particles (compared with *E. coli* and fecal coliforms that did not appear to associate preferentially with any particle size) and were removed from the water column by settling more slowly than the other two indicators	
	Soils				
Temperate	Water column	Alkan et al. 1995	**Marine water** collected from the North Sea Coast of the UK, **away from know sewage outfalls or sludge dumping grounds.** Waters were sterilized and inoculated with setlled sewage and test organisms.	• At turbidity above 0.6 (absorbance at 288 nm), changes in turbidity have a smaller impact on decay rate of both *E. coli* and enterococcithan at turbidity below 0.6	Turbidity: 0.044–0.864 (absorbance at 288 nm)
		Characklis et al. , 2005	**Fresh waters** from three streams in North Carolina; stream catchments were characterized as low-density residential, institutional, and commercial/residential	• Substantial fraction of fecal coliforms, *E. coli*, enterococci, *Clostridium perfringens,* and total coliphages are associated with settleable particles in stormwater • Provides evidence that a substantial fraction of *Clostridium perfringens* spores associate with settleable particles • The fraction of all indicators associated with particles differs between wet- and dry-weather conditions, although the mechanism behind this phenomenon is not known	TSS: 2–157 mg/L
		Krometis et al. 2007	Fresh waters from two streams in North Carolina. The catchment of one stream had low-density institutional use; the second catchment was characterized by low-density residential land use.	• The settleable fraction of *E. coli* and fecal coliforms (fraction of fecal coliforms and *E. coli* associated with settleable particles) remained constant during wet weather events • The settleable fraction of enterococci and *Clostridium perfringens* decreased as wet weather events progressed	TSS: 5–272 mg/L
		Mallin, 2001	**Estuarine** waters from multiple sites on a tidally-influenced creek in North Carolina. Large and small-scale **animal (pig and chicken) operations** were located within the drainage for the creek.	• For data for all sampled streams combined, there was significant correlation (r = 0.768) between turbidity and fecal coliform concentration • For drainages with a high coverage of wetlands, there was no correlation between turbidity and fecal coliforms, despite a correlation between	Not reported

			Portions of the creek drainages had undergone recent rapid development and were characterized by a high fraction of impervious areas.	rainfall and turbidity	
		Tunnicliff and Brickler, 1984	**Fresh water** sampled from the Colorado River within the Grand Canyon; lands within the drainage were protected and the sample sites assumed to be **unimpacted** by human sources of fecal indicator bacteria	• During storm flow conditions, fecal coliforms were positively correlated with turbidity (r = 0.54)	4–589 NTU
	Sediments				
	Soils				

Table 19. Summary of studies examining the influence of rainfall and runoff on persistence and growth of indicator organisms

Climate	Media	Study	Setting	Indicator Organism Response	Rainfall
Tropical	Water column	Bonilla et al. 2007	**Marine waters** sampled at three south Florida beaches near large urban areas and assumed to be influenced by human sewage	• Variations in rainfall and turbidity (combined) could predict 36% of the variation in somatic coliphages from the water column at one site	Not reported
	Sediments	Bonilla et al. 2007	**Marine sediments** sampled at three south Florida beaches near large urban areas and assumed to be influenced by human sewage	• At one site, rainfall and temperature could predict a significant proportion of the variance observed with enterococci in wet sands (sediments)	Not reported
	Soils	Bonilla et al. 2007	**Marine sediments** sampled at three south Florida beaches near large urban areas and assumed to be influenced by human sewage	• At two sites, rainfall or rainfall and turbidity could predict a significant proportion of the variance observed with enterococci, *E. coli*, and fecal coliforms in dry sand	Not reported
Subtropical	Water column	Lipp et al. 2001	**Estuary** waters from 12 sites. Drainages for 6 sites had high off-site disposal system density. Drainages for rivers draining to estuary had low urban use.	• Water column fecal coliform bacteria positively correlated with 7-day antecedent rainfall (correlation coefficient 0.260) • Water column enterococci concentration positively correlated with 7-day antecedent rainfall (r = 0.397)	5.1–28.1 cm/month
		Ackerman and Weisberg, 2003	**Marine waters** from beaches in the City of Los Angeles, CA. Beaches differed in their distance from stormwater runoff conveyances; three beaches were in protected embayments.	• Every storm larger than 25 mm resulted in an increase in the number of beach sites failing water quality standards compared with dry weather and background conditions • 91% of rain events between 6 mm and 25 mm resulted in an increase in the number of beaches failing state water quality standards • There was almost no increase above background of the number of beaches failing state water quality standards for storms <2.5 mm • For large storms, the highest recorded fecal coliform concentrations occurred the day after the storm • For small storms, the highest bacteria concentration occurred on the second day after the storm • Average fecal coliform concentrations returned to	0–ca 90 mm (per rain event)

				background levels within 5 days of a rain event • The period between storms had a minimal effect on the relationship between rainfall and fecal coliforms	
		Dwight et al. 2002	**Marine waters** from 22 beach sites in southern California. Due to the incidence of beach closures and land use, the area was highly-urbanized and **impacted** by **human sewage sources.**	• Precipitation was strongly associated with river discharge and river discharge was associated with high total coliform levels for beaches in the vicinity of the river discharges.	Wet months were classified as those in which more than 25 mm of precipitation was recorded
		Ferguson et al. 1996	**Estuarine waters** from sites along the Georges River, Sydney Australia. The study area was characterized by **urban use** and periodic sewage overflow events.	• Rainfall was associated with significant increases in fecal coliforms, fecal streptococci, *Clostridium perfringens*, *Aeromonas* spp., and F+ RNA bacteriophages.	Not reported
	Sediments	Lipp et al. 2001	**Estuary** waters from 12 sites. Drainages for 6 sites had high off-site disposal system density; drainages for rivers draining to Estuary had low urban use.	• Sediment enterococci concentration positively correlated with 7-day antecedent rainfall (r = 0.353)	5.1–28.1 cm/month
		Ferguson et al. 1996	**Sediments from estuarine waters** from sites along the Georges River, Sydney Australia. The study area was characterized by **urban use** and periodic sewage overflow events. Sites were between 3 and 22.5 km from outfalls.	• Rainfall was not associated with significant increases in fecal streptococci, *Clostridium perfringens*, *Aeromonas* spp., and F+ RNA bacteriophages in sediments • Rainfall was associated with significant increases in fecal coliforms in sediments	Not reported
		Jeng et al. 2005	**Estuarine sediments** from the outfall of a pumped stormwater canal on Lake Pontchartrain, LA and a nearby beach believed to be under the influence of waters from the outfall. Stormwater presumed to be **highly impacted by human sources** such as sewer overflows and dense	• Concentrations of *E. coli*, fecal coliforms, and enterococci increased after rainfall, but the increase was not correlated with the rainfall intensity or stormwater canal pumping volume, likely because of the small number of events sampled. • Sediment enterococci concentrations tended to be higher and recede more slowly after rain events than did fecal coliform and *E. coli* concentrations	Two rainfall events: 4.3 mm and 29.2 mm.

			residential use.		
		LeFevre and Lewis, 2003	**Marine waters** at a beach in a **completely urbanized area** in Auckland, New Zealand	• Increased enterococci concentrations were observed in sediments approximately 1 day after a rain event	Not reported
	Soils				
Temperate	Water column	Ashbolt and Bruno, 2003	**Marine waters** at beaches near **highly urbanized** region. Two beaches received storm waters. One beach receives storm waters, sewer overflow waters and lagoon discharge. Sydney, Australi.a	• Rainfall on the day of sampling (not rainfall in the 24 hours prior to sampling) was the strongest predictor of enterococci counts	Not reported
		Brion et al. 2002	**Fresh** waters in a multi-use reservoir with impacts primarily from **animals (cattle grazing and other activities)** and agriculture. Urban impacts were believed secondary.	• Rainfall increased the frequency in which F+phages were isolated from water samples (odds ratio of 7.9)	8 rainfall events (>0.8 in (20 mm) in the previous 24 hours or >1.0 in (25 mm) in the previous 48 hours) in 2 year study
		Characklis et al. 2005	**Fresh waters** from three streams in North Carolina. Streams catchments were characterized as low-density residential, institutional, and commericial/residential.	• Mean microbial concentrations for fecal coliforms, *E. coli*, enterococci, *Clostridium perfringens,* and total coliphages were at least one order of magnitude higher for wet weather than for background conditions at all sites • There was high variability in indicator organism count between samples for rain event	Wet weather defined as rainfalls that occur after at least 3 days without significant rainfall and that cause at least a four-fold increase in stream discharge
		Krometis et al. (2007)	**Fresh waters** from two streams in North Carolina. The catchment of one stream had **low-density institutional** use. The second catchment was characterized by **low-density residential** land use.	• Fecal coliform and *E. coli* concentrations increased rapidly at storm onset and receded much faster than enterococci and *Clostridium perfringens* during the receding limb of the storm hydrograph	Wet weather defined as rainfalls that occur after at least 3 days without significant rainfall and that cause at least a four-fold increase in stream discharge
		Mallin, 2001	**Estuarine** waters from multiple sites on a tidally-influenced creek in North Carolina. Large and small-scale **animal (pig**	• For data for all sampled streams combined, there was significant correlation (r = 0.601) between rainfall in the 24 hours prior to sample collection and fecal coliform concentration	Not reported

			and chicken) operations were located within the drainage for the creek. Portions of the creek drainages had undergone recent rapid development and are characterized by a high fraction of impervious areas.	• Rainfall was less likely to correlate with fecal coliform concentration for sites whose drainages had 13.8% or higher coverage with wetlands	
		SEPA, 2001	**Marine waters** sampled at beaches in Scotland. Beaches were selected because of a history of non-compliance with recreational water quality standards and are presumed **impacted by human sewage** or runoff from agricultural operations.	• Total and fecal coliform concentrations are correlated with cumulative rainfall prior to sampling; the time period for calculating cumulative rainfall is not stated	0–60 mm
		Seurinck et al. 2006	**Estuarine** water (presumed to be estuarine based on sample site descriptions) at and near bathing beaches in Belgium. Sample sites are **impacted** by wastewater treatment plant effluents, combined sewer overflows, swimmer bacteria loads, and stormwater runoff.	• Rainfall 24 hours prior to sampling and on the day of sampling was significantly associated with detection of human-specific *Bacteroides* marker • Rainfall (it is not stated whether this refers to rainfall on the day of sample, rainfall 24 hours prior to sampling or both) was significantly associated with the detection of fecal coliforms, fecal streptococci, and *E. coli*	0–6 mm rainfall on the day prior to sampling and 0–1.5 mm on the day of sampling
		Tunnicliff and Brickler, 1984	**Fresh water** sampled from the Colorado River within the Grand Canyon. Lands within the drainage were protected and the sample sites were assumed **unimpacted** by human sources of fecal indicator bacteria.	• Exceedance of water quality standards (200 CFU/100 mL) were observed during storm flows. These observations are significant given the lack of human sources of fecal indicator organisms in the drainage.	Not provided
	Sediments	Craig et al. 2002	**Marine sediments** sampled from beaches in the greater Adelaide (Australia) metropolitan area	• A significant correlation was found between sediment fecal coliform concentration and rainfall in the previous 2 days for one site • Significant correlations were found between rainfall in the previous 7 days and fecal coliform concentration at two sites	2-day rainfall range was approximately 0–12 mm; 7-day rainfall ranges ~0–30 mm
	Soils				

Table 20. Summary of studies examining the influence of mixing, currents and tidal effects on persistence and growth of indicator organisms

Climate	Media	Study	Setting	Indicator Organism Response
Tropical	Water column	Shibata et al. 2004	**Marine water** from 2 southern Florida beaches. One beach had no known sewage impacts (although pets are allowed on the beach) but regularly has poor water quality. The second had no known sewage impacts and good water quality.	• Enterococci and *Clostridium perfringens* concentrations were elevated at the shoreline to their highest levels during high tides
	Sediments			
	Soils	Oshiro and Fujioka, 1995	**Sands** at popular ocean bathing beaches that were expected to be contaminated by runoff and waters from showers, but not by stormater or sewage sources	• The results for the various sand samples indicated that the bacterial concentrations in the sand increased as the moisture content of the sand decreased and soil content increased. Thus, the highest bacterial numbers were recovered in dry sand farthest inland from the water line. The relatively lower concentrations of bacteria recovered from the wet sand samples indicate cleaner sand as a result of a washing effect by **wave action**
Subtropical	Water column	Ki et al. 2007	**Marine water and estuarine water** samples were taken from bathing beaches and a tidal wetland in southern California. The surrounding region was highly urbanized.	• Autocorrelation of time series of fecal coliforms, enterococci, and *E. coli* indicates that fluctuations in their concentration in the surf are mainly related to sunlight inactivation, whereas fluctuations in a tidal wetland are primarily due to tidal effects
		Jeong et al. 2005	**Marine water**. Samples taken at Marinas in Newport Beach, CA. Headwaters for the drainage to the study region are an ecological reserve. Other parts of the drainage include significant human inputs of pollutions (presumably some with human sewage).	• During ebb tides salinity at samples sites decreases and fecal indicator bacteria (total coliforms, *E. coli*, and enterococci) counts increase. Because the authors demonstrated a strong negative correlation between salinity and indicator organism concentrations, they attributed the effect of tides on indicator counts to changes in salinity.

		Boehme et al. 2002	**Marine** waters at beaches near **highly urbanized** region in southern California.	• Lunar time-scale variation in enterococci counts were observed. Lunar cycle time scale processes that may influence enterococci concentrations are tidal flushing of estuaries and storm channels, tidally-modulated near-shore circulation patterns, and exfiltration of bacteria-contaminated groundwater via tidal pumping and the horizontal and vertical movement of offshore wastewater fields by internal tides. • Short time-scale fluctuations in enterococci, *E. coli*, and fecal coliform concentrations may be related to rip cell currents
		Grant et al. 2005	**Marine waters** at beaches in southern California. The study region was highly **urbanized** and expected to be **highly-impacted** by human sources of fecal indicators.	• The major source of fecal indicator organisms at beaches is the washout of stormwater from tidal outlets during ebb tides
		Rosenfeld et al. 2006	**Marine waters** at beaches in southern California. The study region was highly **urbanized** and expected to be **highly-impacted** by human sources of fecal indicators.	• The larger the tidal range (lower low tides, higher high tides) the greater the probability of a contamination event. *E. coli*, fecal coliforms, and enterococci counts all exhibited high levels associated with large tidal ranges
		Solo-Gabriele et al. 2000	**Brackish waters** in a highly impacted river in Fort Lauderdale, FL	• The highest *E. coli* measurements made during the study between storm events were at high tides. It was hypothesized that high tides rehydrate *E. coli*-laden soils. Growth in the sediments and release to the water column follow. Growth conditions are favorable, since desiccation reduced the population of *E. coli* predators.
		LeFevre and Lewis, 2003	**Marine waters** at a beach in a **completely urbanized area** in Auckland, New Zealand	• Based on higher observed enterococci numbers in near-bed waters than in near-surface waters in the surf zone and no significant differences between near-bed and near-surface concentration at greater distances off-shore, the authors asserted that washing of enterococci from sediments by waves was likely
	Sediments	Solo-Gabriele et al. 2000	**Brackish waters** in a highly impacted river in Fort Lauderdale, FL	• The highest *E. coli* measurements made during the study between storm events were at high tides. It was hypothesized that high tides rehydrate *E. coli*-laden soils. Growth in the sediments and release to the water column follow. Growth conditions are favorable, since desiccation reduced the population of *E. coli* predators.
		Yamahara et al. 2007	Marine sediments samples drawn from sites along the California Coast of the United States	• Column experiments indicate that enterococci can be mobilized from beach sands readily. Enterococci levels were also examined in exposed and submerged sands and found that enterococci levels were lower in the submerged sands. These findings indicate that rising tides mobilize enterococci from shore sands.

	Soils			
Temperate	Water column	Alkan et al. 1995	**Marine water** collected from the North Sea Coast of the UK, **away from know sewage outfalls or sludge dumping grounds.** Waters were sterilized and inoculated with setlled sewage and test organisms.	• In the absence of mixing of a laboratory mesocosm, die-off (inactivation rate constant) was significantly related to turbidity • Higher levels of mixing yielded higher decay rates for *E. coli* and enterococci • Lower levels of mixing and high levels of turbidity yielded lower rates of inactivation at the bottom of the mesocosm
		An et al. 2002	**Fresh waters** in a multi-use reservoir in Oklahoma with relatively few fecal impacts	• There was a direct relationship between amount of gasoline sold, which was related to recreational boating activity, and the resuspension of *E. coli*. This indicated that recreational boating activity in lake marinas may have resuspended bottom sediments with bound *E. coli*, and the presence of *E. coli* in marinas was not an indication of recent fecal contamination.
		Ashbolt and Bruno, 2003	**Marine** waters at beaches near **highly urbanized** region in Sydney, Australia	• Density currents related to differences in temperature and salinity appeared to direct primary sewage plume waters onshore • Wind effects explain peaks in enterococci counts not related to storm events
	Sediments	Shiaris et al. 1987	**Marine sediments** and overlying **waters** drawn from an intertidal mud flat in Boston Harbor, MA. The harbor received sewage flows, including combined sewer overflows.	• 78% of the variation in levels of enterococci, *E. coli*, fecal coliforms, and *Vibrio parahaemolyticus* in sediments could be accounted for by tidal exposure (minutes per tidal cycle)
	Soils	Whitman and Nevers, 2003	**Fresh** water, foreshore sands, Lake Michigan in Chicago, IL. Water can be assumed to be **impacted by human sources** (runoff) and **impacted by animal sources** (bird feces).	• Wind speed, wave height, and wind direction were useful for predicting *E. coli* concentration in sand. This effect may be direct or may be the result of the impact wind speed and direction on other physical parameters influencing *E. coli* counts.

Table 21. Survey of alternative microbiological and chemical indicators of fecal contamination

Alternate Indicator Organism	Climate	Environmental Medium	Water Type/ Description	Geographic Region	Study
Bacteria					
Bacteroides spp. (*B. fragilis*)	Temperate	Water (culturable)	Lake Washington Ship Canal	Washington	Fiksdal et al. 1985
Bacteroides-Prevotella	Temperate	Water (human- and cow-specific)	Estuarine and inland streams	Oregon	Bernhard and Field 2000
Bacteroides-Prevotella	(Sub)tropical	Water (human-specific molecular marker)	Coastal beach	California	Boehm et al. 2003
Bacteroides	Temperate	Water (total-, human-, and cow-specific markers)	Great Lakes beaches	Wisconsin	Bower et al. 2005
Bacteroides spp.	Subtropical	Water (general- and human-specific markers)	Coastal beach and inland stream	Hawaii	Betancourt and Fujioka 2006
Bacteroides	Temperate	Water	Coastal	Belgium	Seurinck et al. 2006
Bacteroides	(Sub)tropical	Water	Coastal beaches	California	Colford et al. 2007
Bacteroides	(Sub)tropical	Water (human-specific molecular marker)	Coastal beach	California	Santoro and Boehm 2007
Bacteroides	Temperate	Water (general, human-, ruminant-, and swine-specific markers)	Inland streams	Canada	Walters et al. 2007
Bacteroides	(Sub)tropical	Water and sand (human-specific molecular marker)	Coastal beaches	California	Yamahara et al. 2007
Bifidobacterium adolescentis	Tropical	Water (culturable)	River watershed	Puerto Rico	Carillo et al. 1985
Bifidobacterium spp.	Temperate	Water (human- and cow-specific)	Estuarine and inland streams	Oregon	Bernhard and Field 2000
Clostridium perfringens	Tropical	Water (culturable)	Freshwaters	Sierra Leone	Wright 1989

Alternate Indicator Organism	Climate	Environmental Medium	Water Type/ Description	Geographic Region	Study
C. perfringens	(Sub)tropical	Sediment (culturable)	Marine beach and inland stream	Australia	Davies et al. 1995
C. perfringens	(Sub)tropical	Water (culturable)	Coastal surface waters and groundwater	Florida	Paul et al. 1995
C. perfringens	(Sub)tropical	Water and sediment (culturable)	Coastal estuarine	Australia	Ferguson et al. 1996
C. perfringens	Temperate	Water and sediment (culturable)	Marine water and sediments, groundwater	Spain	Lucena et al. 1996
C. perfringens	(Sub)tropical	Water and sediment	Estuary	Florida	Lipp et al. 2001
C. perfringens	(Sub)tropical	Water and sand (culturable)	Inland river	Florida	Desmarais et al. 2002
C. perfringens	(Sub)tropical	Water and sediment	Inland river, pond, lake	Florida	Harwood and Rose 2004
C. perfringens	Tropical	Water and sand (culturable)	Coastal beaches	Florida	Shibata et al. 2004
Bacteriophage					
Bacteroides fragilis (HSP40)	Temperate	Water and sediment (plaque assay)	Marine water and sediments, groundwater	Spain	Lucena et al. 1996
Bacteroides (GB-124)	Temperate	Water (molecular)	Inland river	United Kingdom	Ebdon et al. 2007
F+ and somatic coliphage; F+ RNA coliphage	Temperate	Water (plaque assay)	Great Lakes beaches	Canada	Palmateer et al. 1991
Somatic coliphage	Temperate	Water and sediment (plaque assay)	Marine water and sediments, groundwater	Spain	Lucena et al. 1996
Coliphage	(Sub)tropical	Water	Coastal beach, bay, inland stream and canal	Hawaii	Paul et al. 1997
Somatic coliphage	(Sub)tropical	Water and sediment (plaque assay)	Coastal beach	Australia	Craig et al. 2001

Alternate Indicator Organism	Climate	Environmental Medium	Water Type/ Description	Geographic Region	Study
F+ RNA coliphage and somatic coliphage	(Sub)tropical	Water	Sea, estuary, inland river, stabilization pond, sewage plant	Australia	Sinton et al. 2002
F+ RNA coliphage	(Sub)tropical	Water and sediment	Inland river, pond, lake	Florida	Harwood and Rose 2004
F+ RNA coliphage	(Sub)tropical	Water	Inland streams	Hawaii	Luther and Fujioka 2004
F+ specific coliphage	(Sub)tropical	Water	Coastal beach and bay	California	Noble et al. 2004
F+ and somatic coliphage	(Sub)tropical	Water and sand (plaque assay)	Coastal beach	Florida	Bonilla et al. 2007
Total coliphage; F+ and somatic coliphage; F+ RNA coliphage	Temperate	Water (plaque assay and molecular)	Watershed (stream and reservoir)	Kentucky	Brion et al. 2002
F+ and somatic coliphage	(Sub)tropical	Water	Coastal beaches	California	Colford et al. 2007
Chemical					
Coprostanol	(Sub)tropical	Water and sediment	Coastal	Australia	Nichols et al. 1993
Fecal sterols and linear alkyl benzenes	(Sub)tropical	Sediment	Coastal	California	Phillips et al. 1997
Coprostanol and trialkylamines	(Sub)tropical	Sediment	Coastal	California	Maldonado et al. 2000
10 fecal sterols, including coprostanol	Tropical	Water and sediment	Inland and estuarine	Malaysia and Vietnam	Isobe et al. 2002
Caffeine	Temperate	Water	Coastal bay	Massachusetts	Seigener and Chen 2002
Coprostanol	Tropical and temperate	Water	Inland rivers	Vietnam and Japan	Isobe et al. 2004
Optical brighteners	Temperate	Water	Coastal bays	Virginia	Hagedorn et al. 2005

Alternate Indicator Organism	Climate	Environmental Medium	Water Type/ Description	Geographic Region	Study
10 fecal sterols, including coprostanol	(Sub)tropical	Water	Inland and estuarine river (+ sewage and bird feces)	California	Noblet et al. 2004
Caffeine	(Sub)tropical	Water	Inland watershed, streams, rivers, wetland	Georgia	Peeler et al. 2006
Optical brighteners	(Sub)tropical	Water and sediment	Coastal beach, inland stream	Georgia	McDonald et al. 2006
Optical brighteners	(Sub)tropical	Water	Coastal water, inland stream	Georgia	Hartel et al. 2007