

**LITERATURE REVIEW ON THE SURVIVAL OF
FECAL COLIFORMS IN FRESH AND SALINE WATERS,
AND SEDIMENTS**

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1. INTRODUCTION AND BACKGROUND

The impact of fecal contaminated surface water on shellfish and shellfish harvesting is a health, environmental, and economic concern of the Lummi Nation, tribal members, and the general public. As filter feeders, shellfish such as oysters, mussels, and clams have a potential to carry and transmit enteric pathogens if their environment is polluted with fecal matter. During feeding, shellfish filter large quantities of water and from this process, can accumulate bacterial and viral pathogens in their tissues (Ecology, 2000).

Under the Shellfish Consent Decree (Order Regarding Shellfish Sanitation, *United States v. Washington [Shellfish]*, Civil Number 9213, Subproceeding 89-3, Western District of Washington, 1994), the Washington State Department of Health is accountable to the federal Food and Drug Administration to administer the National Shellfish Sanitation Program (NSSP) on the Lummi Indian Reservation. The NSSP evaluates surface water quality in shellfish harvesting areas using fecal coliform (FC) bacteria as an indicator of fecal pollution. FC bacteria include *Escherichia coli* (*E. coli*) that originate in the gastrointestinal tract of both humans and animals and are displaced in feces.

To ensure public safety, the NSSP shellfish standards affirm that water over shellfish beds may not exceed a FC organism geometric mean value of 14 colonies per 100 milliliters (col./100mL), and the estimated 90th percentile cannot exceed 43 col./100mL. The last thirty consecutive FC values for any given water quality monitoring station are used to calculate these statistics.

Water quality over shellfish beds that does not meeting NSSP standards prevent Lummi tribal members from engaging in lifestyle and cultural practices, and also generating income from shellfish sales. As part of the response to the closure of Portage Bay to shellfishing, and pursuant to an August 2000 intergovernmental Memorandum of Agreement developed as part of the effort to address this closure, the Lummi Indian Business Council has contracted this literature review on FC survival in both fresh and saline waters, and sediments. The purpose of this literature review is to summarize existing research related to FC survival, and provide information that will help the Lummi Nation manage threats to their shellfish resources.

This literature review is organized into five sections. Section 1 is this introduction and background. Section 2 describes the various factors that influence FC survival in the natural environment and summarizes information from scientific papers regarding the impact of each factor on FC survival. Section 3 offers a discussion of the six main factors influencing FC survival. The conclusions of this literature review are presented in Section 4, followed by a listing of references cited in Section 5.

To remain consistent and accurate, the terminology and units used in this literature review (e.g. fecal coliforms, enteric bacteria, *E. coli*, T₉₀, nm) are the same as the terminology and units used by the authors of the research papers included in this document.

2. LITERATURE REVIEW

The survival of FC bacteria in natural environments has been the topic of extensive research due its impact on public health and safety. *Escherichia coli* (*E. coli*) is the most recognized type of FC bacteria (Jamieson et al., 2002). *E. coli* is a normal inhabitant of the intestinal tract of humans and other warm-blooded animals, and is excreted in feces (Auer and Niehaus, 1993). These bacteria are described as non-spore forming, fast growing, gram-negative rods that ferment lactose at 35 °C (Univ. of Kentucky, 1998). The presence of FC bacteria in the natural environment is an indication of fecal pollution from point sources such as municipal wastewater discharges and septic leachate, and nonpoint sources such as agricultural (primarily livestock farms) and/or storm runoff.

The natural environment is a dynamic system influenced by an array of variables. The survival of FC bacteria in the natural environment is influenced by the conditions of its surroundings. Published scientific literature indicates that a variety of physical, chemical, and biological factors impact FC survival in fresh and saline waters, and sediments. Many researchers identify solar radiation, temperature, salinity, nutrients, pH, and predation as factors influencing FC survivability. In conjunction with some of the aforementioned factors, other researchers also acknowledge the effects of turbidity, vertical mixing, sewage concentration, heavy metals, competition, algal toxins, and bacteriophages as factors affecting FC survival.

With the extensive amount of research conducted and available on FC, it is apparent that FC survival in the natural environment is contingent on a variety of physical, chemical, and biological parameters. There is a wide assortment of reported survival times for FC outside the gastrointestinal tract, which reflects the impact of the above-mentioned factors and also probable variation in the analytical methodology and bacterial strains used during experimentation. As a result, this literature review will only present the range of findings reported on the impacts of solar radiation, temperature, salinity, nutrients, pH, and predation on FC survival in saline and fresh waters, and sediments.

2.1 Solar radiation

Of the factors influencing FC survival in the natural environment, solar radiation has been reported as the single most important parameter affecting the die-off of FC bacteria (Alkan et al., 1995; Auer and Niehaus, 1993; Fujioka et al., 1981; Kapuscinski and Mitchell, 1983; Rozen and Belkin, 2001; Solic and Krstulovic, 1992).

Table 1 summarizes the information pertaining to the impact of solar radiation exposure on FC survival.

Table 1. Impacts of solar radiation exposure on fecal coliform survival

REFERENCE	SUMMARY
Alkan et al. (1995)	Solar radiation is a key factor influencing mortality of bacteria in seawater. The time required for a 90% reduction (T_{90}) in <i>E. coli</i> is 0.5 to 8 hours, with mean values ranging from 1.06 and 1.3 hours at a 95% confidence level. The T_{90} for enterococci ranged from 0.5 to 9.8 hours, with mean values ranging from 1.14 to 1.39 at a 95% confidence level. The range of values was dependent on the environmental conditions present such as solar radiation, adsorption to sediments particles, sedimentation, physicochemical factors, nutrients, temperature and biological factors. The impact of sunlight on bacteria mortality is more significant than temperature, when both are present. To a certain degree, turbidity decreased mortality rates (prevents the penetration of light), while vertical mixing enhanced mortality rates (brings bacteria to the water surface).
Auer and Niehaus (1993)	UV light of approximately 260 nm, and visible light has been found to kill coliform bacteria. Dissolved organic matter in aquatic environments mitigates the impact of UV radiation. A decrease in FC survival occurs in the summer. Laboratory experiments conducted in the dark using FC at temperatures of 10 to 35 °C yielded a death rate coefficient ranging from 0.43 to 0.81 day ⁻¹ , with a mean of 0.61 +/- 0.11 day ⁻¹ . The FC death rate coefficient under natural aquatic conditions in the dark is 0.73 day ⁻¹ .
Burkhardt III and Calci (2000)	Study focused on oysters in estuarine water. FC densities in oyster tissue are 4.4 times greater than FC densities measured in the water column. Therefore, oysters harvested from approved waters may have FC densities ranging from less than 6 to 118/100 g in their tissue. Highest concentrations of enteroviruses are found in the winter season, when temperature and sunlight exposure are the lowest.
Burkhardt III et al. (2000)	Inactivation of FC is positively correlated to light energy. Inactivation of FC may be due to UV light damaging nucleic acids. Sunlight has a greater impact on FC at low water temperatures.
Crowther et al. (2001)	Survival of coliform bacteria populations is positively influenced by tide height and onshore winds (which generate turbulence and decrease UV light from penetrating), and negatively influenced by sunlight.
Dan et al. (1997)	Solar radiation (greater than 350 nm) causes <i>E. coli</i> to become inactivated.
Davies et al. (1995)	Sunlight contributes to the mortality of fecal bacteria in water.
Fujioka et al. (1981)	Sunlight influences FC survival in marine environments. Measured T_{90} values for FC in seawater ranged from 21 to 48 hours. Measured T_{90} for FC in seawater exposed to sunlight ranged from

REFERENCE	SUMMARY
	30 to 90 minutes. Indirect sunlight (cloudy day conditions) is capable of inactivating FC after a 1-hour period of stability, and then a gradual decrease in FC occurs with greatest inactivation taking place after 4 hours. FC diluted in seawater, and those diluted in freshwater, both exposed to identical sunlight gave T ₉₀ values of 36 minutes (with complete inactivation occurring within 3 hours), and 114 minutes (with 8% of the population surviving past 3 hours) respectively. Visible light (which penetrated to a measured depth of 3.3 m of clear seawater) and not UV light is primarily responsible for impacting bacterial survival. The impact of visible light on FC survival may be hindered by turbidity, turbulence, and water chemical composition.
Kapusinski and Mitchell (1983)	Sunlight exposure decreases the survival of <i>E. coli</i> in seawater. Mortality lag times of 0.4 to 1.5 hours before die-off of <i>E. coli</i> in seawater exposed to sunlight was observed in this study (T ₉₀ less than 5 hours). Another study has found lag times of 2.7 hours before die off of FC in sewage plumes in coastal surface waters (T ₉₀ less than 5.5 hours). Mortality rates of <i>E. coli</i> in filtered seawater kept in the dark is T ₉₀ greater than 24 hours. Mortality rates of <i>E. coli</i> in seawater exposed to sunlight is T ₉₀ less than 4.9 hours.
Mancini (1978)	Sunlight increases FC mortality in saltwater environments. Wavelengths less than 370 nm are responsible for one-half of the damage sunlight has on FC bacteria. Wavelengths from 370 to 400 nm are responsible for ¼ of the sunlight damage. Wavelengths greater than 500 nm have no impact on FC mortality.
McCambridge and McMeekin (1981)	The combined impact of solar radiation and predation on <i>E. coli</i> mortality was greater than the impact of each factor alone. <i>E. coli</i> in estuarine water subjected to predation by protozoa declined from 5 x 10 ⁸ to 6 x 10 ² organisms per mL after 10 days of incubation in the dark. In a similar experiment without predators, the number of <i>E. coli</i> organisms remained fairly constant. The combined impact of solar radiation and predation on <i>E. coli</i> in estuarine environments caused <i>E. coli</i> to decrease from 6 x 10 ⁸ to zero organisms per mL in 8 days.
Rozen and Belkin (2001)	The survival of enteric bacteria in seawater is influenced by light (radiation). At shallow depths, light greatly impacts enteric bacterial mortality. UV-B radiation (280 to 320 nm) is reported as the most bacteriocidal, inducing DNA damage to FC. FC have been found to be more susceptible to sunlight exposure than enterococci.
Solic and Krstulovic (1992)	Survival of FC bacteria in seawater has ranged from a few hours to a few days depending on experimental conditions. Experiments testing the impact of solar radiation between 510 and 830 Watts/m ² on the survivability of FC found T ₉₀ to decrease by approximately 40% for each 100 Watts/m ² increase in solar radiation. The effect of solar radiation on FC survival was significant to a depth of 30 m.

REFERENCE	SUMMARY
	Solar radiation is more significant than temperature in reducing FC die-off. FC experiments using two different temperatures (12 and 24 °C) under both light and dark conditions found the T ₉₀ of FC kept at 24 °C and exposed to light was approximately 27 times less than the T ₉₀ of FC kept at the same temperature in the dark. FC die-off rates are reported as 0.471 and 1.124 h ⁻¹ for FC subjected to light at 12 and 24 °C respectively. The effect of increasing temperature from 12 to 24 °C under dark conditions resulted in the T ₉₀ value decreasing by a factor of 2.5 times and increased die-off rates of 0.016 and 0.040 h ⁻¹ respectively. T ₉₀ values for FC exposed to varying intensities of solar radiation and temperature ranged from 0.96 to 4.89 hours. T ₉₀ values for FC kept in the dark at variable temperatures, ranged from 51.2 to 145.10 hours. The combined effects of temperature and solar radiation are synergistic. Salinity and solar radiation are also synergistic in terms of FC survival. The die-off rate of FC in sunlight at 10 parts per thousand and 35 parts per thousand salinity was 0.15 and 1.02 h ⁻¹ respectively. The die-off rate of FC in the dark at 10 parts per thousand and 35 parts per thousand salinity was 0.018 and 0.08 h ⁻¹ respectively.
Troussellier et al. (1998)	The presence of sunlight is one of the main stress factors that contribute to bacterial mortality in marine environments. Sunlight impacts the ability of a cell to form colonies.

2.2 Temperature

The optimum growth temperature for enteric bacteria is 37°C, however this temperature is not required for bacterial survival in seawater (Rozen and Belkin, 2001). The low temperature conditions found in fresh and marine waters contribute to a relatively overall increase in FC survival in natural environments (An et al., 2002; Auer and Niehaus, 1993; Rozen and Belkin, 2001; Solic and Krstulovic, 1992). However, other researchers (Buckhouse and Gifford, 1976; Crabill et al., 1999; Univ. of Kentucky, 1998) have reported the opposite outcome, an increase in FC survival in warm temperature environments.

Table 2 summarizes research findings by various authors regarding the impact of temperature on FC survival.

Table 2. Impacts of temperature on fecal coliform survival

REFERENCE	SUMMARY
Alkan et al. (1995)	FC survival was found to be dependent on the environmental conditions present. Increasing temperature from 10 to 30°C had no significant impact on FC survival in seawater because when both temperature and light are present, the impact of light outweighs the impact of temperature.

REFERENCE	SUMMARY
An et al. (2002)	FC mortality increases proportionately with increasing temperature. Lower densities of <i>E. coli</i> were measured at lake marinas during the summer season possibly due to lower loading, warmer temperatures, and ecological conditions such as predation by protozoa. FC mortality increased with increasing temperature. Also, higher temperatures promote the growth of protozoa, which prey on FC and thus contribute to lower FC numbers. The population of total coliforms increased during summer because non-FC bacteria can survive high temperatures. Higher densities of <i>E. coli</i> in bottom waters because FC bacteria were found to be absorbed onto colloid size particles ranging from 0.45 to 10 μm , and secondly, water temperatures are cooler and more favorable for growth.
Auer and Niehaus (1993)	A decrease in FC survival occurs in summer. Laboratory experiments conducted in the dark using FC at temperatures of 10 to 35°C provided a death rate coefficient ranging from 0.43 to 0.81 day^{-1} , with a mean of 0.61 \pm 0.11 day^{-1} . However, this study reported no significant relationship between death rate and temperature.
Buckhouse and Gifford (1976)	Coliform bacteria were found to be viable for one summer under extreme heat and sunlight. Sampling of fresh fecal material (not diluted by fresh or salt water) over 18 weeks, demonstrated that coliforms from the fecal matter were able to survive at densities of 1100 col./mL for 7 weeks. Die-off of fecal material was not detected until 9 weeks into the experiment.
Burkhardt III and Calci (2000)	Study focuses on oysters in estuarine water. Highest concentrations of enteroviruses are found in the winter season, when temperature and sunlight exposure are the lowest.
Burkhardt III et al. (2000)	Inactivation of FC is negatively correlated to temperature. Metabolic activity of FC increases with higher temperatures because FC are mesophilic (like warm environments) bacteria. Sunlight has a greater impact on FC at low water temperatures.
Crabill et al. (1999)	FC densities increased in the summer and stabilized in the fall and winter.
Dan et al. (1997)	Temperature impacts the survival of <i>E. coli</i> in river water.
Delille and Delille (2000)	Survival of enteric bacteria in seawater ranges from a fraction of an hour to weeks depending on bacteria characteristics and environmental conditions such as temperature. Low temperature environments are suitable for enteric bacteria survival.
Dupray and Derrien (1995)	<i>E. coli</i> bacteria have a greater chance of survival in seawater if they have already adjusted to the low temperature conditions in wastewater treatment plants.
Jamieson et al. (2002)	Moist environments, cool temperatures, high nutrient availability, low competition, and pH of 6 to 7 allow for greater survival of fecal bacteria in soils. Fecal bacteria mortality rates increase under hot and dry conditions. Soils act as a reservoir for FC bacteria until

REFERENCE	SUMMARY
	rainfall events transport the bacteria as runoff to water bodies.
Medema et al. (1997)	Cryptosporidium <i>parvum</i> oocysts are capable of surviving several months depending on temperature, predators and exo-enzymes. <i>E. coli</i> and enterococci in untreated river water experienced a die-off rate ten times faster than that of <i>C. parvum</i> oocysts. A 10-degree reduction in <i>C. parvum</i> oocysts in river water took 40-160 days at 15°C and 100 days at 5°C.
Mezrioui et al. (1995)	<i>E. coli</i> from stabilization pond effluent has a longer survival in winter than in summer. <i>E. coli</i> analyzed during summer, fall and winter experiments had a T ₉₀ of 17 to 24 hours, 80 hours, and 125 hours respectively
Nasser and Oman (1999)	At lower temperatures, <i>E. coli</i> inactivation was found to be greater than the inactivation of enteric viruses.
Rozen and Belkin (2001)	The survival of enteric bacteria in seawater is highly influenced by temperature. The optimum growth temperature is 37°C, however this temperature is not required for bacterial survival in seawater.
Solic and Krstulovic (1992)	This study reported a decreasing exponential relationship between seawater temperature and FC to exist. For each 10°C increase in water temperature, the time required to reach a 90% reduction in bacteria (T ₉₀) decreased by approximately 55%. The effect of increasing temperature from 12 to 24°C under dark conditions resulted in the T ₉₀ value decreasing by a factor of 2.5 and increases die-off rates of 0.016 and 0.040 h ⁻¹ respectively. T ₉₀ values for FC exposed to varying intensities of solar radiation and temperature ranged from 0.96 and 4.89 hours. T ₉₀ values for FC kept in the dark at variable temperatures, ranged from 51.2 and 145.10 hours. The combined effect of temperature and solar radiation is synergistic.
Tamburrini and Pozio (1999)	Scientific research reports oocysts of <i>C. parvum</i> can be infective for up to 1 year of incubation in artificial seawater kept under moderate oxygenation and at temperatures of 6 to 8°C. Mice injected with <i>C. parvum</i> oocysts showed <i>C. parvum</i> infections. Other researchers have found <i>C. parvum</i> oocysts to last in artificial seawater at 4°C for over a month, and up to 12 weeks at various saline and temperature conditions.
Univ. of Kentucky, College of Agriculture (1998)	FC favor warm, nutrient rich environments. Higher temperatures lead to an increase in the FC population, while lower temperature lead to a decline.

2.3 Salinity

Saline conditions such as those found in marine environments impact the survival of FC bacteria by posing unwanted stress on cells. As summarized in Table 3, many researchers mention salinity as an important parameter influencing FC survival in the natural environment.

Table 3. Impacts of salinity on fecal coliform survival

REFERENCE	SUMMARY
An et al. (2002)	Factors that impact FC mortality include solar radiation, salinity, lack of nutrients, and predation by other microbes such as protozoa.
Anderson et al. (1979)	Coliform bacteria quickly die in marine environments. High salinity environments cause stress to coliforms. Cells become nonviable within the first 2 days of exposure at 15 to 30 parts per thousand salinity. At salinity levels of 10 parts per thousand or less, the cells experience a slower death rate.
Auer and Niehaus (1993)	Algal toxins, bacteriophages, nutrients, pH, temperature, predation, salinity, and irradiance have been reported to influence the death rate of FC.
Davies et al. (1995)	Sunlight, low nutrient levels, high salinity, toxic agents, and predation and parasitism all contribute to the mortality of fecal bacteria in water. From an experiment that lasted 68 days, the fecal bacteria introduced into seawater gradually lost the ability to form colonies, while the fecal bacteria introduced into marine sediments retained the ability to form colonies for the full extent of the experiment.
Enzinger and Cooper (1976)	Other researchers have found marine water to have a bacteriocidal effect on non-marine bacteria. Bacteria survival is impacted by heavy metals, salinity, competition, antibiosis, and predation. <i>E. coli</i> concentrations dramatically decreased on days 2 to 4 of the experiment as a result of a 10-fold increase in the protozoan population. It has been proposed that <i>E. coli</i> bacteria are not as competitive with indigenous marine protozoa for nutrients as they are with fresh water protozoa.
Fujioka et al. (1981)	High salinity, heavy metals, sunlight, temperature, competition for nutrients, predation by microorganisms, lysis by bacteriophages, aggregation, adsorption by particulate matter are factors which influence FC survival in marine environments. Measured T ₉₀ values for FC diluted in seawater water ranged from 21 to 48 hours. FC bacteria may remain viable for 1 to 3 days in seawater. The T ₉₀ for FC diluted in seawater and then exposed to sunlight ranged from 30 to 90 minutes. Study states that the effects of salinity and the overall marine environment are negligible when sunlight is present. During incubation, FC diluted in freshwater maintained their densities for up to 3 days, whereas those diluted in seawater became inactivated on day 2 or 3. FC diluted in seawater, and those diluted in freshwater, both exposed to identical sunlight gave T ₉₀ values of 36 minutes (with complete inactivation occurring within 3 hours) and 114 minutes (with 8% of the population surviving past 3 hours) respectively.

REFERENCE	SUMMARY
Gerba and McLeod (1976)	This study found that <i>E. coli</i> in a seawater and sediment environment had a greater survival than <i>E. coli</i> in just a seawater environment. <i>E. coli</i> has a greater survival in sediment than in overlaying marine water because the sediments contain more organic matter. The sediment layer contained 10 to 100 times more FC on a volume basis than the aqueous layer. Sediment not only increases <i>E. coli</i> survival in marine environments, but may also promote growth. FC experience a rapid die-off when exposed to marine environments. Natural factors such as salinity, predations, competition, heavy metals, and nutrient limitations impact FC mortality. Die-off curves of <i>E. coli</i> in marine water show T ₉₀ values ranging from 3 to 5 days.
Ghoul et al (1990)	A faster growth rate of <i>E. coli</i> was measured in the presence of marine sediment than in seawater alone. High availability of organic matter and betaines, specifically glycine betaine released by decaying plants, algae, invertebrates, and vertebrates, protects <i>E. coli</i> from salinity effects and increases survival. The uptake of glycine betaine was found to come from sediments, and not the seawater because FC kept in seawater without sediment showed no accumulation of glycine betaine.
Kapusinski and Mitchell (1983)	Inorganic salts in seawater decrease substrate utilization by fecal bacteria and cause bacterial numbers to decline.
Rozen and Belkin (2001)	Exposure to seawater affects the ability of enteric bacteria to form colonies on a solid media. Some scientists have demonstrated that viable but nonculturable cells (VBNC) are either dead or of no significance, while others have shown that they may still be infective. The survival of enteric bacteria in seawater is influenced by temperature (high impact), pH, salinity (low impact), nutrient availability (high impact), and light radiation (high impact). Bacterial survival decreases as saline conditions increase.
Solic and Krstulovic (1992)	Other researchers have reported solar radiation, temperature, salinity, pH, predation, competition, lysis, and algal toxins as factors influencing coliform survival in marine environments. Survival of coliforms in seawater has ranged from a few hours to a few days depending on experimental conditions. Salinity and solar radiation also have a synergistic effect in terms of FC survival. Increasing FC mortality occurs at higher salinity. The die-off rate of FC in sunlight at 10 and 35 parts per thousand salinity was 0.15 and 1.02 h ⁻¹ respectively. The die-off rate of FC in the dark at 10 and 35 parts per thousand salinity was 0.018 and 0.08 h ⁻¹ respectively.
Troussellier et al. (1998)	Main stress factors that contribute to bacterial mortality in marine environments are high salinity, lack of organic material, and presence of sunlight. Enteric bacteria in the stationary phase are less susceptible to hyperosmotic stress than those in the exponential phase. <i>E. coli</i> exposed to other previous stress factors prior to being

REFERENCE	SUMMARY
	exposed to seawater has a greater chance of survival. Experiments lasting less than 48 hours indicated that the combined impact of nutrient deficiency and hyperosmotic shock leads to a decrease in energy charge and inactivation of membrane transport.

2.4 Nutrients

The availability of nutrients is essential for the survival and probable growth of FC bacteria in natural environments. Table 4 summarizes the impact of nutrient availability on FC survival.

Table 4. Impacts of nutrients on fecal coliform survival

REFERENCE	SUMMARY
Alkan et al. (1995)	Survival of enteric bacteria depends on solar radiation, adsorption, sedimentation, physicochemical factors, nutrients, temperature and biological conditions.
An et al. (2002)	Factors that impact FC mortality include solar radiation, salinity, lack of nutrients, and predation by other microbes such as protozoa. Higher levels of FC are found in sediments than water. The removal of FC from water primarily occurs by adsorption onto particles, which later settle and accumulate.
Auer and Niehaus (1993)	Algal toxins, bacteriophages, nutrients, pH, temperature, predation, salinity, and irradiance have been reported to influence the death rate of FC. Dissolved organic matter in aquatic environments mitigates the impact of UV radiation. Sedimentation is a major sink for FC. This study reports that 90.5% of FC bacteria are associated to particles ranging from 0.45 to 10 μm , and the remaining 9.5% associated to particles larger than 10 μm .
Burton et al. (1987)	Several studies have indicated that higher concentrations of pathogenic bacteria are found in the sediment layer than the aqueous phase. This study found prolonged survival of bacteria in fresh water sediments than in overlying waters for as long as several months. Survival of <i>E. coli</i> improved in fresh water sediments containing more than 25% clay due to its high organic content. <i>E. coli</i> survival was lowest in fresh water sandy systems due to its low organic content. The variability in FC concentrations from aqueous systems may be attributed to the resuspension of bacteria living in the sediment layer. The survival of pathogenic bacteria in aqueous systems is dependent on protozoa, antibiotics, organic matter, algal toxins, dissolved nutrients, heavy metals, temperature, and the physicochemical parameters.
Crabill et al. (1999)	FC counts from sediment samples were 2200 times greater than water FC counts. Soluble organics in the sediment region allows FC to survive and stimulates greater heterotrophic activity. The sediment region is typically anaerobic and therefore is not a suitable

REFERENCE	SUMMARY
	environment for protozoa that prey on FC. As a result of both the soluble organics and the low protozoa population in the sediment region, FC are able to survive and possibly even grow in numbers. Other researchers found FC sediment to water ratios varying from 10:1 to greater than 100:1. This study reported FC ratios as high as greater than 2000:1
Davies et al. (1995)	Fecal organisms sorb onto particulate matter that later settles out. Sediment samples have been found to contain 100 to 1000 times as many fecal indicator bacteria than water samples. Sunlight, low nutrient levels, high salinity, toxic agents, and predation and parasitism all contribute to the mortality of fecal bacteria in water.
Delille and Delille (2000)	Survival of FC bacteria in seawater ranges from a fraction of an hour to weeks depending on bacteria characteristics and environmental conditions such as temperature and nutrient availability. Insufficient nutrient supplies decrease the activity of FC bacteria.
Fujioka et al. (1981)	High salinity, heavy metals, sunlight, temperature, competition for nutrients, predation by microorganisms, lysis by bacteriophages, aggregation, adsorption by particulate matter are factors which influence FC survival in marine environments.
Gerba and McLeod (1976)	<i>E. coli</i> has a greater survival in sediment than in overlaying marine water because the sediments contain more organic matter. The sediment layer contained 10 to 100 times more FC on a volume basis than the aqueous layer. This study found that <i>E. coli</i> in a seawater and sediment environment had a greater survival than <i>E. coli</i> in just a seawater environment. Sediment not only increases <i>E. coli</i> survival in marine environments, but may also promote growth. FC experience a rapid die-off when exposed to marine environments. Natural factors such as salinity, predations, competition, heavy metals, and nutrient limitations impact FC mortality. Die-off curves of <i>E. coli</i> in marine water show T ₉₀ values ranging from 3 to 5 days.
Ghoul et al (1990)	A faster growth rate of <i>E. coli</i> was measured in the presence of marine sediment than in seawater alone. High availability of organic matter and betaines, specifically glycine betaine released by decaying plants, algae, invertebrates, and vertebrates, protects <i>E. coli</i> from salinity effects and increases survival. The uptake of glycine betaine was found to come from sediments, and not the seawater because FC kept in seawater without sediment showed no accumulation of glycine betaine. The attachment of FC to particulates in seawater and then sedimentation results in the removal of FC from the aqueous phase.
Hood and Ness (1982)	FC survival in marine environments is a factor of salinity, predation, competition, nutrients, and heavy metals. Increased survival of <i>E. coli</i> in estuarine waters with sediments, than in just estuarine waters.

REFERENCE	SUMMARY
Jamieson et al. (2002)	Moist environments, cool temperatures, high nutrient availability, low competition, and pH of 6 to 7 allow for greater survival of fecal bacteria in soils.
LaLiberte and Grimes (1982)	The presence of FC in water may not be reflective of recent fecal contamination because FC have the ability to survive for several days in sediment. Therefore, FC found in the aqueous phase may come from the resuspension of sediment-bound FC. A greater concentration of <i>E. coli</i> bacteria was found in the autoclaved silty clay system than in the autoclaved sand system. However, the opposite effect occurred in unsterile systems, where greater numbers of <i>E. coli</i> were found in sand. Comparatively, sand does not have as much organic matter as silty clay; therefore, the amount of organic matter in sediment does not dictate bacteria survival in sediment systems.
Rozen and Belkin (2001)	The survival of enteric bacteria in seawater is influenced by temperature (high impact), pH, salinity (low impact), nutrient availability (high impact), and light radiation (high impact). The availability of nutrients promotes bacterial survival in seawater, however a lack of nutrients does not essentially affect colony formation. Ten to 100 fold more FC or enterococci have been measured in sediment samples than in water samples. A higher concentration of organic matter is available to FC and enterococci in the sediment layer, therefore promoting longer survival. Biotic factors such as predation by protozoa, and competition for nutrients also influences the prevalence of enteric bacteria in marine environments.
Troussellier et al. (1998)	Main stress factors that contribute to bacterial mortality in marine environments are high salinity, lack of organic material, and presence of sunlight. Experiments lasting less than 48 hours indicated that the combined impact of nutrient deficiency and hyperosmotic shock leads to a decrease in energy charge and inactivation of membrane transport.
Univ. of Kentucky, College of Agriculture (1998)	FC favor warm, nutrient rich environments. Capable of surviving in soil and sediment. FC can persist for extended periods of time in water and sediment. Resuspension of sediment can reintroduce FC to the water column.

2.5 pH

Compared to other parameters that influence FC survival, such as solar radiation and nutrient availability, the effects of pH are minimal due to small variability in seawater pH (Solic and Krstulovic, 1992). However, experiments with FC and pH have shown FC survival to be influenced by pH (An et al., 2002; Auer and Niehaus, 1993; Jamieson et al., 2002; Rozen and Belkin, 2001; Solic and Krstulovic, 1992).

Table 5 summarizes the literature regarding pH impacts on FC survival.

Table 5. Impacts of pH on fecal coliform survival

REFERENCE	SUMMARY
An et al. (2002)	FC mortality increases proportionately with increasing temperature, increasing pH, and higher dissolved oxygen levels.
Auer and Niehaus (1993)	Algal toxins, bacteriophages, nutrients, pH, temperature, predation, salinity, and irradiance have been reported to influence the death rate of FC.
Jamieson et al. (2002)	Moist environments, cool temperatures, high nutrient availability, low competition, and pH of 6 to 7 allow for greater survival of fecal bacteria in soils.
Rozen and Belkin (2001)	The survival of enteric bacteria in seawater is influenced by temperature (high impact), pH, salinity (low impact), nutrient availability (high impact), and light radiation (high impact). <i>E. coli</i> prefers acidic environments (pH 5). Seawater with a pH range of 7.5 to 8.5 impacts <i>E. coli</i> mortality. FC exposed to lower pH values and temperatures have a higher survival rate.
Solic and Krstulovic (1992)	Other researchers have reported solar radiation, temperature, salinity, pH, predation, competition, lysis, and algal toxins as factors influencing coliform survival in marine environments. The effect of pH was significant in FC mortality both above and below the optimum pH range of 6 and 7. In acidic environments, the T ₉₀ for FC decreased by approximately 40% for each decrease in pH value. In alkaline environments, the T ₉₀ for FC decreased by approximately 30% for each increase in pH value.

2.6 Predation

As summarized in Table 6, predators such as protozoa can greatly impact the survival of FC bacteria in the environment.

Table 6. Impacts of predation on fecal coliform survival

REFERENCE	SUMMARY
An et al. (2002)	FC mortality increases proportionately with increasing temperature, increasing pH, and higher dissolved oxygen levels. Other factors that impact mortality are solar radiation, salinity, lack of nutrients, and predation by other microbes such as protozoa.
Auer and Niehaus (1993)	Algal toxins, bacteriophages, nutrients, pH, temperature, predation, salinity, and irradiance have been reported to influence the death rate of FC.
Davies et al. (1995)	In the absence of predators, fecal organisms can survive for a long time and possibly grow in sediment. Under natural sediment conditions, predation leads to a decline in fecal organisms. Sediment samples have been found to contain 100 to 1000 times as many fecal indicator bacteria than water samples. Sunlight, low

REFERENCE	SUMMARY
	nutrient levels, high salinity, toxic agents, and predation and parasitism all contribute to the mortality of fecal bacteria in water.
Enzinger and Cooper (1976)	Protozoa influence survival of <i>E. coli</i> in estuarine waters. Bacteria survival is impacted by heavy metals, salinity, competition, antibiosis, and predation. <i>E. coli</i> concentrations dramatically decreased on days 2 to 4 of the experiment as a result of a 10-fold increase in the protozoan population. It has been proposed that <i>E. coli</i> bacteria are not as competitive with indigenous marine protozoa for nutrients as they are with fresh water protozoa.
Fujioka et al. (1981)	High salinity, heavy metals, sunlight, temperature, competition for nutrients, predation by microorganisms, lysis by bacteriophages, aggregation, adsorption by particulate matter are factors which influence FC survival in marine environments.
Gerba and McLeod (1976)	Natural factors such as salinity, predations, competition, heavy metals, and nutrient limitations impact FC mortality. Die-off curves of <i>E. coli</i> in marine water show T_{90} values ranging from 3 to 5 days.
Hood and Ness (1982)	FC survival in marine environments is a factor of salinity, predation, competition, nutrients, and heavy metals. Increased survival of <i>E. coli</i> observed in estuarine waters with sediments, than in just estuarine waters.
McCambridge and McMeekin (1981)	The combined impact of solar radiation and predators on <i>E. coli</i> mortality was greater than the impact of each factor alone. <i>E. coli</i> in estuarine water subjected to predation by protozoa declined from 5×10^8 to 6×10^2 organisms per mL after 10 days of incubation in the dark. In a similar experiment without predators, the number of <i>E. coli</i> organisms remained fairly constant. The combined impact of solar radiation and predation on <i>E. coli</i> in estuarine environments caused <i>E. coli</i> to decrease from 6×10^8 to zero organisms per mL in 8 days.
Mezrioui et al. (1995)	Experiments with <i>E. coli</i> and predators (non-filtered water) during summer, fall, and winter had T_{90} values of 24, 80, and 125 hours respectively. Experiments with <i>E. coli</i> in the absence of predators (filtered water) during summer, fall, and winter had T_{90} values of 95, 119, and 53 hours respectively. <i>E. coli</i> survival in summer was high due to water filtration which removed predators greater than 5 μm . <i>E. coli</i> survival in winter showed the opposite effect occurring, with bacterial survival being greater in non-filtered water than in filtered water. Possible reasons for this occurrence may be due to low zooplankton activity during winter, or even filtration of organic nutrients greater than 5 μm in size which support bacterial growth and survival. <i>E. coli</i> survival increases when the bacteria are gradually introduced into more and more saline waters, rather than a rapid introduction. Possible reasons for this phenomenon are the removal of osmotic shock effects, mortality of predator organisms resulting from saline conditions, and available source of organic

REFERENCE	SUMMARY
	nutrients.
Rozen and Belkin (2001)	Biotic factors such as predation by protozoa, and competition for nutrients also influences the prevalence of enteric bacteria in marine environments.
Solic and Krstulovic (1992)	Other researchers have reported solar radiation, temperature, salinity, pH, predation, competition, lysis, and algal toxins as factors influencing coliform survival in marine environments. Survival of coliforms in seawater has ranged from a few hours to a few days depending on experimental conditions.

3. DISCUSSION

This literature review summarizes the results of research regarding factors that affect the survival of FC in fresh and saline waters, and sediments. Published scientific literature identifies various physical, biological, and chemical parameters that influence FC survivability in the natural environment. The six most important parameters are solar radiation, temperature, salinity, pH, nutrients, and predation.

3.1 Solar radiation

Of the six primary factors influencing FC survival in the natural environment, solar radiation is the most important factor. However, an inconsistency exists in the published literature between which portions of the solar spectrum, visible light (400-775 nm) or UV radiation (280-400 nm) results in more harm to FC bacteria. Research by Mancini (1978) indicates that wavelengths less than 370 nm are responsible for one-half of the damage, wavelengths from 370 to 400 nm are responsible for ¼ of the damage, and wavelengths greater than 500 nm have no impact on FC mortality. Later work by Rozen and Belkin (2001) agrees with Mancini (1978) that UV-B radiation (280 to 320 nm) is the most harmful, inducing DNA damage. On a contrary note, Fujioka et al. (1981) studied the effects of sunlight on the survival of FC in seawater and reported that visible light and not UV light primarily affected bacterial survival.

The reviewed literature indicates that under laboratory conditions the death rate coefficients for FC densities in fresh water exposed to solar radiation range from 0.5 to 4.57 day⁻¹ (Auer and Niehaus, 1993), with a 90% reduction in FC occurring in 114 minutes (Fujioka et al., 1981). A 90% reduction in FC in seawater exposed to solar radiation ranges from 30 minutes (Fujioka et al., 1981) to 5 hours (Kapusinski et al., 1983). Impacts of FC survival in sediments is not well documented, but is expected to be more than the T₉₀ of 5 hours reported for FC in the water column because sediments generally have less exposure to sunlight.

3.2 Temperature

Temperature, which is a function of many factors (e.g., time of day, seasonality), has been applied to various experiments to test its affect on FC survival. In general, the results of these experiments have been mixed. Some experiments have concluded that temperature does not have a significant effect on FC survival (Alkan et al., 1995; Auer and Niehaus, 1993). Some researchers found that FC survival in marine waters is highly influenced by temperature (Rozen and Belkin, 2001). Other researchers have found that FC survival decreases with increasing temperature (An et al., 2002; Jamieson et al., 2002; Solic and Krstulovic, 1992). Other researchers found that FC survival increases with increasing temperature (Univ. of Kentucky, 1998).

3.3 Salinity

The survival of FC bacteria is impacted by salinity because inorganic salts in seawater decrease substrate utilization by *E. coli* bacteria, causing a population decline (Kapusinski and Mitchell, 1983). Experiments conducted by Troussellier et al. (1998) report the combined impact of hyperosmotic shock and nutrient deficiency leads to a decrease in energy charge and inactivation of membrane transport. Also, exposure to seawater affects the ability of enteric bacteria to form colonies on a solid media (Rozen and Belkin, 2001).

FC mortality increases at higher salinity, and when sunlight is present the two factors act synergistically to decrease overall cell survival (Rozen and Belkin, 2001; Solic and Krstulovic, 1992). In contrast to Solic and Krstulovic (1992) and Rozen and Belkin (2001), Fujioka et al. (1981) found the effects of salinity and the overall marine environment to be negligible when sunlight is present. According to Solic and Krstulovic (1992), the die-off rate of FC in sunlight at 10 and 35 parts per thousand is 0.15 and 1.02 h⁻¹ respectively, while the die-off rate of FC in the dark at 10 and 35 parts per thousand salinity is 0.018 and 0.08 h⁻¹ respectively.

Anderson et al. (1979) found *E. coli* cells to become nonviable within the first 2 days of exposure at 15 to 30 parts per thousand salinity, but at salinity levels of 10 parts per thousand or less; the cells experienced a slower death rate. Some scientists have demonstrated that viable but nonculturable cells are either dead or of no significance, while others have shown that they may still be infective (Rozen and Belkin, 2001). Therefore, the non-viable *E. coli* cells at 15 to 30 parts per thousand salinity reported by Anderson et al. (1979) may in fact pose a threat depending on how viability is understood and interpreted.

The reviewed literature indicates that the time for a 90% reduction in FC bacteria in seawater ranges from 36 minutes (Fujioka et al., 1981) to 5 days (Gerba and McLeod, 1976). Survival in the marine environments may be increased by the availability of organic matter and betaines, specifically glycine betaine released by decaying plants, algae, invertebrates, and vertebrates, that protect *E. coli* from salinity effects (Ghoul et al., 1990). The uptake of glycine betaine was found to come from sediments, and not

seawater because FC kept in seawater without sediment showed no accumulation of glycine betaine (Ghoul et al., 1990).

3.4 Nutrients

The reviewed literature indicates the availability of nutrients positively influences the survival of FC in fresh and saline waters, and sediments. Numerous FC survival studies have reported higher concentrations of pathogenic bacteria in the sediment layer than the aqueous phase (An et al., 2002; Burton et al., 1987; Crabill et al., 1999; Gerba and McLeod, 1976; Rozen and Belkin, 2001).

A greater concentration of soluble organics in the sediment region allows FC bacteria to survive for extended periods of time (Crabill et al., 1999, Gerba and McLeod, 1976, Ghoul et al., 1990; Rozen and Belkin, 2001). From their experiments, Gerba and McLeod (1976) and Rozen and Belkin (2001) found the marine sediment layer contained 10 to 100 times more FC on a volume basis than marine water. Crabill et al. (1999) found FC counts from freshwater sediment samples to be as much as 2200 times greater than freshwater FC counts.

FC viability also increases in fine soil systems like clay because the soil has a greater ability to retain both moisture and nutrients (Jamieson et al., 2002). Burton et al. (1987) found *E. coli* survival to be greatest in fresh water sediments containing more than 25% clay, and lowest in fresh water sandy systems.

A study by LaLiberte and Grimes (1982) described a greater concentration of *E. coli* in the autoclaved silty clay system than in the autoclaved sand system, however, the opposite trend was noted in unsterile systems, where greater numbers of *E. coli* were found in sand than in silty clay. As a result, LaLiberte and Grimes (1982) conclude that the amount of organic matter in sediment does not control bacteria survival in sediment systems.

3.5 pH

The results obtained from published literature regarding the effects of pH on FC survival are inconsistent.

According to Solic and Krstulovic (1992) and Jamieson et al. (2002), FC bacteria have greater survival in neutral environments in the range of pH 6 to 7. In acidic environments (pH less than 7), the T_{90} for FC decreased by approximately 40% for each decrease in pH value, while in alkaline environments (pH greater than 7), the T_{90} for FC decreased by approximately 30% for each increase in pH value (Solic and Krstulovic, 1992).

In contrast to Solic and Krstulovic (1992) and Jamieson et al. (2002), the report made by Rozen and Belkin (2001) states that *E. coli* prefer acidic environments near pH 5. At pH values of 7.5 to 8.5, which characterized seawater, *E. coli* mortality increases (Rozen and Belkin, 2001).

3.6 Predation

The reviewed literature indicates that predators impact the survival of FC bacteria. Enzinger and Cooper (1976) researched *E. coli* survival in estuarine waters and found *E. coli* densities to dramatically decline on days 2 through 4 of the experiment as a result of a 10-fold increase in the protozoan population. A study by McCambridge and McMeekin (1981) found similar results when *E. Coli* densities in estuarine water subjected to predation by protozoa declined from 5×10^8 to 6×10^2 organisms per mL after 10 days of incubation in the dark. In a similar experiment without predators, the number of *E. coli* organisms remained fairly constant (Cambridge and McMeekin, 1981).

Mezrioui et al. (1995) studied the impact of predation on FC survival in marine environments. *E. coli* subjected to predation during summer, fall, and winter had T_{90} values of 24, 80, and 125 hours respectively. In the absence of predators (filtered water), the T_{90} for *E. coli* during summer, fall, and winter was 95, 119, and 53 hours respectively. The rapid T_{90} measured in the winter-filtered water may be attributed to low zooplankton activity or the filtering off of nutrients, which are required for bacterial survival.

Studies by Enzinger and Cooper (1976), McCambridge and McMeekin (1981), and Mezrioui et al. (1995) indicate that predation greatly contributes to the mortality of FC bacteria in the natural environment.

4. CONCLUSIONS

Numerous factors affect the survival of FC bacteria in fresh and saline waters, and sediments. With the wide range of reported FC death rates and FC T_{90} estimates, it is apparent that FC survival in natural systems is dependent not only on the six parameters discussed above, but also the use of different bacterial strains of FC bacteria and even experimental methodology used to obtain survival data. The information provided in this literature review can be used to better understand the individual and inter-related factors that affect fecal coliform survival in fresh and saline waters, and sediments.

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