Enterococci as Indicators of Environmental Fecal Contamination

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Introduction

Enterococci are found in high concentrations in human feces, usually between 10^4 and 10^6 bacteria per gram wet weight (Layton, Walters, Lam, & Boehm, 2010; Slanetz & Bartley, 1957; Zubrzycki & Spaulding, 1962); see also Enterococcus Diversity, Origins in Nature, and Gut Colonization). Although enterococci usually represent less than 1% of the flora (Tendolkar, Baghdayan, & Shankar, 2003), they are usually present in the fecal consortium, but are outnumbered by other bacteria, including *Escherichia coli*, clostridia, and the *Bacteroidales* (Zubrzycki & Spaulding, 1962). Due to their ubiquity in human feces and persistence in the environment, enterococci have been adopted as indicators of human fecal pollution in water. More recently, their densities on human hands have been used as indicators of hand hygiene. The use of enterococci as indicators of human fecal pollution or contamination can be problematic, however, because enterococci are also found in animal feces (Harwood, Whitlock, & Withington, 2000; Layton, Walters, Lam, & Boehm, 2010), in soils (Byappanahalli & Fujioka, 2004; Goto & Yan, 2011), and on plants (Byappanahalli, Shively, Nevers, Sadowsky, & Whitman, 2003; Imamura, Thompson, Boehm, & Jay, 2011; Müller, Ulrich, Ott, & Müller, 2001). Although there is debate about the extent to which this happens in nature, there is evidence that enterococci are capable of replicating in extra-enteric environments, such as on beach sands (Bahirathan, Puente, & Seyfried, 1998; Zubrzycki & Spaulding, 1962) and in water containing kelp (Byappanahalli, Shively, Nevers, Sadowsky, & Whitman, 2003; Imamura, Thompson, Boehm, & Jay, 2011) and plankton (Mote, Turner, & Lipp, 2012). Identification of human-specific enterococcal species or genotypes could aid in the discrimination of human fecal contamination from other environmental sources of the organisms. Some data suggest

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that *Enterococcus faecium* and *Enterococcus faecalis* may be more prevalent in human feces than other enterococcal species, while *Enterococcus casseliflavus* and *Enterococcus mundtii* may be more abundant in environmental reservoirs (such as on plants) than other species (Bahirathan, Puente, & Seyfried, 1998; Ferguson, Moore, Getrich, & Zhowandai, 2005; Wheeler, Hartel, Godfrey, Hill, & Segars, 2002). However, a number of species of *Enterococcus* have been isolated from human feces (Layton, Walters, Lam, & Boehm, 2010); Enterococcus Diversity, Origins in Nature, and Gut Colonization), so it will be difficult to derive a single host-specific indicator. It has been suggested that *E. faecium* that contains the enterococcal surface protein (*esp*) gene may be human-specific (Scott, Jenkins, Lukasik, & Rose, 2005), but *esp*-containing *E. faecium* can also be found in select animal hosts (Layton, Walters, & Boehm, 2009; Whitman, Przybyla-Kelly, Shively, & Byappanahalli, 2007).

Fecal enterococci from the GI tract consortia of healthy humans are generally not virulent. Nevertheless, multidrug-resistant *Enterococcus* strains have emerged as leading causes of hospital-acquired infections (Tendolkar, Baghdayan, & Shankar, 2003). Vancomycin-resistant enterococci are particularly important pathogens (Willems, et al., 2005), as are *esp*-containing *E. faecalis* (Shankar, Baghdayan, Huycke, Lindahl, & Gilmore, 1999) and *E. faecium* (Willems, et al., 2001), as well as other types of *E. faecalis* and *E. faecium* (Wisplinghoff, Bischoff, Tallent, Seifert, Wenzel, & Edmond, 2004). It is estimated that there are 800,000 cases of enterococcal infection in the US each year, adding $500,000,000 to annual healthcare costs (Tendolkar, Baghdayan, & Shankar, 2003). Therefore, the presence of enterococci in the environment and on hands may have important direct health implications.

Detection of Enterococci

Because of their importance as indicators of fecal pollution, a great deal of effort has gone into developing methods for detection of enterococci in the environment. Selective solid and liquid media are available for one-step detection and isolation of the organisms. Quantitative PCR (QPCR) assays that target the 23s rRNA operon have also been developed (Haugland, Siefring, Wymer, Brenner, & Dufour, 2005; Hou, et al., 2006; Ludwig & Schleifer, 2000). Standardized methods have been approved for the detection of enterococci in water, including the United States Environmental Protection Agency (USEPA) Method 1600 (United States Envinronmental Protection Agency, 2006), the USEPA Method A (United States Environmental Protection Agency, 2010), and the International Organization for Standardization (ISO) methods 7899-2 (International Organization for Standardization, 2000) and ISO 7899-1 (International Organization for Standardization, 1998). IDEXX (Westbrook, ME) defined-substrate assays Enterolert and Enterolert-E are also approved for detection of enterococci in water in the United States (US) and the European Union (EU), respectively. Readers are directed to Edge and Boehm (Edge & Boehm, 2010) for a full description of environmental enterococcal enumeration methods.

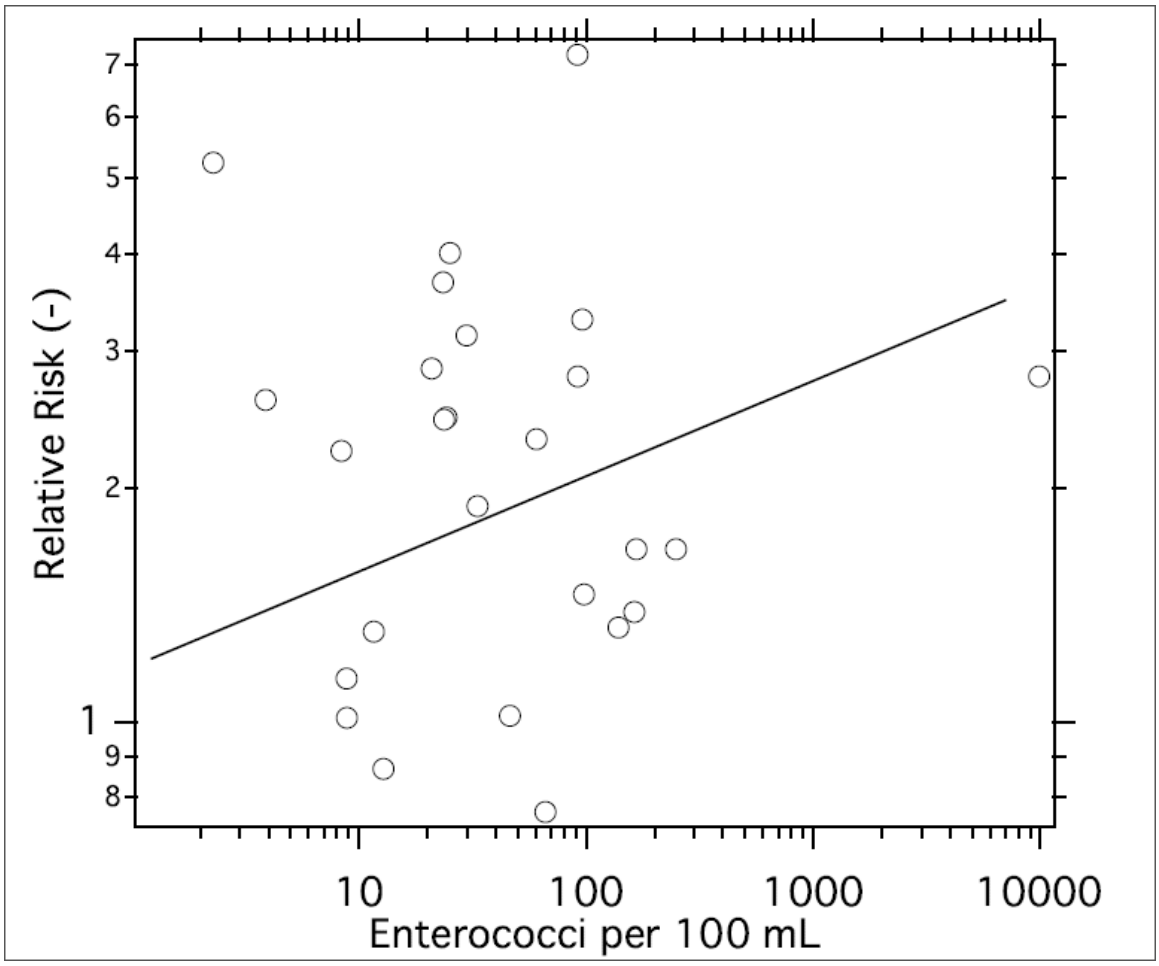

Figure 1. Enterococccci concentrations in marine waters versus relative risk of acquiring gastrointenstinal illness, as reported in the meta-analysis of Wade et al. (Wade, Pai, Eisenberg, & Colford Jr., 2003). The line shown is the weighted best-fit line (r=0.37, p=0.051) and has a slope of 0.3, as reported by the authors. The figure is created using data extracted from Figure 1 of Wade et al. (Wade, Pai, Eisenberg, & Colford Jr., 2003).

Enterococci as Indicators of Fecal Contamination in Recreational Water

Both drinking and recreational waters are monitored for microbial quality. In drinking water, coliforms, including total and fecal coliforms (and *Escherichia coli* in particular) are the primary method of assessing contamination. In the European Union (EU), enterococci are used as indicators of drinking water contamination (The Council of the European Union, 1998). In the EU, enterococci are not permitted in a 100 mL sample of tested drinking water that flows from a tap, and they are not permitted in a 250 mL sample of bottled water.

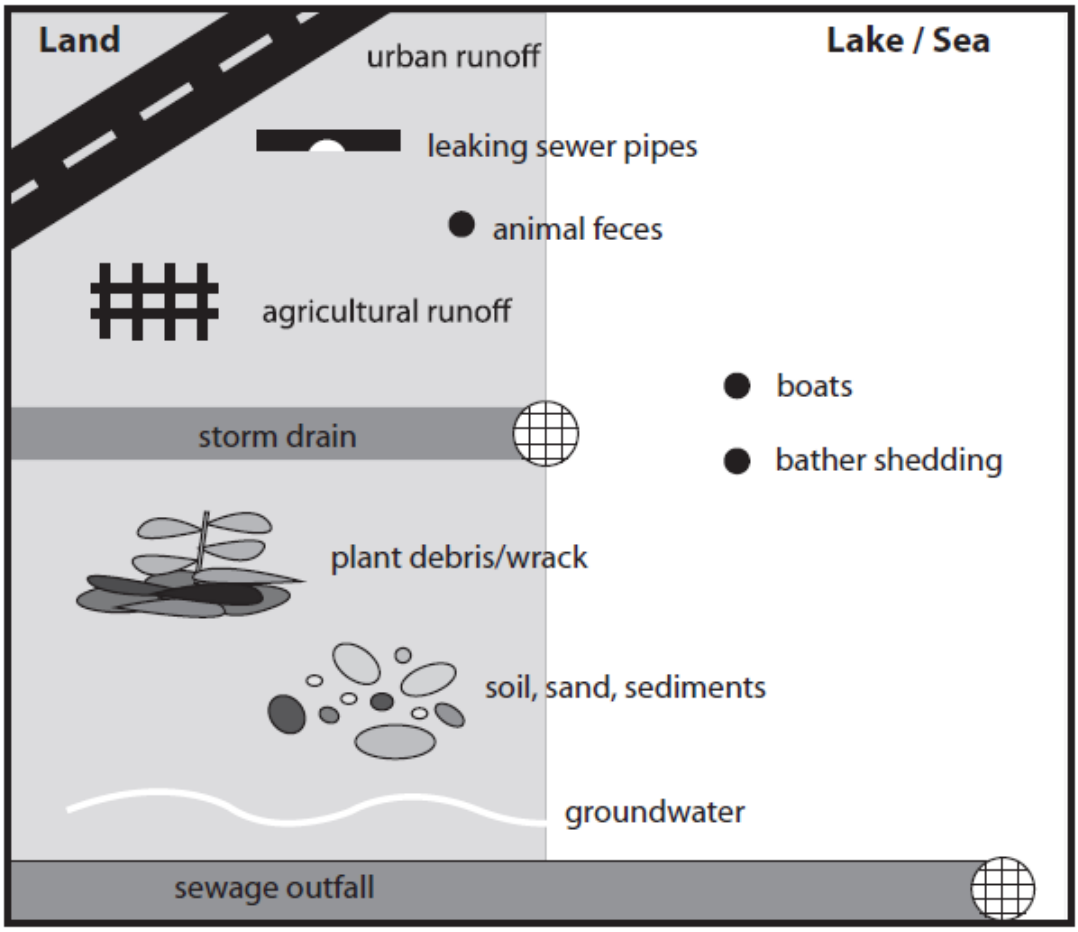

Figure 2. Sources of enterococci in recreational waters. See Table 1 for example concentrations associated with the sources.

Enterococci are also used as indicators of fecal contamination of recreational waters throughout the world. In the US, the fecal pollution standard for recreational bathing waters was originally set using concentrations of total coliforms, based on the results of a US Public Health Service study of swimmer health on Lake Michigan in Chicago, IL in 1948 (Stevenson, 1953). In recognition of the fact that related Gram-negative bacteria are naturally present in water, that standard was subsequently revised to a "fecal" coliform standard, which assumes that only a fraction of total coliforms were of fecal origin. In the late 1970s and early 1980s, swimmer health studies were carried out to aid in the identification of new fecal indicator organisms that may be more reliable than fecal coliforms (Cabelli, 1983; Dufour, 1984). Researchers determined that concentrations of enterococci concentrations measured in recreational marine waters polluted by treated wastewater were strongly correlated to the number of swimmers becoming sick with gastrointestinal illness (Cabelli, 1983). Similar results were obtained in other studies

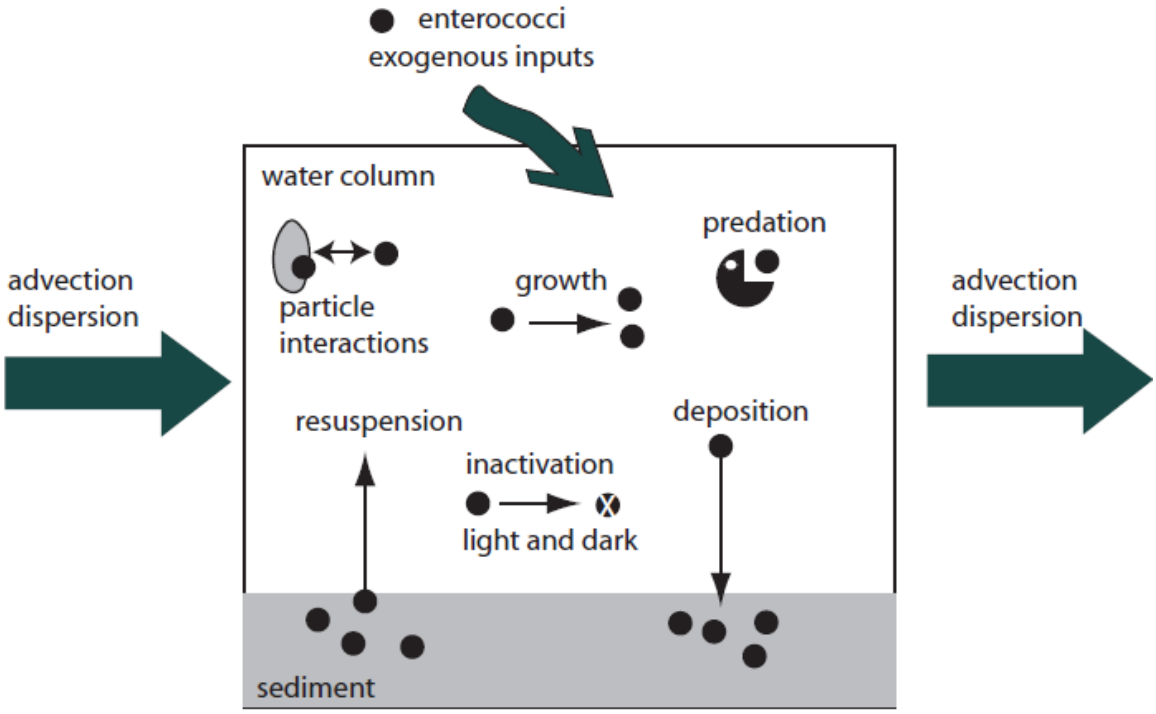

Figure 3. Processes that affect concentrations of enterococci in surface waters.

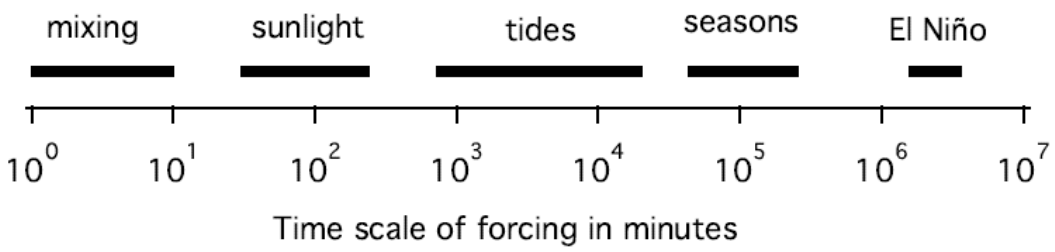

Figure 4. Time scales over which enterococci vary in marine waters, due to natural forcing mechanisms. Figure adapted from Boehm et al. (Boehm, et al., 2002).

around the world (Boehm & Soller, 2011). A meta-analysis of these results (Wade, Pai, Eisenberg, & Colford Jr., 2003) found evidence for a positive association between enterococcal concentrations and swimmer gastrointestinal illnesses (Figure 1). The associations were further confirmed in a suite of epidemiological studies carried out in the EU (Wiedenmann, Krüger, Dietz, López-Pila, Szewzyk, & Botzenhart, 2006) and the US

(Wade, et al., 2006) in the 2000s, which supported an association between enterococcal concentrations and swimmer health in recreational freshwaters, as well as marine waters. Given this evidence, the US, EU, and the World Health Organization (WHO) recommend that enterococci be adopted as an indicator of recreational water quality and risk of swimmer illness.

Recreational water quality standards now vary by country, but they generally relate bacterial counts to a geometric mean and a statistical threshold value (also referred to as a single-sample standard). The WHO compiled recreational water quality standards for countries around the globe in 1999 (World Health Organization, 1999). The policy for each country requires the use of a specific enterococcal enumeration method. In all cases, the method is culture-based, and involves the use of selective and differential media in solid or liquid form (Edge & Boehm, 2010). In the US, a new standard method for measuring enterococci in water has been developed by the USEPA, which uses quantitative polymerase chain reaction (QPCR) in conjunction with a hydrolysis probe (United States Environmental Protection Agency, 2010). However, inter- and intra-laboratory variation of this method, as well as the relationship between QPCR results and those of culture-based assays, are still being debated (Shanks, et al., 2012; Whitman, et al., 2010).

In the US, EPA marine recreational water quality criterion for enterococci in water is not more than 104 colony forming units (CFU) / 100 mL (single-sample standard) and 35 colony forming units / 100 mL (geometric mean standard) (United States Environmental Protection Agency, 2011). These values may soon be revised to extend to fresh waters and to include a standard for enterococcal detection by quantitative PCR. In the EU, bathing water standards for enterococci range from limits of 100-400 CFU/100 mL, depending on whether the beach is marine or fresh, and whether the beach is rated as excellent or sufficient (The Council of the European Union, 2006).

In epidemiological studies used to establish enterococcal standards, the etiologies of swimmer illness were not confirmed, but are not believed to have been directly caused by enterococci. Rather, it is thought that the main etiologies of recreational waterborne illness are viral. One study suggests norovirus-like agents as the main cause of disease (Soller, Bartrand, Ashbolt, Ravenscroft, & Wade, 2010). Thus, the correlative relationship between enterococci concentrations and health might suggest that enterococci in recreational waters are indicative of human viruses. There is a striking lack of data to support an association between enterococcal and virus concentrations, or the concentrations of pathogens in general, in recreational waters (Hellein, Battie, Tauchman, Lund, Oyarzabal, & Lepo, 2011; Jiang & Chu, 2004; Noble & Fuhrman, 2001; Pusch, et al., 2005; Viau, Lee, & Boehm, 2011). A few reports have found positive associations between enterococci and the bacterial enteric pathogens *Campylobacter* and *Salmonella* in surface waters (Viau, et al., 2011; Walters, Thebo, & Boehm, 2011), but the causative link between enterococci and swimmer illness remains unknown. As pathogen detection techniques advance to allow for the more sensitive and specific detection of human viruses, as well as

enteric bacterial and protozoan pathogens in water, the basis for associations between waterborne enterococci and pathogens may be discovered.

Sources of enterococci in recreational waters include sewage, agricultural and urban runoff, stormwater, direct input by animals via defecation, bather shedding, boats, plant debris (for example, wrack), polluted groundwater, soils, sediments, and sands (Figure 2). In developed countries, sewage is typically well-treated prior to discharge through an outfall that is usually located far from recreational waters. Direct inputs of untreated sewage, however, can impact recreational waters during storm events in regions that have combined sewer overflows and in regions with leaking sewer lines (Sercu, Van De Werfhorst, Murray, & Holden, 2009). Runoff, generated by storms, urban activities like car washing and irrigation, and agricultural activities can contain extremely high concentrations of enterococci, sometimes that surpass concentrations measured in raw sewage (Olivieri, Boehm, Sommers, Soller, Eisenberg, & Danielson, 2007; Reeves, Grant, Mrse, Copil Oancea, & Boehm, 2004). The source of enterococci in runoff can include soil, animal feces, exfiltrated raw sewage, and decaying plant material. Recent research indicates that decaying vegetation on both fresh and marine beaches can contain enterococci (Byappanahalli, Shively, Nevers, Sadowsky, & Whitman, 2003; Imamura, Thompson, Boehm, & Jay, 2011), while sediments and soils may also harbor enterococci (Byappanahalli & Fujioka, 2004; Mote, Turner, & Lipp, 2012). Additionally, beach sands have been shown to harbor enterococci which can grow (Zubrzycki & Spaulding, 1962) and be transported into the adjacent waters (Yamahara, Layton, Santoro, & Boehm, 2007). Table 1 shows example concentrations of enterococci in various sources mentioned here; however, it should be noted that concentrations in these sources can be quite variable in time and space.

When the source of enterococci to surface waters is not fecal, their presence may not indicate a health risk. Epidemiology studies have investigated the correlation between enterococci and swimmer illness in recreational waters not impacted by wastewater, and the results are equivocal (Boehm & Soller, 2011). For example, in Mission Bay, California (CA), a site where enterococci are believed to be from birds and runoff, swimmer illness did not correlate to enterococci (Colford Jr., et al., 2007). At Doheny Beach, CA, a site polluted with urban runoff, swimmer illness correlated to enterococci when runoff was discharging into the ocean, but the correlation did not persist when runoff was not discharging (Colford Jr., et al., 2012).

Surface waters throughout the world are plagued by high concentrations of fecal bacteria. In the US, 24% of surface water bodies are listed as impaired, due to elevated levels of fecal indicator bacteria, and a subset of these are impaired because of high concentrations of enterococci (United States Environmental Protection Agency, 2012). When a surface water is known to contain concentrations of enterococci that exceed regulatory standards, actions must be taken to reduce their concentrations. Microbial source tracking (MST) using animal host-specific gene markers in *Bacteroidales* has become an increasingly popular tool for identifying sources of enterococcal contamination in water. There are *Bacteroidales* assays that identify the presence of dog, horse, human, cow, and ruminant

feces (Dick, et al., 2005; Kildare, Leutenegger, McSwain, Bambic, Rajal, & Wuertz, 2007; Shanks, et al., 2008; Shanks, Kelty, Sivaganesan, Varma, & Haugland, 2009). Unfortunately, it may be difficult, if not impossible, to allocate enterococci sources using *Bacteroidales* genetic markers, due to the differential fate and transport of bacterial DNA and culturable enterococci in the environment (Jeanneau, et al., 2012; Walters, Yamahara, & Boehm, 2009). Ongoing work is assessing the feasibility of this approach and investigating the possibility of source tracking with enterococcal genetic markers.

Another approach to reducing enterococcal concentrations in water is to identify inputs based on an understanding of the fate and transport of enterococci. Figure 3 shows the various processes that control the concentrations of enterococci in surface waters. Upon entering a surface water, enterococci concentrations vary due to dispersion and advection, which are controlled by concentration gradients and fluid velocities, respectively. Enterococci concentrations are further influenced by sedimentation/deposition, resuspension, particle interactions, growth, predation, and light and dark inactivation due to environmental stresses, such as sunlight and oligotrophy, respectively.

Enterococci are subject to light and dark inactivation and potentially to growth in the environment. Of these processes, the photoinactivation of enterococci has perhaps been the most extensively studied. Sunlight can cause direct damage to nucleic acids or other cellular components, or catalyze the formation of reactive oxygen species, which can cause photooxidative damage to enterococci. Enterococcal sunlight decay rates range between 0.1 and 6 h^{-1} (Fisher, Iriarte, & Nelson, 2012; Marracini, Ferguson, & Boehm, 2012). Enterococci species that contain a yellow carotenoid pigment decay at slower rates when subjected to photostress, due to the ability of the carotenoid to quench reactive oxygen species within the cell (Marracini, Ferguson, & Boehm, 2012). Recent work (Sassoubre, Nelson, & Boehm, 2012) suggests that enterococci are inactivated primarily by endogenously-produced reactive oxygen species generated by cellular chromophores, when illuminated by sunlight in seawater that does not contain colored dissolved organic matter. Dark decay rates are reported between 0.005 and 0.03 h^{-1} (Boehm, Keymer, & Shellenbarger, 2005); dark decay may be caused by stress from exposure to variable salinities, non-ideal temperatures, or a lack of carbon or essential vitamins. While there is no evidence showing that enterococci can grow in ambient oligotrophic waters, experiments showed enterococci can grow in sands (Yamahara, Walters, & Boehm, 2009) as well as in water augmented with decaying kelp (Byappanahalli, Shively, Nevers, Sadowsky, & Whitman, 2003; Imamura, Thompson, Boehm, & Jay, 2011). Growth in oligotropic water has been observed for *E. coli* O157:H7, which is also an enteric bacterium (Vital, Hammes, & Egli, 2008), so this might be possible for enterococci as well. Finally, some researchers have reported that enterococci can enter a viable but non-culturable (VBNC) state in water from which they may be resuscitated (Heim, Del Mar Lleo, Bonato, Guzman, & Canepari, 2002; Lleò, Bonato, Benedetti, & Canepari, 2005). Most studies of enterococcal decay and growth have used culture-based assays for enterococci enumeration; these assays would not be able to detect the VBNC population. A more thorough understanding of the VBNC population would be useful to more fully

understand the extra-enteric lifestyle of enterococci (Lleò, Bonato, Benedetti, & Canepari, 2005).

Enterococcal predation can occur by bacterivorous protozoa (such as amoebas, forams, nanoflagellates, and ciliates) and various other zooplankton (hereafter collectively referred to as grazers). An additional possible biological removal mechanism for enterococci is through infection by lytic bacteriophages (Purnell, Ebdon, & Taylor, 2011); however, to our knowledge, this removal mechanism has not been thoroughly evaluated in natural waters. Two studies have documented grazing rates of enterococci in natural waters. Boehm et al. (Boehm, Keymer, & Shellenbarger, 2005) used a dilution method that is typically used in oceanography to measure grazing rates on phytoplankton, and measured a grazing rate of 0.02 h^{-1} for enterococci. Menon et al. (Menon, Billen, & Servais, 2003) reported enterococci mortality rates due to grazing of 0.01 to 0.03 h^{-1} by observing the disappearance of radioactivity from the enterococcal DNA labeled with tritiated thymidine.

A comparatively small number of studies have considered the interaction between particles and enterococci in natural waters. It has been assumed that the bacteria-particle interactions are in equilibrium, and an isotherm model has been applied. Liu et al. (Liu, et al., 2006) assumed that 10% of the total enterococci in Lake Michigan waters were associated with a particle. Jeng et al. (Jeng, England, & Bradford, 2005) assumed that 9% of enterococci were associated with particles in stormwater. Characklis et al. (Characklis, Dilts, Simmons 3rd, Likirdopulos, Krometis, & Sobsey, 2005) showed that 20%–55% of enterococci were associated with settlable particles in stormwater and background water samples. Mote et al. (Mote, Turner, & Lipp, 2012) found that enterococci in an estuary were associated with particles greater than 30 μm. More research is needed to understand if kinetic models of bacterial attachment to particles in surface waters are needed, and to understand the mechanisms by which the attachment of enterococci to particles occurs. A single study has characterized the surface properties of *E. faecalis* and found the bacterium is negatively charged at all pHs, even in the presence of ions (Schinner, Letzner, Liedtke, Castro, Eydelnant, & Tufenkji, 2010). A mechanistic understanding of how electrostatic, hydrophopic, and other surface-surface interactions control enterococci adhesion to particles would be useful.

The deposition of enterococci to sediments at the base of the water column can occur if planktonic enterococci settle, or if they are attached to larger particles that settle to the base of the water column. Settling velocity is a function of particle (in this case, bacterial) size, shape and density, and fluid density and viscosity. Schinner et al. (Schinner, Letzner, Liedtke, Castro, Eydelnant, & Tufenkji, 2010) determined that *E. faecalis* has an equivalent spherical diameter of about 0.8 μm, but assumptions about the exact shape and density of enterococci must be made in order to infer a settling rate. Liu et al. (Liu, et al., 2006) estimate that enterococci settle at a rate of 0.023 m/d in Lake Michigan when they are not associated with particles. Enterococcal association with particles of different sizes, shapes, or densities will affect the settling rate. Resuspension of enterococci that have previously been deposited in the sediments can occur when the sediment is disturbed and

experiences shear stresses greater than the critical shear stress. Readers are directed to Nevers and Boehm (Nevers & Boehm, 2010) for more discussion on these processes. Deposition and resuspension of *E. coli* have been studied in a stream using an antibiotic resistant strain not typically found in the environment (Jamieson R. , Joy, Lee, Kostaschuk, & Gordon, 2005; Jamieson R. C., Joy, Lee, Kostaschuk, & Gordon, 2005), but no similar study with enterococci has been carried out. Rather, mathematical formulations for deposition and resuspension of enterococci have primarily been used in models of enterococci in water (Sanders, Arega, & Sutula, 2005; Steets & Holden, 2003).

Long-term spatially and temporally intense studies of enterococci concentrations in marine waters indicates that they vary at predictable time scales, due to various fate and transport processes mentioned above (Figure 4). Rainfall, which is heavier in some areas during El Niño events, leads to higher concentrations of enterococci in ambient waters, due to inputs of stormwater (Boehm, et al., 2002). Enterococci concentrations also vary due to the tides—fortnightly and semi-diurnal signals in enterococci concentrations can be found that correspond to the spring-neap and ebb-flood tidal cycles (Boehm, et al., 2002; Yamahara, Layton, Santoro, & Boehm, 2007). The tides control transport of enterococci in marine waters through tidal currents, and tides also can modulate enterococci inputs (Boehm & Weisberg, 2005). For example, only during falling ebb tides will tidal lagoons that contain high concentrations of enterococci from bird feces discharge to coastal waters (Grant, et al., 2001). Sunlight suppresses enterococci concentrations near high noon, due to photoinactivation (Boehm, Yamahara, Love, Peterson, McNeill, & Nelson, 2009). Finally, enterococci concentrations vary at high frequencies in marine waters, due to mixing processes that generate patches and ligaments of enterococci in waters free of enterococci (Boehm A. B., 2007).

Knowledge of enterococci sources, fate, and transport can inform the creation of models that predict enterococcal concentrations in surface waters. Process-based models of enterococci in surface waters (Boehm, Keymer, & Shellenbarger, 2005; Cho, et al., 2010; Liu, et al., 2006) have been used to better understand sources of contamination and implement pollution control strategies through total daily maximum loads (TMDLs). Relatively simple statistical models of enterococci concentrations are used for beach management in some regions of the US and EU (Francy, 2009; Hou, Rabinovici, & Boehm, 2006; Stidson, Gray, & McPhail, 2012); the main goal of these models is to identify conditions when health risks are high and conditions are unsafe for swimmers. Predictors like rainfall, tide, time of day, and wave height are used as independent variables in statistical models to predict concentrations of enterococci. If models predict concentrations over a specific threshold, a beach warning is issued so that the public knows that swimming conditions may not be safe. The USEPA has recently developed software for creating statistical models of enterococci concentrations in surface waters, called Virtual Beach (Frick, Ge, & Zepp, 2008).

Table 1. Concentrations of enterococci measured in common sources to recreational waters.

Source	Concentration	Reference
Kelp wrack	10^1-10^4 CFU/ dry g	(Imamura, Thompson, Boehm, & Jay, 2011)
Sand	1-10^4 CFU/g	(Halliday & Gast, 2011; Yamahara, Layton, Santoro, & Boehm, 2007)
Bather shedding	10^6 CFU/person	(Elmir, et al., 2007)
Urban runoff	10^3 MPN/100 ml	(Reeves, Grant, Mrse, Copil Oancea, & Boehm, 2004)
Stormwater	0-10^6 MPN/100 ml	(Olivieri, Boehm, Sommers, Soller, Eisenberg, & Danielson, 2007)
Dog feces	10^4-10^8 CFU/g feces	(Wright, Solo-Gabriele, Elmir, & Fleming, 2009)
Bird feces	10^2-10^6 CFU/g	(Wright, Solo-Gabriele, Elmir, & Fleming, 2009)
Groundwater	10^2 MPN/100 ml	(Boehm, Shellenbarger, & Paytan, 2004)
Raw sewage	10^5 MPN/100 ml	(Ahmed, Stewart, Gardner, & Powell, 2008)
Agricultural runoff	10^3 MPN/100 ml	(Díaz, O'Geen, & Dahlgren, 2010; Reeves, Grant, Mrse, Copil Oancea, & Boehm, 2004)

Note that these are representative concentrations only, as they are quite variable in most sources, particularly in secondary sources, like runoff and sewage. CFU is colony forming units and MPN is the most probable number.

Enterococci as Indicators of Fecal Contamination on Hands

Contaminated hands are believed to be a vector for infectious diseases, particularly enteric and respiratory illnesses. For this reason, hand washing has been promoted as a way to save millions of lives (Curtis, 2003). Studies to document the prevalence of hand washing in hospitals, child care centers, and in developing countries where sanitation infrastructure is poor and hand washing facilities are rare, have sought to identify good, unbiased hand washing indicators. The presence of enterococci on hands has been investigated as such an indicator.

When used as an indicator of hand washing and hygiene, the source of enterococci is primarily believed to be fecal in origin. Enterococci can also be found in the mouth (Gold, Jordan, & van Houte, 1975), so oral secretions may also be a source. Soil represents an additional enterococcal source to hands. Limited work has been done to confirm the sources of enterococci found on hands.

Several studies have used both enterococci (or fecal streptococci) and *E. coli* (or fecal coliforms) as indicators of hand hygiene in developing and developed countries. These studies found that enterococci are superior indicators (Kaltenthaler, Elsworth, Schweiger,

Mara, & Braunholtz, 1995; Kaltenthaler & Pinfold, 1995; Pickering, Julian, Mamuya, Boehm, & Davis, 2011; Pinfold & Horan, 1996) because they strongly correlated to other hygiene indicators (such as good hygiene knowledge), or that their accumulation on hands could be traced to specific activities (like defecating) during structured observations. The superior performance of enterococci over coliforms has been attributed to the prolonged survival of enterococci on inoculated clean hands (Pinfold, 1990) and inanimate surfaces (Kramer, Schwebke, & Kampf, 2006).

Most studies of enterococci on hands have reported the presence or absence of the organism in hand rinse, fingertip rinse, or fingertip impression samples (Judah, Donachie, Cobb, Schmidt, Holland, & Curtis, 2010; Kaltenthaler, Elsworth, Schweiger, Mara, & Braunholtz, 1995; Kaltenthaler & Pinfold, 1995; Pinfold, 1990; Pinfold & Horan, 1996). Only a few studies have reported concentrations. Pickering et al. (Pickering, et al., 2010; Pickering, Julian, Mamuya, Boehm, & Davis, 2011) found between 1000 and 10000 CFU enterococci per 2 hands on women and children under 5 years old in peri-urban Dar es Salaam, Tanzania; every person tested had measurable enterococci on their hands. A survey in the United Kingdom (UK) detected enterococci on the hands of 28% of commuters who used public transit (Judah, Donachie, Cobb, Schmidt, Holland, & Curtis, 2010). While these results suggest a difference in enterococcal prevalence on the hands of individuals in regions with good (UK) and poor sanitation infrastructure (Tanzania), enterococcal enumeration was carried out using different sampling and cultivation methods, so care should be taken in comparing the two studies.

Measuring enterococci on hands may be useful for understanding post-collection stored water contamination in developing countries, as well as the spread of infectious disease in both developing and developed countries. Pickering et al. (Pickering, et al., 2010) reported a positive correlation between enterococci on hands and enterococci in stored drinking water in households in peri-urban Dar es Salaam, suggesting that post-collection contamination of stored waters in areas with low levels of sanitation could be facilitated by hands contaminated during defecation or other activities (Pickering, Julian, Mamuya, Boehm, & Davis, 2011). The authors also found a correlation between enterococci on hands and gastrointestinal and respiratory symptoms. A similar correlation was found between enterococci on hands of children in child care centers in California, US and respiratory illness (Julian, Pickering, Leckie, & Boehm, 2013).

There are no current regulations or standard methods for measuring enterococci on hands. Based on research conducted over the last 20 years, it appears that the presence and concentration of enterococci may be good indicators for hygiene and health. However, further work will need to be done to confirm this.

Future Research Needs

In water, enterococci are used as indicators of environmental contamination, because they are found in high concentrations in feces, and exposure to enterococci is linked to adverse health effects in swimmers. Recreational water quality standards are based on enterococci

concentrations, so understanding their sources, fate, and transport in the environment is central to assessing and maintaining good water quality. A method to allocate sources of enterococci found in a surface waters would be beneficial to the community of water quality managers. This could be in the form of a molecular microbial source tracking tool, analogous to the tools used to track sources of *Bacteroidales*, or perhaps it could be in the form of a process-based model that links concentrations to particular sources, given spatial-temporal variation in the enterococci concentration signal.

Researchers have just recently started to use enterococci on hands as indicators of hand hygiene. Investigation into sources of enterococci on hands, the time scale of their survival, and their potential to grow on skin will add to the understanding of the strengths and limitations of this hand hygiene indicator. Additional studies that link enterococci density on hands to hand hygiene practices (like hand washing) and health outcomes such as respiratory disease and gastrointestinal illness will further lend credence to their use as hygiene indicators.

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