Transport of enterococci and F+ coliphage through the saturated zone of the beach aquifer

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ABSTRACT

Coastal groundwater has been implicated as a source of microbial pollution to recreational beaches. However, there is little work investigating the transport of fecal microbes through beach aquifers where waters of variable salinity are present. In this study, the potential for fecal indicator organisms enterococci (ENT) and F+ coliphage to be transported through marine beach aquifers was investigated. Native sediment and groundwaters were collected from the fresh and saline sections of the subterranean estuary at three beaches along the California coast where coastal communities utilize septic systems for wastewater treatment. Groundwaters were seeded with sewage and removal of F+ coliphage and ENT by the sediments during saturated flow was tested in laboratory column experiments. Removal varied significantly between beach and organism. F+ coliphage was removed to a greater extent than ENT, and removal was greater in saline sediments and groundwater than fresh. At one of the three beaches, a field experiment was conducted to investigate the attenuation of F+ coliphage and ENT down gradient of a septic leach field. ENT were detected up to 24 m from the leach field. The column study and field observations together suggest ENT can be mobile within native aquifer sediments and groundwater under certain conditions.

Key words | coliphage, enterococci, groundwater, recreational water quality

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INTRODUCTION

At the land-sea interface, one potentially important pathway for non-point pollution from land to the sea is groundwater. Submarine groundwater discharge (SGD) is the flow of coastal groundwater, both meteoric water and recirculated seawater directly into the ocean (Burnett *et al.* 2006). It has the potential to transport various elements to the coastal ocean and thus plays an important role in biogeochemical cycling (Burnett *et al.* 2006; Kroeger *et al.* 2007; Bone *et al.* 2007). In some urban locations, it has been shown to be an important source of macronutrients, nitrogen and phosphorous, which can cause coastal algal blooms (Hwang *et al.* 2005; de Sieyes *et al.* 2008). It has also been indirectly linked to high concentrations of fecal indicator bacteria (FIB) at Huntington Beach, California (Boehm *et al.* 2004), and has been implicated as a potential source of FIB to other marine beaches (Boehm *et al.* 2003, 2009; Izbicki *et al.* 2012; Russell *et al.* 2013).

Epidemiology studies show an association between FIB concentrations and swimmer health risk at beaches (Boehm & Soller 2011). Local laws dictate allowable levels of FIB in coastal waters used for recreation (beaches) to prevent waterborne illness. Routine monitoring in the USA resulted in 20,120 beach advisories and closures in 2012 owing to elevated levels of microbial pollutants; this number is up from 6,200 in 1999 (Dorfman & Haren 2013). Sixty-three percent of the advisories and closures in 2012 were due to unknown sources of contamination (Dorfman & Haren 2013). At the same time, nearly one-third of the shellfish harvesting waters in the USA are classified as being 'fecal impaired' by the National Shellfish Registry (NOAA 1998).

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There is a clear need to identify the sources of and remediate microbial pollution in coastal waters. Although studies have implicated SGD as a source of fecal organism to coastal waters, there has been limited work to investigate the potential for fecal organisms to be transported through the beach aquifer. The present study evaluates the potential for SGD to be a source of FIB to the coastal ocean using laboratory column experiments and a field study.

Sources of FIB to the beach aquifer may be exogenous or endogenous. Exogenous sources to the beach aquifer include leaking sewer lines and septic tanks and their leach fields. Endogenous FIB sources include sands and beach wrack which are both known to contain elevated FIB at some beaches (Yamahara *et al.* 2007; Imamura *et al.* 2011), and can be mobilized by seawater and infiltrate to the beach aquifer (Russell *et al.* 2012). Although the transport of FIB through unsaturated beach sands via intermittent flow of seawater has been studied (Russell *et al.* 2012), little work has examined the transport of FIB through saturated sands of the beach aquifer (Boehm *et al.* 2004), thus it is not well understood how mobile FIB are within this portion of the aquifer. The goal of the present study is to examine the mobility of enterococci (ENT), Gram-positive FIB, as well as a fecal indicator virus, F+ coliphage, through saturated beach sands. This information will provide insight into the potential for SGD to be a source of FIB to coastal waters.

Bacterial and viral transport through saturated porous media has been well studied in simplified media like quartz sands (Redman *et al.* 2004; Torkzaban *et al.* 2008), as well as more complex soils (Schijven & Hassanizadeh 2000; Schinner *et al.* 2010). The removal of the organisms by media during saturated flow can be conceptualized as a two-step process where the organisms first come into close proximity to the grains via diffusion, interception, and gravity, and then may attach due to surface-surface interactions including electrostatic, Van der Waal, and hydrophobic interactions (Ginn *et al.* 2002). The transport of organism to grain surface is quantified as the collision frequency (η_0) while the attachment of the organism to the grain is quantified as the attachment efficiency (α) (Tufenkji & Elimelech 2004). Organisms may additionally be removed by physical straining (Bradford *et al.* 2002) or inactivation. Attachment of organisms to grain surfaces depends on solution chemistry, including ionic strength, pH, and concentration of organic matter (Yee *et al.* 2000; Kuznar & Elimelech 2007; Foppen *et al.* 2008); organism surface features such as macromolecules and/or flagella (Jucker *et al.* 1998; Rijnaarts *et al.* 1999; McClaine & Ford 2002); and composition of the grain surface, for example, the presence of metal oxides and organic carbon (Johnson & Logan 1996; Truesdail *et al.* 1998).

Most studies on organism transport through porous media are conducted with single strains of laboratory grown organisms. Unlike laboratory grown organisms, indigenous organisms can be acclimatized to environmental conditions. The inactivation rate (John & Rose 2005) and surface properties (Senoo *et al.* 1992) of indigenous organisms can be different from those grown in the laboratory. However, there have been limited studies on the transport of indigenous organisms in porous media (Harvey & Garabedian 1991).

In the present study, we characterize the transport of indigenous wastewater ENT and F+ coliphage suspended in raw beach aquifer groundwater through columns of native beach aquifer sediments from three beaches. We also conducted a field study of ENT and F+ coliphage concentrations down gradient of a septic leach field in a beach aquifer. A mechanistic understanding of the processes whereby organisms are removed from the groundwater by native beach sediments is not necessarily a goal of this study as this would require a reductionist approach and a more simplified system (Redman *et al.* 2004; Walker *et al.* 2004; Abudalo *et al.* 2005; Chen & Walker 2012). Rather, the aim is to assess whether health-relevant bacteria and viruses can be transported through the saturated portion of the beach aquifer at three diverse beaches and thus provide insight into whether SGD can potentially be a source of microbial pollution to coastal waters.

METHODS

Column experiments

Sediments and groundwaters were collected from three beaches: Stinson Beach (37.899 °N, 122.646 °W), Los Osos Beach (35.3168 °N, 120.836 °W), and Carpinteria Beach

(24.399 °N, 119.516 °W), all located along the California coast. The three beaches were sampled on 10 August, 23 August, and 13 September 2011, respectively. Five to six boreholes were dug using a 4′ hand auger across the width of the beach from the water's edge, past the high tide berms. At each boring location, 2,000 cm^3 of sediment were collected from the top 12" of the saturated zone using an ethanol-sterilized, air-dried stainless steel auger and placed in acid-washed high-density polyethylene (HDPE) bottles. Groundwater was then sampled using a low density polyethylene (LDPE) sampling tube fit with a screened tip using a peristaltic pump. The sampling tube was rinsed with deionized water before sampling each borehole. The tube was lowered into each borehole approximately 15 cm below the water line and purged for 10 min at 500 mL/min prior to sampling. Three liters of unfiltered groundwater for column experiments were collected into 3.8 L autoclaved polypropylene bottles and stored on ice. The salinity and pH of groundwater was measured in the field by pumping to a sensor (Hydrolab, Loveland, CO, USA) emplaced in a flow-through cell. Only sediment and groundwater samples from the most saline and fresh boreholes of the subterranean estuary were retained for the column experiments. All samples were stored on ice during transportation to the laboratory.

Saturated column tests were conducted as follows. Fresh and saline beach sediments from each of the three beaches were packed into separate 4 cm diameter, 10 cm length glass columns fit with polytetrafluoroethylene (PTFE) end caps to construct six columns total. All 1/8" (outer diameter) tubing and connectors used in the experiments were PTFE. Prior to each test, all equipment was acid washed in 10% hydrochloric acid, rinsed with deionized (DI) water, and air-dried. Sediments were homogenized and wet-packed into columns. Packed columns were flushed with three pore volumes of site groundwater (fresh or saline to match the sediments) prior to beginning each experiment. Flow through each column was opposed to gravity to ensure saturated flow conditions. Care was taken to avoid introducing air bubbles into packed columns. After flushing with site water, columns were dosed continuously at 0.35 mL/min for 18 h with influent solution delivered via peristaltic pump. Considering an estimated porosity of 0.4 for clean beach sands, this dosing rate equates to a linear velocity of 1 m day^{-1}. The influent solution was composed of a mixture of 10% 200 μm sieved primary treated wastewater from the Palo Alto, California wastewater treatment plant and 90% raw, site groundwater, either fresh or saline depending on the sediment being tested. The influent solution was constantly stirred on a stir plate, and was sampled four times over each 18-h experiment. Experiments were carried out at 21 °C. Fractions were collected from the column outlet at 20-min intervals and the influent was sampled approximately every 6 h during the experiment. ENT in the influent and effluent were quantified using membrane filtration and incubation on mEI agar following EPA method 1600 (United States Environmental Protection Agency 2006). Male-specific (F+) coliphage was quantified using the double agar layer method of EPA method 1602 (United States Environmental Protection Agency 2001). Following each experiment, a tracer test using 500 mg/L potassium bromide solution dissolved in the native groundwater used for the experiment was conducted in each saturated column under the same flow conditions used for the microbial tests. Bromide was measured in the influent and effluent using a bromide electrode (NexSens, Fairborn, OH, USA).

The pore volume of each column was determined by calculating the volume of water passing through the columns at which the concentration of bromide was 50% the influent concentration (Yeh *et al.* 2000). The grain size distribution of the sediments was determined using standard methods (ASTM C136, http://www.astm.org/Standards/C136.htm). Elemental iron (Fe) and manganese (Mn) content of the sand was determined by X-ray Fluorometry (Spectro Xepos HE XRF Spectometer). C_{org} was determined using loss-on-ignition at 450 °C for 4 h on duplicate or triplicate samples. The average log removal ($\log_{10} C/C_o$) observed in each of the six sediments (3 beaches × 2 sediments – fresh and saline) was determined as follows. We normalized the concentration of the column effluent (C) by the concentration of the column influent (C_o), averaging across all time points after breakthrough, and then $\log_{10}$ transforming the average (Mohanty *et al.* 2014). If the organism was not detected in the effluent, the lower limit of detection (~100 colony forming units (CFU) or plague forming units (PFU)/100 mL) was used in the calculation of average C/C_o. The collision frequency η_0 between organisms and grains and the attachment efficiency α of the organisms to

the grains in each column was estimated following the procedures outlined in Tufenkji & Elimelech (2004). As described by those authors, the procedure requires an assumption of steady-state conditions. Correlations between α and sediment and influent characteristics were investigated using Pearson's *r*.

Field study

The field study was conducted at Stinson Beach, California (37.899 °N, 122.646 °W), a residential beach community that uses septic systems exclusively for wastewater disposal. Experiments quantified contaminant flux adjacent to a large septic system servicing visitors and staff at Stinson Beach Park (Figure 1). In the system, effluent is piped from five septic tanks servicing various buildings into a large central 18.9 m^3 tank for temporary storage before being pumped to a 200 m^2 leach field near the beach. Float-triggered pumps deliver 3.8 m^3 pulses of septage to the leach field; the timing of each pumping event is logged.

Eight 2" polyvinyl chloride (PVC) water table wells and an array of 116 nested ¼" LDPE and ¾" PVC samplers were installed (Figure 1). The 2" wells were installed up gradient or within 200 m of the leach field, depending on the well, to monitor hydraulic head and background groundwater quality. Continuous head measurements were collected during November 2008, April 2009, and August 2009 using data logging pressure transducers (Solinst, Georgetown, ON, Canada) installed into a subset of the 2" well array (wells S12, S10, S08, S20, and S21; Figure 1). The nested samplers each consisted of three ¼"-diameter LDPE tube samplers, each with 1.5 m long screens made from ¼"-diameter slotted LDPE tubing covered in polypropylene mesh with 0.025-cm openings, nested around a fourth central ¾" rigid PVC sampler with a 1.5 m long screen with 0.025-cm slots. The four samplers in each cluster were staggered vertically at 1.5 m vertical intervals for depth-discrete sampling. Three transects of samplers, Transects A, B, and C, were installed approximately orthogonal to groundwater flow 5, 10, and 15 m down gradient from the leach field, as measured along the centerline of the transect.

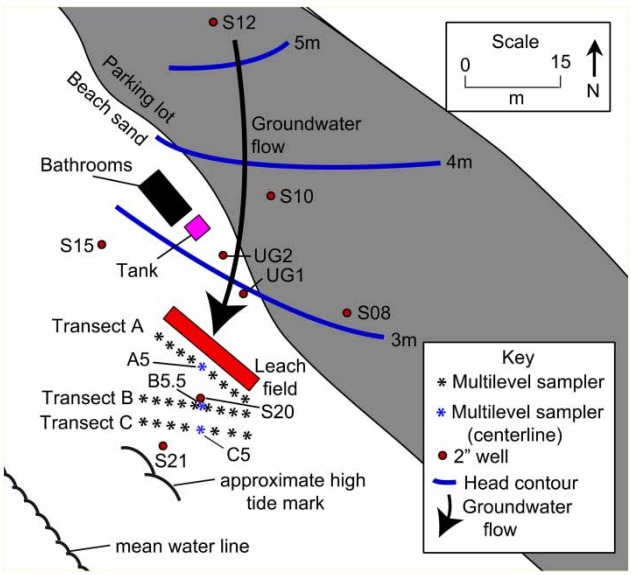

Figure 1 | Plan view of Stinson Beach field site including bathrooms, dirt parking lot (gray zone), tank (pink box), leach field, 2" wells, November 2008 head contours based on water levels measured in all 2" wells shown, groundwater flow direction, and multilevel well transects A, B, and C with each multilevel sampler location delineated by an asterisk. The full color version of this figure is available in the online version of the paper.

In Figure 1, the 'centerline' multilevel samplers delineate our best estimate of the plume centerline; additional monitoring was conducted at these locations. The ocean mean water line is shown at the bottom left corner of the figure. The centerline of Transects A, B, and C are 5, 10, and 15 m down gradient, respectively, from the center of the leach field, measured along the direction of groundwater flow. The high tide line and mean water line were approximately 20 and 32 m down gradient of the center of the leach field measured along the direction of groundwater flow.

Hydraulic gradients at the site were calculated from monthly average hydraulic heads in wells S12, S10, S08, S20, and S21 (Figure 1). Site-wide aerial contour maps of hydraulic heads were created from these monthly average hydraulic head datasets, and the distance from the water line to each well was measured along flow lines perpendicular to groundwater head contours. The curvature of the piezometric surface (shown in Figure 1 for November 2007) was relatively constant throughout the experiment, so the distances measured from the November 2007 contour map were used for subsequent hydraulic gradient calculations. Average head elevations, measured relative to mean sea level, were then plotted against distance from the water line for each well for each month, and linear regression was used to calculate site-wide hydraulic gradient (*i*) with estimates of error.

Hydraulic conductivities (K) were estimated using the tidal efficiency method, which is based on the concept of tidal wave propagation and dampening with distance in a coastal aquifer with a tidal boundary (Ferris 1963). We measured tidal efficiency at multiple wells during two periods of the study with little or no precipitation. The periods analyzed were 20 April 2008 to 18 June 2008 for wells S10, S12 and S15 and from 12 November 2008 to 10 January 2009 for wells S20 and S21. No open-ocean tide gauge exists at the site; as in previous research (de Sieyes *et al.* 2008, 2010), we assumed that tides were well represented by the tide gauge at Point Reyes, California (Sta. ID 9415020, 37 °59'48"N, 122 °58'30"W). For the purposes of this analysis, we assume a uniform beach aquifer thickness of 32 m (Stinson Beach County Water District 1998) and a specific yield of 0.25, a value typical of well-sorted, fine-grained sands (Fetter 2001). Head records were detrended and high pass filtered using a cutoff of 2.5 days to remove variations not associated with semidiurnal tides. Tidal efficiency was then calculated at distances from the approximate mean water line as estimated from survey data. A second set of tidal efficiency analyses was carried out as follows. Inland distances were measured from well S21, the most seaward monitoring well, and tidal efficiency was calculated at each of the other wells using the S21 head record as a tidal boundary. Results from the two methods agreed reasonably well, and were combined for use in further calculations.

Sampling of the septage collection tank was conducted approximately every 2 months between June 2008 and August 2009. The multilevel samplers in the monitoring network and the 2' monitoring wells up gradient from the leach field (UG1 and UG2) were sampled during three site-wide, day-long 'snapshot' sampling events on 22 November 2008, 5 April 2009, and 9 August 2009. New, dedicated LDPE sample tubes and 10% HCl acid-washed all-plastic syringes were used to purge three well volumes from and sample each well. Care was taken to sample slowly to avoid bubbling and cross-contamination from vertical flow in the multilevel samplers. When the tank and the multilevel samplers along the centerline of the septage plume (wells A5, B5.5, and C5, Figure 1) were sampled, measurements of temperature, salinity, dissolved oxygen (DO), and pH were also made using a peristaltic pump, a flow-through cell and sonde (Hydrolab, Loveland, CO, USA). For any given snapshot, many of the shallowest samplers were dry; thus the total number of samples collected during any event was never equal to the total number of samplers in the network ($n = 116$).

Two hundred milliliter samples of unfiltered water were collected into sterile centrifuge tubes and stored on ice for analysis of fecal indicator organisms. ENT were enumerated by membrane filtration using EPA method 1600 (United States Environmental Protection Agency 2006). F+ coliphage was enumerated using the single agar layer method following EPA method 1602 (United States Environmental Protection Agency 2001).

Microbiological fluxes associated with septage pumped to the leach field were calculated based on mean and standard deviation values of septage discharge rate (logged continuously) and of the log-transformed ENT and F+ coliphage concentrations in the collection tank, which were measured 14 times throughout the study. To propagate the collective variability of the two measures, a model iterating 1,000 times selected values of flow rate and log (concentration) from normal distributions about each mean value. The log transform of the concentration was transformed to linear space and multiplied by the flow rate to yield a flux for that iteration. Fluxes from all iterations were log-transformed and summarized as mean ± standard deviation.

Microbiological fluxes associated with groundwater flowing through control planes made up of multilevel well transects installed orthogonal to flow downgradient from the septic system were calculated via the Theissen polygon transect approach (Einarson & Mackay 2001) using the following equation:

$$w_{cp} = i_{cp} \sum_{n=1}^{n_{well}} \sum_{m=1}^{m_{ver}} (A_{n,m} K_{n,m} C_{n,m}) \quad (1)$$

where w is total flux (micro-organisms time^{-1}), K is hydraulic conductivity (length time^{-1}), A is the area of sampled interval (length^2) for each zone of aquifer monitored by a given monitoring well for which a contaminant concentration C (micro-organisms volume^{-1}) is representative, i is hydraulic gradient (-), subscript cp denotes the entire control plane, and subscripts n and m delineate the horizontal and

vertical position of each well in that control plane, respectively. In short, total contaminant flux in each component section of a transect is calculated as the product of the concentration C flowing through the component section, the section area A, and a Darcy velocity K^*i, where K is hydraulic conductivity and i is hydraulic gradient orthogonal to the section. The method assumes that well transects bisect the entire plume such that all plume mass flows through each transect. A Monte Carlo model was used to calculate contaminant flux through each transect in each of 10,000 iterations for each of three monitoring snapshots. This method was used to account for the uncertainty in the hydraulic gradient (i) and the hydraulic conductivity (K). For each iteration, random normally distributed values of i and $\log_{10}$-transformed K were chosen based on monthly binned i values for the month of the snapshot being analyzed and all K estimates. Each iteration also incorporated direct surveyed measurements of A and field measurements of C collected during the snapshot. For the purposes of flux calculations, concentrations below the detection limit were assigned a value of zero. The main assumptions in this approach are that the system is in steady state with respect to constituent input, microbial and physico-chemical removal mechanisms, and advection-dispersion within the aquifer, and that the monitoring network captures the entire plume emanating from a source of constant, steady input. Net transformation rates for microbial constituents were quantified using a linear-least squares approach (Borden *et al.* 1997).

RESULTS

Column experiments

Over the duration of the column experiments (~18 h), there was no change in ENT or F+ coliphage concentrations in the influent solution (data not shown) suggesting no inactivation of the organisms in the aqueous phase. ENT concentrations in the influent solution was $\sim 10^5$ CFU/100 mL and F+ coliphage concentrations were $\sim 10^4$ PFU/100 mL (Table 1). The detection limit (dl) of the organisms in the effluent fractions was ~100 CFU or PFU/100 mL which allowed us to observe at most 2–3 log reductions

Table 1 | Characteristics of influent solutions during the column experiments including the initial concentration (C_o) of ENT and F+ coliphage (F+) in units of CFU and PFU per 100 mL, respectively, native raw site GW pH and salinity (Sal, unitless per UNESCO guidelines), and calculated influent salinity (based on the 90/10% v/v mixture of native GW and sewage)

Experimental site	C_o ENT	C_o F+	GW pH	GW sal	Calculated influent sal
Stinson Fresh	1.3×10^5	3.4×10^4	6.67	0.46	0.41
Stinson Saline	5.0×10^5	3.5×10^4	7.66	32.20	28.98
Los Oso Fresh	1.4×10^5	2.8×10^4	6.80	2.89	2.60
Los Oso Saline	2.4×10^5	1.2×10^4	7.06	31.70	28.53
Carpinteria Fresh	1.2×10^5	3.9×10^4	7.18	1.19	1.07
Carpinteria Saline	1.5×10^5	2.0×10^4	7.49	32.76	29.48

after transport through the column (Figure 2). Key attributes of the grain size distributions, porosities, percent fines, and

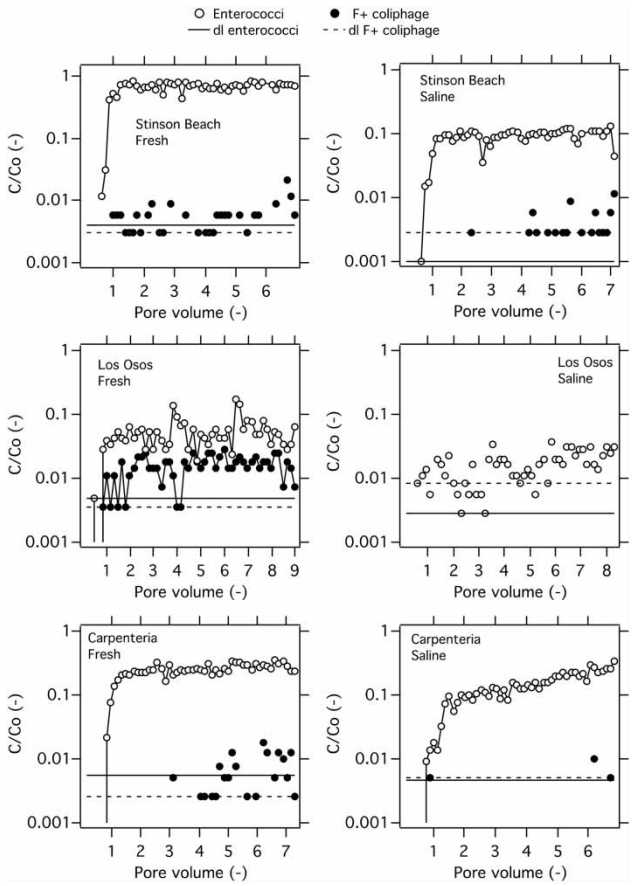

Figure 2 | Breakthrough curves of ENT (solid circles) and F+ coliphage (open circles) for the six tested sediment columns. A line connects the data points if most are above the dl. The dl for *C/Co* (controlled by the lower limit of detection for *C*) is shown in each panel (solid line for ENT dl, dashed line for F+ coliphage dl).

Fe, Mn, and organic carbon (C_{org}) content of the sediments are provided in Table 2. Sediments were well graded silica sands with d_{50} between 0.27 and 0.34 mm. Fe content varied from 8 to 40 mg/g while Mn varied from 95 to 950 μg/g. Organic carbon was low and varied between 0.27 and 1.43% within a range previously reported for beach sands in this region (Yamahara *et al.* 2007). The groundwater (Table 1) and sewage chemistry (pH = 6.75, salinity = 0.1) were measured separately and the calculated salinities of influent solutions are provided (Table 1); they varied from 0.4 to 29.5.

ENT and F+ coliphage were removed to different extents in the sediment columns from the three different beaches (Figure 2, Table 3). ENT removal was greatest at Los Osos Beach (removal of nearly 2 logs in saline and 1.3 logs in fresh), and lowest at Stinson Beach (removal of 0.2 logs in fresh and 1 log in saline). F+ removal was near our limit of removal detection in most of the sediments (~2 log reductions). In some of the columns, F+ coliphage breakthrough occurs more toward the end of the experiment which suggests a longer duration experiment could provide more information on breakthrough.

Comparing microbes in the same sediment columns, removal of F+ coliphage was greater than ENT in each column (greater by, on average, 1.2 logs, $p < 0.05$ paired *t*-test). Comparing saline to fresh columns at the same beach, removal was greater in the saline relative to the fresh water columns, although the differences were significant at the alpha = 0.1 and 0.2 levels only (greater by, on average, 0.5 log for ENT, $p = 0.1$, and 0.1 log for F+ coliphage, $p = 0.2$, paired *t*-tests).

The attachment efficiency, α, of ENT and F+ coliphage was calculated using the methods outlined by Tufenkji & Elimelech (2004) and thus steady state was assumed. α varied from 0.026 to 0.19 for ENT and 0.014 to 0.035 for F+ coliphage depending on the sediment (Table 3). Because many of the F+ coliphage measurements in the column effluent were at the limit of detection (Figure 2), F+ coliphage α may be underestimates. According to the correlation equations of Tufenjki & Elimelech (2004), diffusional transport is the most important mechanism via which organisms and grains come into cross proximity (data not shown) with coliphage coming into contact with grains more frequently than the bacteria (collision frequency η_o is ~0.1 for F+

Table 2 | Sediment characteristics including coefficient of uniformity (C_u), coefficient of curvature (C_c), grain size diameter of the 50% tile grain (d_{50}), pore volume of the columns, porosity, % fines, and Fe, Mn, and C_{org} content

Experimental site	C_u [-]	C_c [-]	d_{50} (mm)	Porosity	% fines	Fe (mg/g)	Mn (μg/g)	C_{org} (%)
Stinson Fresh	2.00	1.04	0.34	0.41	0.08	7.86	101.2	0.61
Stinson Saline	2.27	0.95	0.31	0.40	0.00	13.57	95.8	1.43
Los Oso Fresh	1.94	0.98	0.28	0.31	0.25	29.85	473.9	0.78
Los Oso Saline	1.82	1.00	0.29	0.33	0.08	39.43	949.4	0.83
Carpinteria Fresh	1.88	1.01	0.27	0.38	0.04	24.37	823.5	0.27
Carpinteria Saline	1.88	0.97	0.29	0.40	0.01	14.84	311.4	0.57

Table 3 | Results from column experiments including average C/C_o of ENT and F + coliphage (F+) during breakthrough (see text for calculation details), $\log_{10}$ removal, α of ENT and F + , and deposition rate (k_d) (Tufenkji & Elimelech 2004) of ENT and F +

Experimental site	C/C_o ENT	C/C_o F +	Log removal ENT	Log removal F +	α ENT	α F +	k_d (1/s) ENT	k_d (1/s) F +
Stinson Fresh	6.9×10^{-1}	5.9×10^{-3}	0.2	2.2	0.026	0.029	4.14×10^{-5}	5.73×10^{-4}
Stinson Saline	9.5×10^{-2}	4.2×10^{-3}	1.0	2.4	0.194	0.035	2.69×10^{-4}	6.25×10^{-4}
Los Oso Fresh	5.4×10^{-2}	1.5×10^{-2}	1.3	1.8	0.121	0.014	4.30×10^{-4}	6.19×10^{-4}
Los Oso Saline	1.6×10^{-2}	8.3×10^{-3}	1.8	2.1	0.194	0.017	2.69×10^{-4}	6.63×10^{-4}
Carpinteria Fresh	2.6×10^{-1}	6.9×10^{-3}	0.6	2.2	0.069	0.020	1.62×10^{-4}	5.98×10^{-4}
Carpinteria Saline	1.6×10^{-1}	6.7×10^{-3}	0.8	2.2	0.110	0.024	2.09×10^{-4}	5.72×10^{-4}

coliphage and ~0.01 for ENT). α can be translated into an organismal deposition rate, k_d, in each of the columns (Tufenkji & Elimelech 2004). k_d ranged from 4×10^{-5} to 4×10^{-4} s^{-1} for ENT and was consistently 6×10^{-4} s^{-1} for F+ coliphage (Table 3).

The correlation between α and sediment characteristics (d_{50}, porosity, percent fines, Fe, Mn, C_{org}) and aqueous chemistry (groundwater pH, groundwater salinity, calculated influent salinity) was investigated. F+ coliphage α was positively correlated with porosity ($r_p = 0.84$, $p < 0.05$) suggesting that removal was greater in sediments with higher porosity. F+ coliphage α was also negatively correlated with Fe density ($r_p = -0.81$, $p < 0.05$) so sands with high Fe had lower attachment efficiency; F+ coliphage α was also negatively correlated with Mn density at the alpha = 0.1 level ($r_p = -0.76$, $p = 0.08$). Porosity was negatively correlated to Fe ($r_p = -0.89$, $p < 0.05$), and Mn and Fe were correlated ($r_p = 0.88$, $p < 0.05$), so the reported correlations between these parameters and F+ coliphage α are not independent. ENT α was positively correlated with GW salinity and influent salinity at the alpha = 0.1 level (both $r_p = 0.78$, $p = 0.07$) indicating increased removal with higher salinity groundwater. ENT α was correlated positively with sediment C_{org} content at the alpha = 0.1 level ($r_p = 0.74$, $p = 0.095$), but F+ coliphage α was not. C_{org} was not correlated to other grain or aqueous chemical parameters. No other correlations were significant.

Field experiment

Sediment conductivities K based on tidal efficiency ranged from 2 to 36 m day^{-1} with a geometric mean of 5 m day^{-1}, all of which fall in the typical values for sands. Average ± standard deviation seaward hydraulic gradients at the field site in November 2008, April 2009, and August 2009 were 0.0322 ±0.0018, 0.0314 ±0.0010, and 0.0287 ±0.0001. Considering a mean annual hydraulic gradient i of 0.0310, hydraulic conductivity K of 5 m day^{-1}, and effective porosity estimated at 0.25 for clean (clay free) beach sands (Fetter 2001), the estimated annual average groundwater velocity is 0.625 m day^{-1}. This value agrees well with the 1 m day^{-1} linear velocity used in the column experiments.

Septage discharged to the leach field was low in DO (<1 mg L^{-1}), neutral in pH (7.1), fresh in salinity (0.3), and had a mean temperature of 19.5 °C based on 14 measurements from the wet-well between April 2008 and August 2009 (data not shown). Geometric mean ENT ($n = 18$) concentrations in septage were 4.1×10^3 (range: 8.6×10^2–1.5×10^5) CFU/100 mL. Geometric mean F+ coliphage ($n = 12$) concentrations in septage were 34 (range: 20–95) PFU/100 mL. The analysis of both F+ coliphage and ENT was not possible for six samples, hence the discrepancy in total sample analyses.

Average septage discharge rates to the leach field between June 2008 and August 2009 were 4.9×10^3 $\pm 1.9 \times 10^3$ L /day. Average microbial fluxes to the leach field during the same period were 6.2 ±0.3 log PFU/day F+ coliphage and 8.3 ±0.6 log CFU/day ENT.

Groundwater temperature, salinity, DO, and pH along the plume centerline during each of the three spatial snapshots were found to vary between 16 °C and 20 °C, 0.4–0.6 mg/L, 0.3–0.8 mg/L, and 6.36–7.16, respectively.

During each snapshot, all samples from Transect A (Figure 1), the closest transect to the leach field, were analyzed for F+ coliphage while all samples from the sampler network were analyzed for ENT. In November 2008, Transect A samples were negative for coliphage, and ENT were not detected in the well network except in the two multilevel samplers up gradient from the leach field, UG1 and UG2. Here, the maximum ENT concentration was 28 CFU/100 mL, respectively. These ENT could be from a variety of sources including other leach fields in the area. During the April 2009 snapshot, samples from Transect A for F+ coliphage data were discarded due to a faulty negative control. Two samples from Transect A contained 4 CFU/100 mL ENT, but no other samples in the network were positive. In August 2009, all Transect A samples were negative for F+ coliphage. However, 15 of 87 samples (some of the 116 wells were dry during this snap shot, hence only 87 samples) within the well network were positive for ENT with concentrations ranging from 2 to 74 CFU/100 mL. ENT were detected in each transect, and the geometric mean of positive samples was 8 CFU/100 mL. No ENT or coliphage were detected in the two wells (UG1 and UG2) up gradient from the leach field during this snap shot.

Fluxes of ENT at the three downstream multi-level well transects are plotted in Figure 3. Fluxes of F+ coliphage were not calculated due to lack of detection. ENT were

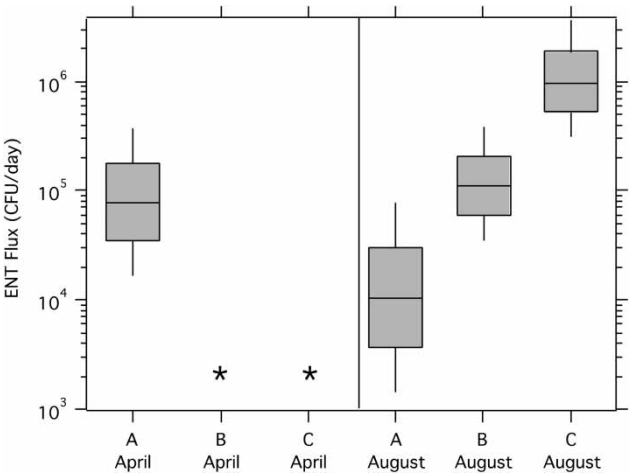

Figure 3 | Fluxes of ENT associated with groundwater discharging through each transect during the April 2009 and August 2009 snapshots when ENT was detected. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the 10th and 90th percentiles. Fluxes were not calculated when no samples in a given transect during a snapshot were above the dl; an asterisk (*) has been substituted at these locations and times. Transects B and C are 5 and 10 m down gradient from Transect A measured along the estimated plume centerline.

detected in Transect A in April 2009 and in all transects in August 2009, allowing for the calculation of fluxes at these locations and times only. In April 2009, flux (log-mean and standard deviation of log-transformed fluxes) at Transect A was 1.9 ±0.5 log CFU/day. In August 2009, fluxes at Transects A, B, and C were 4.0 ±0.7, 5.0 ±0.4, and 6.0 ±0.4 log CFU/day, respectively. The maximum ENT flux in the sampler network occurred in August 2009 at Transect C, the farthest down gradient transect; an increase in ENT flux with distance was observed during this snapshot. The calculation of these fluxes requires the assumption of steady-state conditions. Given the available data collected during the study, we believe this assumption is reasonable. However, continuous monitoring in the network of wells was not feasible so there is some uncertainty in this assumption.

DISCUSSION

This study investigated the transport of ENT and F+ coliphage through beach sands from the subterranean estuary of three beaches. Column experiments documented removal capacity of the sands. A field study at one beach documented the attenuation of ENT and F+ coliphage from a septage leach field, down gradient within the freshwater portion of the beach aquifer. We generally found that removal capacity of the beach sands was high for F+ coliphage, but lower for ENT.

In column experiments, F+ coliphage was removed approximately 2 logs (the most we could detect) by most sands over the length of the column (10 cm). However, we still detected F+ coliphage near our limit of detection in some of our effluent in five of the six sediments. Thus under most of the conditions we tested, F+ coliphage was not 100% removed. The mechanism of removal from the aqueous phase was due to interaction with sediment as there was no inactivation in the aqueous phase over the course of the study, consistent with conclusions from other studies of coliphage filtration (Schijven & Hassanizadeh 2000). We estimate attachment efficiencies between 0.014 and 0.035 for F+ coliphage in the sands, within the range of values reported by others for F+ coliphage (Schijven & Hassanizadeh 2000; Hijnen *et al.* 2005). These values are a function of sediment and aqueous phase chemical and physical characteristics (Elimelech & Omelia 1990) which affect electrostatic and hydrophobic forces between viruses and grains. In the present study, we included multiple beaches and groundwater chemistries characteristic of beach aquifers in this region. Thus, this range of α values could be used to estimate regional F+ coliphage beach-relevant deposition rates (Tufenkji & Elimelech 2004) in a future modeling application. Within our sands, F+ coliphage attachment efficiency was positively correlated to porosity, and negatively correlated with Mn and Fe content, but because the three sediment characteristics were themselves correlated, the results are difficult to interpret. Metals such as Fe and Mn are likely in the form of metal oxides on the sands. Iron oxides are typically positively charged and have different affinities for viruses (Abudalo *et al.* 2005) whereas Mn-oxides are usually negatively charged and do not typically sorb viruses (which tend to be negatively charged at near neutral pH) (Harvey & Ryan 2004). One might expect that F+ coliphage attachment efficiency would be higher in sands with greater Fe. However, without knowing more about the Fe-containing minerals in the sands, and whether positively charged attachment sites were already occupied by other organisms

or coated with natural organic matter (Pieper *et al.* 1997), it is not possible to explain mechanistically why attachment would be reduced in sands with higher Fe content. It should be noted that we used F+ coliphage indigenous to wastewater in our study, so numerous different types of coliphage were enumerated in the effluent. Thus it is not possible to describe more thoroughly their surface characteristics.

ENT were removed to a lesser extent that F+ coliphage in the column studies, but had higher attachment efficiencies than the coliphage (between 0.026 and 0.19). Although this may seem counterintuitive, it can be explained by the lower collision frequency of ENT relative to the coliphage with the grain surfaces; removal is the product of collision frequency and attachment efficiency (Tufenkji & Elimelech 2004). Attachment efficiency was higher at higher salinities consistent with the understanding of the effect of ionic strength on electrostatic interactions between like-charged surfaces (Franchi & Omelia 2003). Bacteria are negatively charged at near neutral pH and a study of the zeta potential and charge of *Enterococcus faecalis* indicates it is negatively charged under environmentally relevant conditions (Schinner *et al.* 2010; Chen & Walker 2012). Quartz sands are also negatively charged at environmentally relevant pHs. None of the six columns showed complete removal of ENT suggesting that under field-relevant conditions, ENT can remain mobile in the aqueous phase over the scale of our columns. In particular, we saw limited removal of ENT in the freshwater sediment at one of the sites (Stinson Beach); effluent concentrations in this column were nearly the same as those in the influent. The reason for this lack of removal is presently unknown.

Two previous studies examined the transport of *Enterococcus faecalis* through porous media columns and both report larger α than those reported herein (Schinner *et al.* 2010; Chen & Walker 2012). Both studies examined transport of *Ent. faecalis* through idealized columns of quartz sand suspended in simplified influent fluid whereas our study examined transport of indigenous wastewater ENT suspended in real groundwater through unaltered sediments. Our system contains metals and organic matter, as well as other bacteria and biofilms that could dramatically change surface-surface interactions relative to those in the previously studied simplified systems and compete for attachment sites (Pieper *et al.* 1997). Schninner *et al.* (2010) also examined transport of *Ent. faecalis* through unaltered agricultural soil and found an α greater than the ones reported herein presumably due to the agricultural soil having very different characteristics to our sands. Therefore, it is not surprising that results differ between our study and those previously done with ENT.

The field study at Stinson Beach was consistent with the results from the column experiments in two ways. First, F+ coliphage, although present in the septage discharged to the leach field, was never detected down gradient of the leach field suggesting efficient removal within the leach field or within the beach aquifer between the leach field and the first transect. However, the initial concentrations in the septage were quite low (~10 PFU/100 mL) and our field methods assayed 40 mL of groundwater for the phage which corresponded to a dl of ~1 PFU/100 mL. Clearly, this limited our abilities to observe changes in concentration in the field. Previous work in sandy soils in Florida showed several log reductions of coliphage PRD1 in unsaturated sands beneath a septic leach field (Nicosia *et al.* 2001), suggesting that F+ coliphage could have been completely removed in the unsaturated sands under the Stinson leach field. It would be useful to test the removal of viruses in a beach aquifer field system where their concentrations are initially higher to confirm removal capacity of the beach aquifer.

Second, in the field we observed ENT within the saturated portion of the beach aquifer down gradient of the leach field. ENT were detected within the well network during two of the three sampling events, albeit at concentrations ~2 orders of magnitude lower, on average, than those in the septage collection tank. This region of the beach aquifer contained primarily fresh groundwater and during the column studies with aquifer material from this region, we saw minimal ENT removal. This could suggest most of the ENT removal in the septage occurred within the leach field. We observed a net increase in ENT flux through the well network in August 2009, which may suggest exogenous inputs of ENT to the well network or enterococcal growth along the flowpath. Assuming the downgradient increase is due to ENT growth, the data are consistent with a first-order growth rate of 0.28 day^{-1}. This rate is in good agreement with ENT growth rates observed in intermittently wetted beach sands from Monterey,

California (0.20–0.63 day^{-1}) (Yamahara *et al.* 2009). The increase could also be due to unsteady inputs of ENT from the leach field.

Previous field studies have explored the subsurface migration of introduced microbial tracers in sandy aquifers, although most of these have been conducted inland (Harvey 1997; Harvey & Harms 2003). Bales *et al.* (1995) examined the transport of bacteriophage PRD-1 and stained, indigenous bacteria in the saturated zone of a sandy aquifer in Cape Cod. They found nearly 7 logs of removal of the injected virus and only 2 logs of bacterial removal in various downgradient wells, consistent with our observations that viruses were removed more so than bacteria in native aquifer materials. Harvey & Garabedlan (1991) calculated the attachment efficiency of indigenous aquifer bacteria using field data from the same sandy aquifer in Cape Cod and found α was between 0.3×10^{-3} and 9.7×10^{-3}, smaller than values reported herein.

CONCLUSION

Although sand filters have long been used as a unit process for water and wastewater treatment (Tchobanoglous *et al.* 2003), our work illustrates that within the beach aquifer, sand does not always remove microbial contaminants completely. Evidence from this study indicates that ENT may migrate through the beach aquifer and may even grow there while F+ coliphage may be more readily removed by aquifer materials. We derived a range of beach aquifer-relevant attachment efficiencies, which may be used to derive deposition rates in future studies of microbial migration through both the saline and freshwater portions of the beach aquifer. However, caution should always be taken in extrapolation attachment efficiencies determined at the bench scale to the field scale as scale dependent heterogeneity, ripening, and microbe surface heterogeneities could render attachment efficiencies scale dependent (Schijven & Hassanizadeh 2000; Ginn *et al.* 2002).

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