

Contribution of Sand-Associated Enterococci to Dry Weather Water Quality

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S Supporting Information

ABSTRACT: Culturable enterococci and a suite of environmental variables were collected during a predominantly dry summer at a beach impacted by nonpoint source pollution. These data were used to evaluate sands as a source of enterococci to nearshore waters, and to assess the relationship between environmental factors and dry-weather enterococci abundance. Best-fit multiple linear regressions used environmental variables to explain more than half of the observed variation in enterococci in water and dry sands. Notably, during dry weather the abundance of enterococci in dry sands at the mean high-tide line was significantly positively related to sand moisture content (ranging from <1–4%), and the daily mean ENT in water could be predicted by a linear regression with turbidity alone. Temperature was also positively correlated with ENT abundance in this study, which may indicate an important role of seasonal warming in temperate regions. Inundation by spring tides was the primary rewetting mechanism that sustained culturable enterococci populations in high-tide sands. Tidal forcing modulated the abundance of enterococci in the water, as both turbidity and enterococci were elevated during ebb and flood tides. The probability of samples violating the single-sample maximum was significantly greater when collected during periods with increased tidal range: spring ebb and flood tides. Tidal forcing also affected groundwater mixing zones, mobilizing enterococci from sand to water. These data show that routine monitoring programs using discrete enterococci measurements may be biased by tides and other environmental factors, providing a flawed basis for beach closure decisions.



INTRODUCTION

The indicator bacteria *Enterococcus* (ENT) are used as a proxy for fecal contamination in marine recreational waters, and is routinely monitored at beaches to prevent bather contact with contaminated waters in compliance with U.S. federal guidelines.¹ However, contrary to the assumption that ENT isolated from the environment must be associated with the presence of fecal matter, observations from many beaches have documented that ENT can persist in the environment and often can be found at high densities in beach sands.² Understanding how the abundance of ENT in sands may be impacted by natural variation in environmental conditions, and whether ENT in sand covary with ENT in water, is important. Beach sands may be a source of ENT to bathing waters, so quantifying the abundance and characterizing the environmental conditions that promote or reduce the exchange of bacteria between sand and water will help guide management of the public health risk.

Effects of environmental conditions on the survival of ENT in seawater have been experimentally observed^{3–5} as well as modeled based on field observations.⁶ Collectively, the results show that higher temperatures, greater salinities and increased UV enhance ENT inactivation in water. Additional studies suggest ENT is able to persist longer in environmental waters if they are particle-associated.^{7–9} ENT die-off rates have been shown to be reduced in sand compared to water,¹⁰ and rates

can differ in sand and water between strains of ENT from different sources.¹¹ Growth of ENT has also been observed in sterilized and natural sands subjected to varied experimental conditions, including rewetting.^{12,13} Predation and competition with the indigenous microbial communities can also significantly affect ENT survival and adds an additional layer of complexity to questions of ENT persistence and human health risk.^{14–16}

The majority of water quality violations stem from unknown sources of enterococci bacteria.¹⁷ Observations of elevated concentrations of bacteria in upper intertidal beach sands motivated our hypothesis that sands may be a source of bacteria to waters during high tides, when waters come into contact with this relatively enriched reservoir of bacteria. This hypothesis is supported by water quality data collected at different beaches, including observations of ENT concentrations that were significantly greater during high-tides.^{18,19} A meta-analysis of California water quality data that showed water samples collected during higher spring tides had significantly greater concentrations of ENT and a higher probability of

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exceeding the single sample maximum of 104 colony forming units (CFU)/100 mL.²⁰

Our study investigated spatial and temporal variation of ENT in water, intertidal sand, and high-tide (dry) sand during a three-month period of predominantly dry weather in 2010. The study site is a 500 m stretch of Commercial Street beach adjacent to Macmillan Pier in Provincetown Harbor, Massachusetts (Supporting Information (SI) Figure 1). This beach has a history of both wet and dry weather water quality violations stemming from nonpoint source pollution, which likely includes runoff from nearby impervious surfaces and storm drains, fecal matter from birds (gulls and pigeons at the beach, and cormorants on the breakwater), dogs on the beach and swimming in the water, boats that discharge waste illegally in the harbor, and improperly maintained septic systems. In the northeastern U.S. the bathing season is relatively short, and water and air temperature, precipitation, and humidity change dramatically between May and September and from year to year. A suite of environmental variables were collected during sampling to evaluate potential correlations with elevated enterococci. Sand from the mean high tide line, sand at the water's edge, and water were sampled at different tidal stages for enterococci throughout the summer to examine the relationship between elevated enterococci in the water and contact with sand.

MATERIALS AND METHODS

Following preliminary sampling during wet weather in June and July of 2009, five sites along Provincetown Harbor (SI Figure 1) were sampled between 8am and 9am every Sunday, Monday and Tuesday from the end of May through the beginning of August, 2010. The five sites along the beach were chosen to reflect the presence of different potential sources of fecal bacteria, including municipal stormwater pipes buried in the sands at two of the five sites. Sites were approximately 100m apart (SI Figure 1). One liter of water was collected at each site by submerging an acid-washed, autoclaved 1L Nalgene bottle underwater at midcalf (approximately 30 cm) depth. Duplicate samples of sand were collected 0.5 m apart by using sterile 50 mL Falcon tubes to take small cores of approximately 5 cm in length/depth; wet sand was collected at the water line and dry sand at the mean high tide line. Samples were transported on ice to the lab within 2 h of collection where the U.S. EPA Method 1600²¹ was used to enumerate ENT per 100 mL water and was modified to enumerate ENT in sands (detailed in SI). Water temperature, salinity, dissolved oxygen, conductivity and turbidity were measured for each sample at the time of collection. Currents were examined by the deployment of two acoustic Doppler current profilers (ADCPs) within the harbor from June 10th to August 10th. Air temperature, wind speed and direction, solar insolation and relative humidity data were collected continuously at Macmillan Pier from June 10 to August 10, 2010; the record of local precipitation at the Provincetown Municipal Airport (station KPCV, located approximately 5km from Macmillan Pier) was accessed through NOAA's NCDC Climate Data Online.

Statistics. ENT abundance data were log₁₀ transformed, after which samples approximated a normal distribution. One-way ANOVA was used to test for significant differences in ENT abundance between the five sites, and no significant difference was found between the five sites for water, wet or dry sand. As such, daily mean ENT for water, wet sand, and dry sand were used for correlation and multiple linear regression analyses. Air

temperature, winds, solar insolation and relative humidity data collected on 15 min intervals were averaged over 24 h prior to the sampling event (8am-8am), and from 5 am to 9 am the morning of the sampling event. Water temperature, salinity, dissolved oxygen, conductivity, and turbidity were collected with each of the five water samples, and the daily along-beach mean was used for data analysis of these variables. Pearson's correlation coefficients and stepwise selection of best-fit Multiple Linear Regression models were calculated using R.²² Two multiple linear regression models were built using stepwise selection to predict the dependent variable of mean ENT in water and in dry sand. All variables collected during sampling as well as time-averaged (day and morning prior to sampling) environmental variables collected from Macmillan Pier were offered as potential predictive variables. For water, daily ENT abundance in sand was also offered as a potential predictor and vice versa. The regression models were selected based on highest adjusted R² and acceptable distribution of residuals. Variance Inflationary Factors were calculated to control for multicollinearity and all variables included in the models had VIF < 2. Data visualization and one-way ANOVA tests for differences in tidal range, tidal stage, etc. were conducted using R.²² In all tests, $p < 0.05$ was the cutoff for significance.

RESULTS AND DISCUSSION

Environmental Measurements. The ability of the harbor breakwater to retain polluted water within Provincetown Harbor was considered, but current profilers deployed within the area suggested this would be an unlikely scenario since the residence time of water within the harbor was less than a day. Winds primarily blew from the southwest during sampling days, water temperature (ranging from 16.3 to 24.6 °C) and air temperature (ranging from 15.8 to 26.1 °C) increased over time through the summer, and salinity of water samples (ranging from 23.2 to 30.63 PSU) was significantly linearly related to tidal level of water.

2010 was drier and warmer than 2009. Comparing the month of July from each year, 2009 had 12.7 cm total precipitation and an average air temperature of 20 °C, whereas 2010 was drier and warmer with 4 cm total precipitation (none of which immediately preceded sampling) and average air temperature of 23.4 °C. The lack of wet-weather sampling made 2010 ideal for study of dry-weather exceedance events.

Spatial Distribution of ENT CFU in Water, Intertidal Wet Sand, and Dry Sand along the Beach. Samples collected along the beach on any given day exhibited highly variable ENT concentrations, ranging from undetectable to hundreds of CFU/100 mL in water and thousands of CFU per 100 g dry-weight sand (SI Table 1). There were 6 days that had water quality violations (>104 CFU/100 mL), but rarely was more than one water sample in violation of the standard on the same day, and only once were three of the five samples above the single-sample maximum.

As other studies have shown at beaches in Florida,^{23,24} California,¹⁹ and along the Great Lakes,^{25,26} beach sands at Provincetown were enriched in ENT relative to the water, and the average concentration of ENT in high-tide sands was comparable to concentrations documented in these other environments (on average, greater than 10²CFU/100 g and ranging into 10³CFU/100 g). These distributions and the difference between wet and dry seasons are illustrated in Figure 1. Over the sampling season, dry sand was significantly more

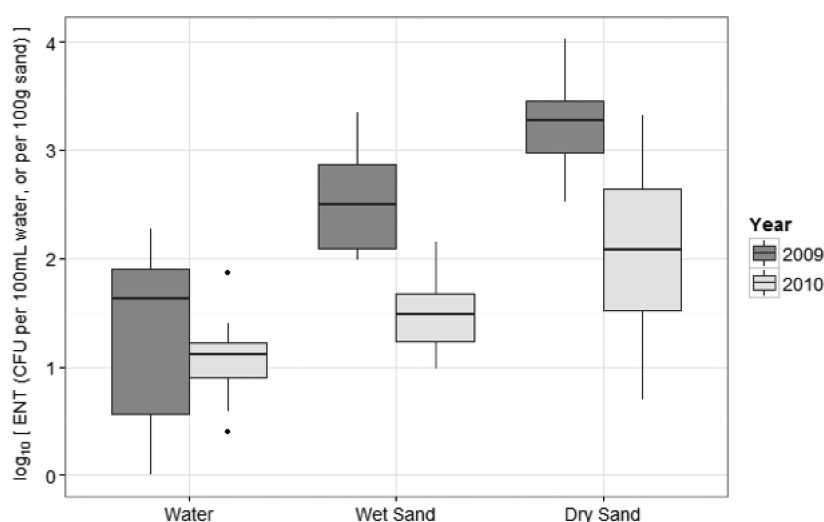


Figure 1. Wet and dry weather impact total abundance of ENT at a Provincetown beach, but patterns of distribution in water and sands are consistent. Comparing the same period in July, ENT in water, wet sand and dry sand were significantly higher during preliminary sampling in 2009 wet weather ($n = 12$ days, 12.7 cm precipitation, often preceding sampling) than was observed in 2010 ($n = 15$ days, 4 cm precipitation, none preceding sampling). Boxplots show the 25th, 50th, and 75th percentiles of data as the lower edge, middle and upper edge of the box. Whiskers extend to the most extreme data point (if it is within $1.5 \times \text{Interquartile Range}$ —if points fall outside this range, they are considered outliers and plotted individually).

enriched in ENT than wet sand (student's paired t test, $p=0.02$), and wet sand was significantly more enriched in ENT than water ($p < 0.001$). In dry sands, the daily along-beach average of the five samples was significantly negatively correlated to the variance of the five samples (Pearson's $r = -0.45$, $p = 0.01$) indicating that on days when higher amounts of ENT were observed in sands, the trend was not driven by a few patches of extreme ENT concentration. This trend between daily geometric mean and variance was directionally similar but insignificant in wet sand ($r = -0.25$, $p = 0.16$), and nonexistent in water ($r = 0.1$, $p = 0.58$).

Environmental Variables Are Associated with the Abundance of ENT in Dry Sand, Wet Sand and Water. Significant correlations ($p < 0.05$) were found between the daily mean $\log(\text{ENT})$ and a number of the environmental variables collected during sampling and at Macmillan Pier. Notably, daily mean ENT abundance in dry sand was significantly correlated to tidal range (Pearson's $r = 0.66$, $p < 0.0001$), and dry sands collected during spring tides had significantly higher mean ENT than sands collected during neap tides (Student's t test, $p < 0.001$). We hypothesize the primary mechanism by which sand is moistened during dry weather is from inundation during high tides, and the greater tidal range of spring tides produced moister sand at the high tide line. The data support this hypothesis, as daily tidal range was significantly positively correlated to daily mean dry sand moisture content ($r = 0.71$, $p < 0.0001$).

ENT in individual samples of dry sands was significantly related to moisture content in the sample ($r = 0.4$, $p < 0.0001$), with dry sand moisture content ranging from $<1\%$ to 4% over the course of the study. Daily mean ENT populations in high-tide dry sands were also correlated to the 4 h averaged (5am–9am) solar insolation ($r = -0.42$, $p = 0.015$) and relative humidity ($r = 0.54$, $p = 0.002$). Solar insolation and relative humidity are strongly inversely related to one another and affect the rate of moisture evaporation from sands, thus extending or curtailing the positive impact of tidal wetting on ENT populations.

In contrast to dry sand, daily mean ENT abundance in wet sand was not correlated to tidal range, and only weakly related to air temperature ($r = -0.31$, $p = 0.08$) and previous 48 h precipitation ($r = 0.32$, $p = 0.07$). Measurements of wind speed and direction at Macmillan Pier ($n = 26$ days) did yield two significant and positive correlations with wet sand ENT: 24 h averaged wind speed ($r = 0.51$, $p = 0.008$) and direction ($r = 0.60$, $p = 0.001$). This suggests the possibility of wind increasing wave-induced runup, resulting in recirculation of ENT within the swash zone and exiting at the water line where wet sand was being sampled.

The daily mean culturable ENT in water were strongly and significantly correlated to mean turbidity of the samples collected ($r = 0.74$, $p < 0.0001$). Temperature of the water sample ($r = 0.45$, $p = 0.01$) and the air at the time of sampling ($r = 0.36$, $p = 0.03$) were also significantly correlated to ENT abundance. ENT in the water samples were not significantly correlated to ENT in dry sand ($r = 0.25$, $p = 0.16$) or in wet sand ($r = 0.15$, $p = 0.40$).

Step-Wise Multiple Linear Regression Successfully Utilizes Environmental Variables to Describe ENT Abundance in Sands and in Water. The variations in turbidity measurements were small (ranging from <1 to 4 NTU) but significant; over half of the variation in the daily mean ENT in waters along the beach could be predicted by a linear regression with turbidity alone (adjusted $R^2 = 0.53$). For the subset of days during which wind speed and direction were collected at Macmillan Pier ($n = 26$), variation in the daily mean $\log(\text{ENT})$ could be predicted by a combination of water temperature, tidal height at the time of sample collection (related to tidal stage), and 4-h averaged wind direction measured at the Pier (adj $R^2 = 0.58$, Figure 2A). It is important to consider that these regressions represent variation in water quality during dry weather. In contrast, the preliminary samples collected at the same site during 6 weeks in the summer of 2009 had much higher ENT (Figure 1), and a greater amount of variation in water ENT concentrations could be explained by

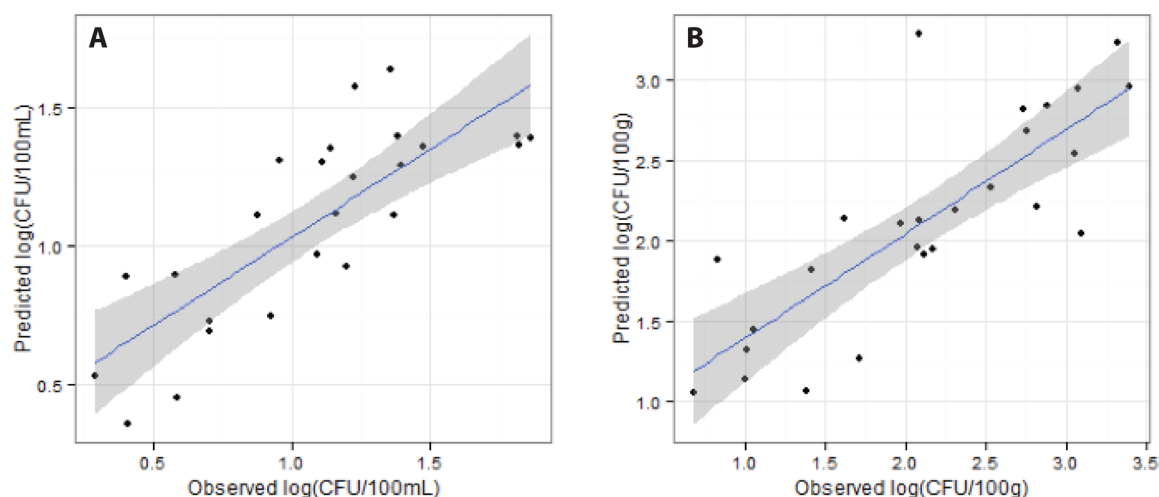


Figure 2. Observed culturable ENT vs multiple linear regression model predictions. Testing all environmental variables collected locally during sampling and at Macmillan wharf ($n = 26$ days over 8 weeks), the variables selected for best-fit multiple linear regression predicting ENT in water (adj. $R^2 = 0.58$, panel A) were water temperature, tidal height, and 4 h averaged A.M. wind direction at Macmillan Pier. The variables selected for the best-fit multiple linear regression model describing ENT in dry sand (adj. $R^2 = 0.62$, panel B) were tidal range and morning solar irradiance. Shading indicates the 95% confidence interval around the line.

a linear combination of 24 h precipitation and air temperature ($R^2 = 0.78$).

No linear combination of environmental variables could adequately predict a significant portion of the variation of ENT in wet sand, which likely reflects the fact that wet sand sampling was not sampled at a fixed spatial location but rather migrated with the location of the water line at 8am throughout the study.

Abundance of ENT in Upper Intertidal Sands Is Strongly Influenced by Moisture Availability. The population of ENT in dry sand was best described by a linear combination of tidal range and 4-h averaged solar insolation from Macmillan Pier ($R^2 = 0.65$, Figure 2B). ENT populations in high-tide dry sands that are rarely submerged are strongly, positively correlated to the moisture content of sands, which in this dry sampling season were tightly coupled to wetting from extreme spring tides. As moisture content decreased following spring tides and in the absence of rainfall, the abundance of culturable ENT decreased as well. Although many studies have documented the sensitivity of *E. coli* to moisture content of sands, ENT have often been described as resistant to desiccation and/or insensitive to moisture content. In part this has been due to observations from microcosm experiments that found inactivation of ENT to be no different in wet and dry sands.^{10,12} In a survey of ENT in sands conducted over a full day at Lover's Point in CA, the moisture content of sand samples collected hourly ranged from <1% to saturated at 19%, and abundance of ENT was negatively correlated to moisture content. Other studies have documented a similar range of moisture content and ENT densities in dry and intertidal sands at different field sites, and similarly drawn conclusions that moisture content was either negatively²⁷ or not correlated²⁸ to ENT abundance in sands. These observations were upheld by our overall observations that dry sands were enriched in ENT relative to wet sand (Figure 1). However, the data presented here represent the first field sampling strategy to capture the small variations in moisture content in dry sands over weeks and months due to tidal wetting. Considering that ENT have been shown in lab experiments to initially decrease as moisture content in sands decreased, and then to regrow with simulated tidal wetting,¹³ it is not surprising that significantly higher

amounts of enterococci are recovered from dry sand field samples after they have been subjected to tidal wetting. The strong, significant relationship that we observe confirms the importance of moisture content to ENT abundance and persistence at beaches.

Tides Modulate Abundance of ENT in the Intertidal.

Including the daily concentration of culturable ENT in sands as potential predictive variables for the water quality stepwise selection process did not improve the final multiple linear regression model describing ENT in water, but tidal height was an important variable for the water quality regression. To specifically test the hypothesis that sands may be a source of bacteria to waters during high tides, the samples were binned based on when they were collected within the tidal cycle. Tidal categories were low, flood, high, and ebb, with each category covering a 3 h period. For example, low and high categories included samples collected within ± 1.5 h from predicted high and low tide, respectively. The results are illustrated in box-and-whisker plots (Figure 3), and revealed significant patterns that suggest a mechanism for exchange of bacteria between sand and water in the intertidal.

Contrary to our hypothesis that the concentration of ENT in water would increase at high tide due to interaction with dry sands hosting the highest concentrations of ENT, water samples collected during high tide had significantly lower concentrations of bacteria than samples collected during flood and ebb tides (Figure 3, right panel). ANOVA between water samples grouped by tidal stage (low-flood-high-ebb) shows a significant difference between the mean ENT of these groups ($p = 0.0001$). More specifically, the Tukey HSD test shows significant differences between ENT in flood and high tides ($p < 0.01$), and between high and ebb tides ($p < 0.01$), but not between any other combination of tidal phases.

Likewise, the turbidity data show that suspended particles had significantly greater concentrations during the flood and ebb tides. Turbidity shows the same distribution and differences as was found between ENT in water and tidal stage (ANOVA, $p = 0.04$). According to the Tukey HSD, flood tide turbidity was significantly greater than the mean of each of the other groups ($p < 0.01$), and ebb tide turbidity was significantly

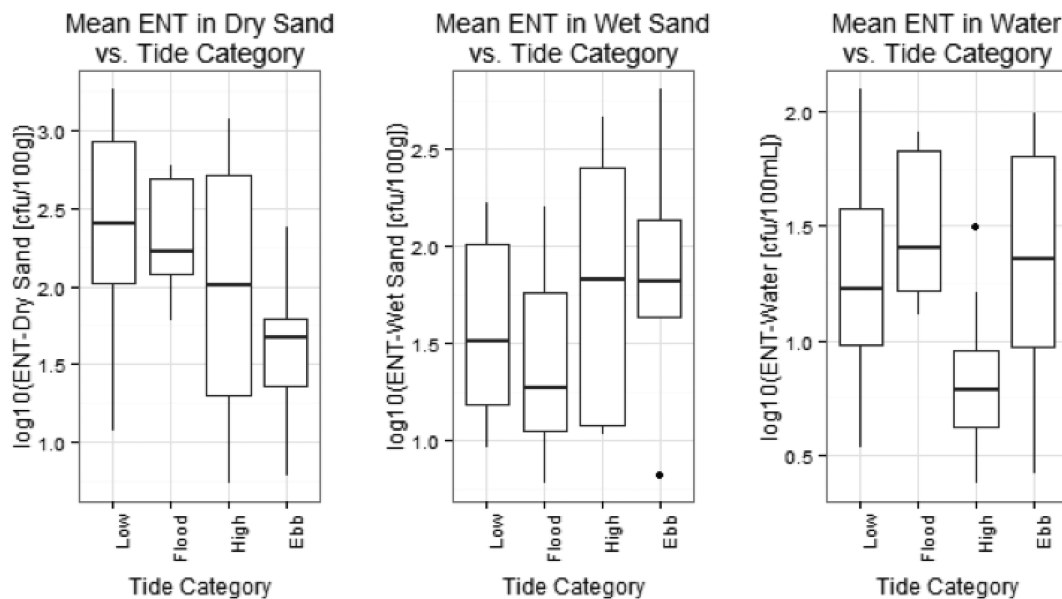


Figure 3. ENT abundance in the intertidal is modulated by tides. Because ENT were collected at 8 am every morning, over the course of the summer sands and waters were repeatedly sampled during different tidal stages. Box-and-whisker plots presented here bin ENT samples by tidal stage, and illustrate how tides are associated with different patterns of ENT abundance in each of the environmental matrices. For example, midintertidal wet sands sampled in the same spatial zone but during flood and ebb tides, show significantly higher ENT during ebb tides (middle panel). This may reflect drawdown of bacteria from the high-tide dry sands, which were found to have significantly less ENT when sampled during ebb tides (left panel). Contrary to our hypothesis, waters (right panel) contacting ENT-enriched dry sands during high tide have significantly less ENT than waters collected during ebb and flood tides. Boxplots show the 25th, 50th, and 75th percentiles of data as the lower edge, middle and upper edge of the box. Whiskers extend to the most extreme data point (if it is within 1.5*Interquartile Range—if points fall outside this range, they are considered outliers and plotted individually).

greater than high tide ($p < 0.05$). Increased turbidity during flood and ebb tidal phases suggests tidal resuspension as a mechanism for mobilization of ENT and other organic material from beach sands. Increased turbidity has been related to increased bacterial abundance in other systems,^{7,8,29,30} but more often in relationship to storm events³¹ or in estuaries with much higher turbidities than those observed at Provincetown.⁹

The high-tide dry sands also had a distinct trend in ENT with tidal stage (Figure 3, left panel), with significantly less bacteria in sands collected as the tide was ebbing. The data aggregated in this manner suggest that from ebb until the next high tide, the bacteria either regrow in the high tide sands, or the exposed sand is repopulated by ENT from people, birds, dogs, or other sources. The low concentration in dry sands collected during the ebb tide combined with the observation of relatively higher concentration of bacteria in wet sands collected during the ebb tide (Figure 3, middle panel) suggest there may be drawdown of bacteria from high dry sands to intertidal wet sands or through groundwater mixing zones (Figure 4). The “same” wet sands (that is, sands collected in the same spatial zone of the intertidal) are more enriched in ENT as the water retreats from the dry sand on ebb tides than they are when the water approaches from the lower intertidal during the flood tide.

Evidence of ENT Mobilization through Groundwater Flux. While groundwater was not directly sampled at Provincetown, the extensive sampling of surfzone salinity highlighted how groundwater may play an important role in this system. Daily mean salinity ranged from 23.2 to 30.6 PSU, and was strongly linearly related to tidal height. Samples collected at low tides were fresher and samples collected at midtide had intermediate salinities.

Provincetown is underlain by a lens of fresh groundwater bounded laterally by the Atlantic Ocean and Cape Cod Bay; the

groundwater is recharged by rainfall as well as effluent from the wastewater treatment plant, and discharges 12.5 million gallons per day into the surrounding coastal waters.³² Studies at other sites have illustrated how groundwater can be a direct source of pollution to coastal waters by transporting and discharging land-based contaminants.³³ However, there was no correlation between salinity and ENT abundance in water or sand in this study. Contaminated groundwater itself does not appear to be the primary source of ENT at this beach.

Alternately, Russell et al.³⁴ showed that ENT were transported by wave associated events downward through surface sands to the groundwater, and could potentially then be carried seaward by submarine groundwater discharge (illustrated in Figure 4). In other studies, models and physical observations of tidal forcing on sloping beaches have documented the importance of groundwater subterranean estuaries,³⁵ which are composed of the fresh groundwater from natural and anthropogenic recharge and recirculating seawater from the intertidal. As seawater washes up on the beach it infiltrates the sands and mixes with fresh groundwater to create the subterranean estuary mixing zone that is identifiable by its intermediate salinity. The circulation cell that develops and flux of “estuarine” groundwater into the ocean is tightly coupled to the tidal cycle. Net inflow to the mixing zone occurs in the upper intertidal zone during mid to high tide periods, and net outflow occurs in the lower intertidal during mid to low tides, with discharge of fresh, unmixed groundwater potentially occurring during low tides. The volume and cross-shore extent of the mixing zone varies as the tidal range expands and contracts with the spring - neap tidal cycle.³⁶ Estuarine groundwater mixing zones are more extensive and the total groundwater flux to the ocean is greater during spring tides, because the flux is dominated by the

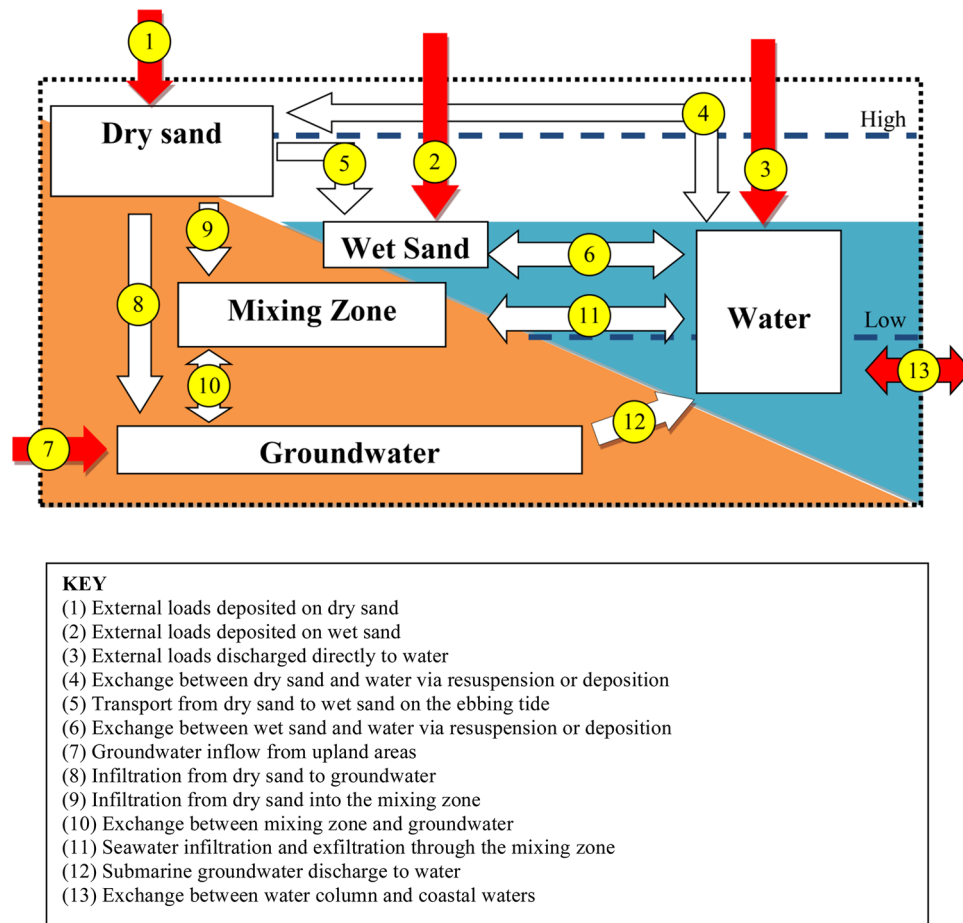


Figure 4. Conceptual model of ENT in coastal bathing waters. Depicted below are five storage compartments for ENT within the coastal zone. Red arrows represent fluxes into and out of the system from external sources, and white arrows represent fluxes between the compartments within the system. The dry sand compartment is representative of the surficial sands (upper 10 cm) between mean high and high–high tide lines, and the wet sand compartment is representative of surficial sands between mean high and mean low tide lines. The mixing zone compartment represents intertidal sands below the surface that are impacted by seawater infiltration and may interact with fresh groundwater. ENT growth/death rates are specific to and important within each compartment. By sampling the dry sand, wet sand, and water compartments for changes in salinity, moisture content, and enterococci abundance over time and across tidal cycles, we observed evidence of fluxes 5, 6, 9, 11, and 12.

recirculating seawater. In cases where groundwater is contaminated, neap tides actually produce a stronger signal of surfzone contamination because the terrestrial groundwater constitutes a greater percentage of the total flux.³⁷

At Provincetown, ENT and turbidity were more abundant in water during low tide compared to high tide (e.g., Figure 3 right panel); the low tides correspond to the period of greatest expected flux from the groundwater mixing zone, which could drive the mobilization of both ENT and particles from sands (Figure 4). Likewise, the ENT and turbidity were elevated during spring tides, consistent with increased seawater recirculation and groundwater fluxes from the subterranean estuary. Salinities in lower intertidal zone samples were slightly higher during spring tides than during neap tides, which also is consistent with increased groundwater mixing in the intertidal.

Implications for Predicting Dry-Weather Water Quality Violations. In terms of rapidly determining the likelihood of an exceedance event, turbidity could be a useful measurement in this system, as it was strongly linearly related to mean water ENT and reflects the important tidal forcing factors in this system. Turbidity is easy to measure at the time of sampling and could be monitored autonomously, which may provide a means for rapid early assessment of water quality in

dry weather. With regard to wet weather water quality prediction, turbidity was not measured in 2009, so it is not possible to say whether its predictive value would be confounded by runoff, which would increase both turbidity and water ENT.

The physical forcing mechanisms and transport of ENT described in this study could bias routine monitoring results. Single-sample water quality violations were detected in 10/153 samples, corresponding to violations occurring somewhere along the beach on 7 of the 32 days measured at 8 am. To move beyond the effect of tides on ENT distribution and specifically test impact on exceedance events, samples were binned based on the rate of change in water level at the time of sampling. This metric incorporates information about whether the sample was collected during a rising (flood), falling (ebb), or slack (high/low) tide, as well as contribution of tidal amplitude from spring to neap tides. A logistic regression ($R^2 = 0.88$, $p < 0.001$) using presence/absence of samples exceeding the single sample maximum as the dependent variable and binned rate of change in water level (<0.5 , >0.5 but <1.0 , >1.0 but <1.5 , >1.5 but <2.0 , and >2.0 ft/h) shows that the probability of having an exceedance event when absolute change in water level is <0.5 ft/h (high/low tides) is 0.08, and

the probability increases logarithmically to 0.551 when it is >2 ft/h (spring ebb/flood tides). Thus, for ENT concentrations approaching the regulatory limit, sampling at a particular tidal stage could push the sample over or under the limit. Health risks associated with resuspended sand-associated bacteria require more study.

Multiple linear regressions were not predictive of water quality violations during the sampling season because water quality never exceeded standards at all five sites on the same day, but larger data sets that capture more interannual variability could train robust predictive models, such as those models currently operating at beaches on the Great Lakes.³⁸ The utility of these regressions is the insight they provide into how bacterial abundance at a beach relates to the environment conditions and changes over time. Overall, the data from 2010 demonstrate the importance of tides during dry weather, and can be contrasted to the smaller data set collected in 2009 (Figure 1), when stormwater dominated the ENT signal. Temperature was included in water quality regression model in both wet and dry years, and in both years warmer temperatures were associated with increased ENT. This is in contrast to studies that have documented increased ENT inactivation with higher temperatures.^{29,39,40} The increase in ENT abundance with temperature may reflect enhanced growth rates for ENT in this temperate environment. Alternatively, temperature increases during the summer also correspond with increased human impact at the site (more visitors, boaters, and bathers), and potentially additional sources of ENT. At temperate beaches, seasonal changes in temperature may play a larger role in the abundance of enterococci than at beaches in regions where temperatures are more consistently warm throughout the year.

Contaminated sands at beaches and local forcing mechanisms are important and predictable factors for water quality. Recent modeling work on sediment-related transport of enterococci at an embayed beach in Florida found that tides and waves played a significant role in the abundance of ENT in the near shore water.⁴¹ Ge et al. also found that onshore waves and sediment resuspension were significant in the explanation for *E. coli* variability at a Chicago beach on Lake Michigan.⁴² Experimentally, ENT and other bacteria have been shown to move easily through unsaturated intertidal sands.⁴³ Together, these modeling, experimental, and field studies all show that the mobilization of ENT to water from sands is a common driver of beach water quality issues, and should especially be considered as a source of dry-weather water quality violations.

■ ASSOCIATED CONTENT

● Supporting Information

Expanded Methods, Study Site Map (SI Figure 1), and daily ENT mean, max and min for each of the environmental matrices (SI Table 1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) U.S. EPA *Recreational Water Quality Criteria*; U.S. EPA Office of Water: Washington, DC, 2012; <http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/>.
- (2) Halliday, E.; Gast, R. J. Bacteria in beach sands: An emerging challenge in protecting coastal water quality and bather health. *Environ. Sci. Technol.* **2011**, *45* (2), 370–379.
- (3) Sinton, L. W.; Hall, C. H.; Lynch, P. A.; Davies-Colley, R. J. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl. Environ. Microbiol.* **2002**, *68* (3), 1122–1131.
- (4) Fujioka, R. S.; Hashimoto, H. H.; Siwak, E. B.; Young, R. Effect of sunlight on survival of indicator bacteria in seawater. *Appl. Environ. Microbiol.* **1981**, *41* (3), 690–696.
- (5) Noble, R. T.; Lee, I. M.; Schiff, K. C. Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. *J. Appl. Microbiol.* **2004**, *96* (3), 464–472.
- (6) Boehm, A. B.; Yamahara, K. M.; Love, D. C.; Peterson, B. M.; McNeill, K.; Nelson, K. L. Covariation and photoinactivation of traditional and novel indicator organisms and human viruses at a sewage-impacted marine beach. *Environ. Sci. Technol.* **2009**, *43* (21), 8046–8052.
- (7) Characklis, G. W.; Noble, R. T.; Fries, J. S. Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, NC. *J. Environ. Eng.* **2006**, No. 10, 1338–1345.
- (8) Suter, E.; Juhl, A. R.; O'Mullan, G. D. Particle association of enterococcus and total bacteria in the Lower Hudson River Estuary, USA. *J. Water Resour. Prot.* **2011**, *3* (10), 715–725.
- (9) Kay, D.; Stapleton, C. M.; Wyer, M. D.; McDonald, A. T.; Crowther, J.; Paul, N.; Jones, K.; Francis, C.; Watkins, J.; Wilkinson, J.; Humphrey, N.; Lin, B.; Yang, L.; Falconer, R. A.; Gardner, S. Decay of intestinal enterococci concentrations in high-energy estuarine and coastal waters: Towards real-time T90 values for modelling faecal indicators in recreational waters. *Water Res.* **2005**, *39* (4), 655–667.
- (10) Mika, K. B.; Imamura, G.; Chang, C.; Conway, V.; Fernandez, G.; Griffith, J. F.; Kampalath, R. A.; Lee, C. M.; Lin, C. C.; Moreno, R. Pilot-and bench-scale testing of faecal indicator bacteria survival in marine beach sand near point sources. *J. Appl. Microbiol.* **2009**, *107* (1), 72–84.
- (11) Anderson, K. L.; Whitlock, J. E.; Harwood, V. J. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Appl. Environ. Microbiol.* **2005**, *71* (6), 3041–3048.
- (12) Hartz, A.; Cuvelier, M.; Nowosielski, K.; Bonilla, T. D.; Green, M.; Esiobu, N.; McCorquodale, D. S.; Rogerson, A. Survival potential of *Escherichia coli* and Enterococci in subtropical beach sand: Implications for water quality managers. *J. Environ. Qual.* **2008**, *37* (3), 898–905.
- (13) Yamahara, K. M.; Walters, S. P.; Boehm, A. B. Growth of enterococci in unaltered, unseeded beach sands subjected to tidal wetting. *Appl. Environ. Microbiol.* **2009**, *75* (6), 1517–1524.
- (14) Feng, F.; Goto, D.; Yan, T. Effects of autochthonous microbial community on the die-off of fecal indicators in tropical beach sand. *FEMS Microbiol. Ecol.* **2010**, *74* (1), 214–25.
- (15) Korajkic, A.; Wanjugi, P.; Harwood, V. J. Indigenous microbiota and habitat influence *Escherichia coli* survival more than sunlight in simulated aquatic environments. *Appl. Environ. Microbiol.* **2013**, *79* (17), 5329–5337.
- (16) Halliday, E.; McLellan, S. L.; Amaral-Zettler, L. A.; Sogin, M. L.; Gast, R. J. Comparison of bacterial communities in sands and water at

beaches with bacterial water quality violations. *PloS One* **2014**, 9 (3), e90815.

(17) Dorfman, M.; Rosselot, K. S. *Testing the Waters: A Guide to Water Quality at Vacation Beaches*; National Resource Defense Council, 2011.

(18) Wright, M.; Abdelzaher, A.; Solo-Gabriele, H.; Elmir, S.; Fleming, L. The inter-tidal zone is the pathway of input of enterococci to a subtropical recreational marine beach. *Water Sci. Technol.* **2011**, 63 (3), 542–549.

(19) Yamahara, K. M.; Layton, B. A.; Santoro, A. E.; Boehm, A. B. Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. *Environ. Sci. Technol.* **2007**, 41 (13), 4515–4521.

(20) Boehm, A. B.; Weisberg, S. B. Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. *Environ. Sci. Technol.* **2005**, 39 (15), 5575–5583.

(21) U.S. EPA, *Method 1600: Membrane Filter Test Method for Enterococci in Water*; U.S. EPA: Washington, DC, 2002.

(22) Team, R. C. R. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013.

(23) Abdelzaher, A. M.; Wright, M. E.; Ortega, C.; Solo-Gabriele, H. M.; Miller, G.; Elmir, S.; Newman, X.; Shih, P.; Bonilla, J. A.; Bonilla, T. D. Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. *Appl. Environ. Microbiol.* **2010**, 76 (3), 724–732.

(24) Bonilla, T. D.; Nowosielski, K.; Cuvelier, M.; Hartz, A.; Green, M.; Esiobu, N.; McCorquodale, D. S.; Fleisher, J. M.; Rogerson, A. Prevalence and distribution of fecal indicator organisms in South Florida beach sand and preliminary assessment of health effects associated with beach sand exposure. *Mar. Pollut. Bull.* **2007**, 54 (9), 1472–82.

(25) Wheeler Alm, E.; Burke, J.; Spain, A. Fecal indicator bacteria are abundant in wet sand at freshwater beaches. *Water Res.* **2003**, 37 (16), 3978–3982.

(26) Whitman, R. L.; Nevers, M. B. Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. *Appl. Environ. Microbiol.* **2003**, 69 (9), 5555–5562.

(27) Oshiro, R.; Fujioka, R. Sand, soil, and pigeon droppings: Sources of indicator bacteria in the waters of Hanauma Bay, Oahu, Hawaii. *Water Sci. Technol.* **1995**, 31 (5), 251–254.

(28) Piggot, A. M.; Klaus, J. S.; Johnson, S.; Phillips, M.; Solo-Gabriele, H. M. Enterococci levels are related to sediment biofilms at recreational beaches in South Florida. *Appl. Environ. Microbiol.* **2012**, 78 (17), 5973–5982.

(29) Ortega, C.; Solo-Gabriele, H. M.; Abdelzaher, A.; Wright, M.; Deng, Y.; Stark, L. M. Correlations between microbial indicators, pathogens, and environmental factors in a subtropical Estuary. *Mar. Pollut. Bull.* **2009**, 58 (9), 1374–1381.

(30) Mallin, M. A.; Johnson, V. L.; Ensign, S. H. Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream. *Environ. Monit. Assess.* **2009**, 159 (1), 475–491.

(31) Lee, C. M.; Lin, T. Y.; Lin, C. C.; Kohbodi, G. N. A.; Bhatt, A.; Lee, R.; Jay, J. A. Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments. *Water Res.* **2006**, 40 (14), 2593–2602.

(32) Masterson, J. P.; Portnoy, J. W. *Potential Changes in Ground-Water Flow and Their Effects on the Ecology and Water Resources of the Cape Cod National Seashore, Massachusetts*; U.S. Department of the Interior, U.S. Geological Survey, 2005.

(33) Boehm, A. B.; Shellenbarger, G. G.; Paytan, A. Groundwater discharge: Potential association with fecal indicator bacteria in the surf zone. *Environ. Sci. Technol.* **2004**, 38 (13), 3558–3566.

(34) Russell, T.; Yamahara, K. M.; Boehm, A. B. Mobilization and transport of naturally occurring enterococci in beach sands subject to transient infiltration of seawater. *Environ. Sci. Technol.* **2012**, 46 (11), 5988–5996.

(35) Robinson, C.; Gibbes, B.; Li, L. Driving mechanisms for groundwater flow and salt transport in a subterranean estuary. *Geophys. Res. Lett.* **2006**, 33 (3), L03402.

(36) Robinson, C.; Li, L.; Barry, D. Effect of tidal forcing on a subterranean estuary. *Adv. Water Res.* **2007**, 30 (4), 851–865.

(37) de Sieyes, N. R.; Yamahara, K. M.; Layton, B. A.; Joyce, E. H.; Boehm, A. B. Submarine discharge of nutrient-enriched fresh groundwater at Stinson Beach, California is enhanced during neap tides. *Limnol. Oceanogr.* **2008**, 53 (4), 1434–1445.

(38) Olyphant, G. A.; Whitman, R. L. Elements of a predictive model for determining beach closures on a real time basis: The case of 63rd Street Beach Chicago. *Environ. Monit. Assess.* **2004**, 98 (1), 175–190.

(39) McFeters, G. A.; Stuart, D. G. Survival of coliform bacteria in natural waters: Field and laboratory studies with membrane-filter chambers. *Appl. Environ. Microbiol.* **1972**, 24 (5), 805.

(40) Sinton, L. W.; Davies-Colley, R. J.; Bell, R. G. Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in seawater chambers. *Appl. Environ. Microbiol.* **1994**, 60 (6), 2040–2048.

(41) Feng, Z.; Reniers, A.; Haus, B. K.; Solo-Gabriele, H. M. Modeling sediment-related enterococci loading, transport, and inactivation at an embayed nonpoint source beach. *Water Resour. Res.* **2013**, 49 (2), 693–712.

(42) Ge, Z.; Nevers, M. B.; Schwab, D. J.; Whitman, R. L. Coastal loading and transport of *Escherichia coli* at an embayed beach in Lake Michigan. *Environ. Sci. Technol.* **2010**, 44 (17), 6731–6737.

(43) Boehm, A. B.; Yamahara, K. M.; Sassoubre, L. M. Diversity and transport of microorganisms in intertidal sands of the California Coast. *Appl. Environ. Microbiol.* **2014**, 80 (13), 3943–3951.