

## Enterococci Concentrations in Diverse Coastal Environments Exhibit Extreme Variability

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Received July 20, 2007. Revised manuscript received September 25, 2007. Accepted September 26, 2007.

Fecal indicator bacteria (FIB) concentrations in a single grab sample of water are used to notify the public about the safety of swimming in coastal waters. If concentrations are over a single-sample standard, waters are closed or placed under an advisory. Previous work has shown that notification errors occur often because FIB vary more quickly than monitoring results can be obtained (typically 24 h). Rapid detection technologies (such as quantitative polymerase chain reaction) that allow FIB quantification in hours have been suggested as a solution to notification errors. In the present study, I explore variability of enterococci (ENT) over time scales less than a day that might affect interpretation of FIB concentrations from a single grab sample, even if obtained rapidly. Five new data sets of ENT collected at 10 and 1 min periodicities for 24 and 1 h, respectively, are presented. Data sets are collected in diverse marine environments from a turbulent surf zone to a quiescent bay. ENT vary with solar and tidal cycles, as has been observed in previous studies. Over short time scales, ENT are extremely variable in each environment even the quiescent bay. Changes in ENT concentrations between consecutive samples (1 or 10 min apart) greater than the single-sample standard (104 most probable number per 100 mL) are not unusual. Variability, defined as the change in concentration between consecutive samples, is not distinct between environments. ENT change by 60% on average between consecutive samples, and by as much as 700%. Spectral analyses reveal no spectral peaks, but power-law decline of spectral density with frequency. Power-law exponents are close to 1 suggesting ENT time series share properties with  $1/f$  noise and are fractal in nature. Since fractal time series have no characteristic time scale associated with them, it is not obvious how the fractal nature of ENT can be exploited for adaptive sampling or management. Policy makers, as well as scientists designing field campaigns for microbial source tracking and epidemiology studies, are cautioned that a single sample of water reveals little about the true water quality at a beach. Multiple samples must be taken to gain a snapshot into the patchy structure of microbial water quality and associated human health risk.

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### Introduction

The United States Clean Water Act and BEACH Act require coastal states to monitor recreational waters for fecal indicator bacteria (FIB) to assess water quality. Exposure to FIB from municipal wastewater and urban runoff in marine waters correlates to adverse health outcomes in swimmers according to formal epidemiology studies (1–3). Monitoring results are used for public notification of water quality via beach advisories and closures. In the United States, 98% of agencies conducting monitoring use a single-sample exceedance criteria for issuing advisories and closures (4). If FIB concentrations in a single grab sample of water exceed the criteria, public notification of poor water quality is required. For enterococci (ENT), the preferred FIB for monitoring marine waters (5), the recommended single sample standard for beaches is 104 most probable number (MPN) of colony forming units (CFU)/100 mL (6).

United States Environmental Protection Agency approved methods to measure FIB require an 18–96 h incubation period as they are culture-based. Several studies have shown that temporal changes in FIB concentrations in beach water occur at shorter time scales (7, 8). Thus, out-of-compliance beaches remain open during the laboratory incubation period and may be in compliance by the time warnings are posted (8, 9). Rapid detection technologies are culture independent, allowing FIB quantification in under 4 h (10, 11). Transitioning to rapid methods has been proposed as a means for addressing management errors resulting from the delay associated with culture-based assays.

However, there is strong evidence that no matter how rapidly a test result can be obtained, a single sample of water will not adequately describe water quality for an entire day. It is now known that FIB vary at time scales less than a day. In particular, FIB vary with tidal and solar cycles (12, 13) which modulate their transport and inactivation in coastal waters, respectively. Fortunately, the manner in which FIB vary with tides and sunlight is predictable, so health-protective monitoring can be conducted (for example, periods with highest FIB can be sampled). A single study has documented FIB variability at time scales less than an hour in a turbulent surf zone and attributed this to rip cell mixing (14). In this case, variation did not appear to be predictable. More work is needed to examine FIB variability at short time scales (less than an hour) at diverse beach environments to determine if short-period variability is present along all coastlines or only present in turbulent surf zones. Such extreme variability could have profound influence on the policy outcomes (i.e., beach advisories and closures), monitoring plans, and usefulness of rapid detection technologies.

There is reason to believe that FIB variability at time scales less than an hour will be common based on work with other physical, chemical, and biological parameters in the coastal environment (15–18). For example, temporal variability in temperature, nitrite, and fluorescence has been documented at scales of seconds to hours in coastal waters (15, 16, 19). These studies found that parameter variability, or “patchiness”, is not confined to a set of frequencies, nor did they find that the variability is random (i.e., white noise). Rather, they found that extreme variability of many coastal parameters is fractal in nature. That is, variability is observed at all time scales and there is no characteristic time scale associated with the signal.

Fractal time series are identified from a power-law decay in spectral density ( $E$ ) with frequency ( $f$ ) (16). The power

**TABLE 1. Descriptions of Experiments Included in the Study (Freq. is the Frequency at Which Samples Were Collected during the Experiments; "Building" Indicates that the Waves Increased from 0 to 1 m over the Course of the Experiment)**

site	location	start	end	freq. (1/min)	tide range (m)	breaker height (m)
HSB02	Huntington Beach	4/12/02 16:00	4/13/02 10:00	0.1	1.4	1
LPS05	Lovers Point, South	10/22/05 11:00	10/23/05 9:00	0.1	1.5	0-1 (building)
LPS07	Lovers Point, South	2/3/07 11:00	2/4/07 11:00	0.1	1.7	0
LPN07	Lovers Point, North	2/3/07 11:00	2/4/07 11:00	0.1	1.7	0
LPmin	Lovers Point, South	10/23/05 2:00	10/23/05 3:00	1	0.2	1

law-exponent  $\beta$  in  $E(f) \sim f^{-\beta}$  can be related to the fractal dimension  $D$  as follows:  $D = 2 - 0.5(\beta - 1)$  where  $D$  varies between 1 and 2 (16).  $D$  and  $\beta$  are useful for describing how energy in a time series varies from one time scale to the next. Their magnitudes are controlled by physical (e.g., turbulent velocities and dispersion) and biological (e.g., variation in growth and grazing rates) processes (15, 18). If  $\beta = 0$ , the signal in the time domain is referred to as white noise because  $E(f)$  is constant. In this case, the signal is not fractal, but is considered random because variability at every frequency contributes equally to the time series. If  $\beta = 1$ , the signal is fractal and classified as  $1/f$  noise which is ubiquitous in nature (for example, flow in streams (20) and DNA sequences (21)). In this case, the energy associated with each frequency falls off as frequency increases. Because  $E(f)$  and  $f$  are related, the signal in the time domain is considered structured. When turbulent velocities are responsible for advecting a passive scalar,  $\beta = 5/3$  as described by Kolmogorov (15).

In the present study, I examine extreme temporal variations (periods between 1 min and 24 h) in FIB concentrations in diverse marine coastal environments ranging from wave-sheltered to wave-exposed open ocean beaches. I report five new ENT data sets, collected at 10 and 1 min periodicities. A goal of this paper is to determine if ENT variation at short time scales is dictated by the physical environment in which they were measured (i.e., a quiescent, wave-sheltered cove or a turbulent surf zone). In addition, I examine how variation at different time scales or frequencies contributes to the overall ENT signal using Fourier analysis. In particular, I examine if high frequency variability is random or fractal in nature. The implications of the results for monitoring beaches for ENT and human health risk are discussed.

## Materials and Methods

Enterococci (ENT) are the focus of this study because they correlate best to human health outcomes in marine waters (5). ENT concentrations were measured every 10 min for 18 h at Huntington State Beach (HSB, 33°38' N, 117°58' W) in 2002, and every 10 min for 22 and 24 h in 2005 and 2007, respectively, at Lovers Point, CA (LP, 36°37' N, 121°55' W). In 2005, ENT concentrations at LP were measured every 1 min for approximately an hour during the longer duration 10-min study (Table 1). During each experiment, samples were taken at a fixed location, and thus sampling was Eulerian in nature.

Tides and waves are major factors affecting mixing and transport in the very nearshore and might explain heterogeneity in ENT variability between experiments. To characterize the tides and waves during each experiment, tide level and range were obtained from XTide (<http://www.flaterco.com/xtide/files.html>) and breaker heights were recorded visually by the author (Table 1). In 2002, water samples were collected from HSB at station 6N (22) (hereafter referred to as experiment HSB02). HSB is characterized by a well-developed surf zone, and during HSB02 breakers were 1 m high. During 2005 and 2007, samples were collected at LP, which is sheltered from waves under the majority of conditions except during long-period northwest (NW) swell.

During 2005, I sampled LP at a single location on the beach once every 10 and 1 min, as described above (hereafter referred to as LPS05 and LPmin for 10 and 1 min period experiments, respectively, Table 1). The experiments began under quiescent conditions with no waves, and over the course of the study a NW swell built until 1 m waves were breaking on the beach. During 2007 at LP, I collected samples at two locations on the beach, approximately 50 m apart (sites N and S) (hereafter referred to as LPN07 and LPS07, respectively). Waves were absent during the entire study, and the water was extremely quiescent. The tide range during all studies was similar, with the exception of the study where samples were collected every minute for 1 h at LP (LPmin) during which the water level barely changed.

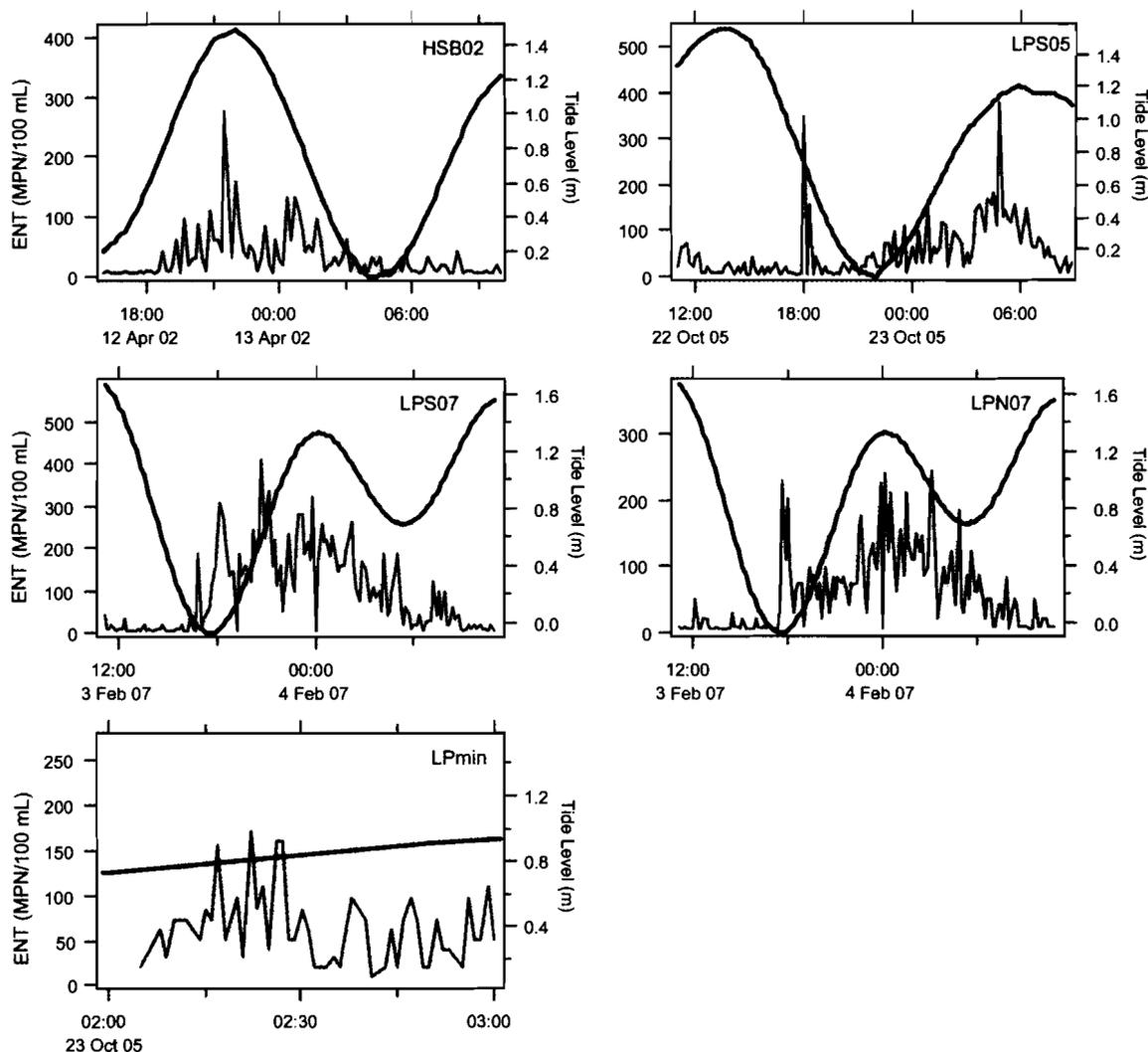
Fifty mL of water was collected in sterile containers and immediately stored on ice and analyzed within an hour of collection. Prior to analysis, containers were mixed by inverting three times. Ten mL subsamples were assayed for ENT using Enterolert defined chromogenic substrate assays implemented in a 97-well format (IDEXX, Westbrook, ME). An interlaboratory comparison study in southern California using waters adjacent to HSB found that Enterolert yielded results consistent with traditional methods of membrane filtration and multiple tube fermentation with low error rates (23). Therefore, Enterolert is expected to perform well in the present study. Ten mL of well-mixed sample water and reagent were dispensed into 90 mL of Butterfields buffer. This allowed detection of ENT between 10 and 24192 most probable number (MPN)/100 mL. Concentrations and 95% confidence intervals were determined from MPN tables. The 95% confidence intervals represent a measure of the method uncertainty. For data analysis purposes, ENT concentrations below the lower limit of detection (10 MPN/100 mL) were assigned a value of 5 MPN/100 mL.

Data were analyzed using SPSS v.11 (SPSS) and Matlab v7.0.4 (Mathworks). Kruskal-Wallis tests were used to compare ENT concentrations measured between sites or conditions. Following Whitman and Nevers (24), the number of samples ( $n$ ) required during the experiments to achieve a specific level of certainty, or coefficient of variation (CV), about the experiment average ( $\bar{x}$ ) given the standard deviation ( $s$ ) was calculated as  $n = (s/CV\bar{x})^2$ . CV values of 20% and 50% were chosen for simplicity, although any CV could have been used.

Fourier transforms were applied to detrended ENT data series. To determine whether spectral densities decayed as power laws with frequency and were thus fractal, spectral density estimates were averaged within equal logarithmically spaced intervals following Lovejoy et al. (15). Linear regressions were applied to determine power-law exponents  $\beta$  and their 95% confidence intervals. This approach assumes that a single fractal dimension can be used to describe data (16).

## Results

**Ten Minute Time Series.** Time series of ENT measured once every 10 min are illustrated in Figure 1 along with tide level (HSB02, LPS05, LPS07, LPN07). High frequency variability is evident that cannot be explained by measurement uncer-



**FIGURE 1.** ENT time series analyzed in the present study. Shaded areas represent 95% confidence intervals about each measurement as determined from MPN tables, black line is measured ENT. Heavy black line is tide level (shown on right axes). The code in the upper right corner describes the location and time of experiments (see Table 1).

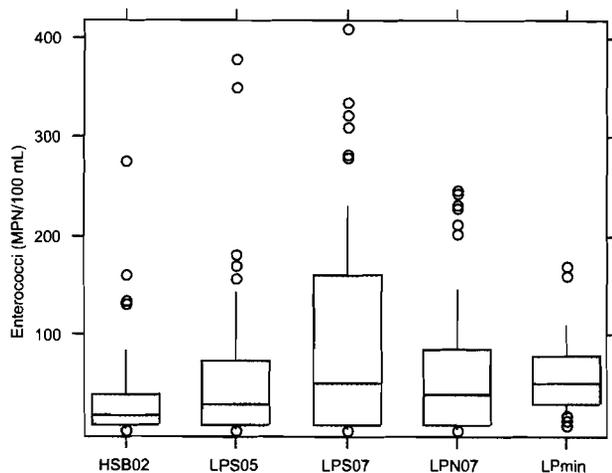
tainty. This is based on the fact that measurements do not fall within the 95% confidence bounds of one-another (gray shading in Figure 1). Confidence intervals varied according to concentration as determined by the MPN tables and ranged from 37 to 311 MPN/100 mL (Figures S1 and S2 in the Supporting Information). ENT distributions measured during the experiments are significantly different from each other ( $p < 0.05$ ) with the exception of LPN07 and LPS05 which are similar ( $p > 0.05$ ) (Figure 2). The highest ENT concentrations were measured at site LPS07, followed by LPN07 and LPS05, and HSB02 (Figure 2, Table 2). The number of samples with ENT below the lower detection limit of 10 MPN/100 mL is reported in Table 2. No measured concentration was over the upper detection limit.

All sites display significant diurnal patterns: ENT concentrations are significantly higher at night compared to the day ( $p < 0.05$ ). This supports reports of the sunlight inactivation of indicator organisms in natural waters (25). All sites show significant variation with tide. ENT concentrations at LP sites (LPS05, LPS07, LPN07) are higher during flood compared to ebb tides ( $p < 0.05$ ). In contrast, ENT concentrations at HSB02 are higher during ebb compared to flood tides ( $p < 0.05$ ). These results are in agreement with previous reports of semidiurnal variation of ENT at these beaches and are likely due to tidal modulation of ENT sources (26, 27).

The average change in ENT concentration between consecutive samples during the experiments ranges from 26 (HSB02) to 45 (LPS07) MPN/100 mL per 10 min (Table 2). The maximum change in ENT concentration between samples is 345 MPN/100 mL per 10 min measured at LPS05. At all sites, the maximum change in ENT concentration between consecutive samples is greater than the California single-sample ENT standard of 104 MPN/100 mL. This indicates that changing the sampling time by as little as 10 min could result in a change in the posting or advisory status of the beach. There are instances when there is no change between ENT measurements between consecutive samples. Many of these (approximately 40%) occur when 5 MPN/100 mL is assigned as the lower limit of detection and thus may be an artifact of our detection limit.

The difference between ENT concentrations measured in consecutive samples relative to the experiment average ( $\delta$ ) was calculated (Table 2). The distributions of  $\delta$  are not different between experiments ( $p > 0.05$ ) and range from 0 to 7 (10 min)<sup>-1</sup> and average 0.6 (10 min)<sup>-1</sup>. This means that overall, ENT concentrations typically vary by 60% every 10 min.

Using the standard deviations and means reported in Table 2, a beach manager would need to collect 39 (HSB02), 31 (LPS05), 25 (LPS07), and 25 (LPN07) samples to obtain an estimate of concentration within a coefficient of variation of



**FIGURE 2.** Box and whisker plots show the range of ENT concentrations measured during each study. The lower, middle, and top box edges correspond to the 25th, 50th, and 75th percentiles of the indicated set of measurements, the "whiskers" indicate the 10th and 90th percentiles, and the symbols show measurements lower and greater than the 5th and 95th percentiles, respectively.

**TABLE 2.** ENT Concentration Measurement Results<sup>a</sup>

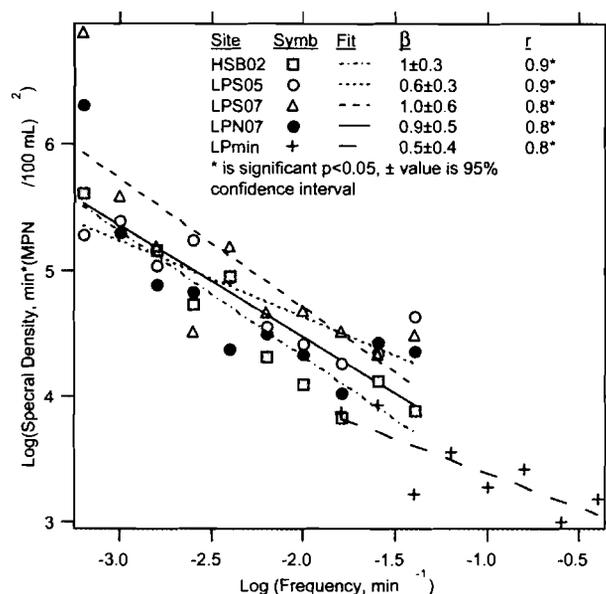
experiment	N	UD	ave	SD	GM	ave change (min-max)	ave $\delta$ (min-max)
HSB02	102	24	33	41	19	26 (0-234)	0.8 (0-7.1)
LPS05	131	14	54	60	31	35 (0-345)	0.6 (0-6.4)
LPS07	144	22	96	95	44	45 (0-318)	0.5 (0-3.3)
LPN07	144	28	60	59	32	36 (0-238)	0.6 (0-4.0)
LPmin	49	0	62	39	51	34 (0-140)	0.5 (0-2.3)

<sup>a</sup> N is the number of samples collected and UD is the number of samples with ENT below the lower detection limit of 10 MPN/100 mL; ave is arithmetic average, SD is standard deviation, GM is geometric mean, all with units of MPN/100 mL; ave change is the average change between consecutive samples with minimum and maximum given in parentheses and units of MPN/100 mL per 10 min except for LPmin where units are MPN/100 mL per min; ave  $\delta$  is the average change between samples relative to the experiment average with units  $(10 \text{ min})^{-1}$  except for LPmin where units are  $(\text{min})^{-1}$ .

20% about the experiment mean. If a coefficient of variation of only 50% were desired, 6 (HSB02), 5 (LPS05), 4 (LPS07), and 4 (LPN07) samples would be required.

There are no peaks in the spectral densities at specific frequencies (Figure 3). Rather, spectral densities decay as power-laws with frequency. Power-law exponents  $\beta$  for each spectra are within 95% confidence of 1 with the exception of LPS05.  $\beta$  for LPS05 ranges between 0.3 and 0.9 with 95% confidence. All linear regressions were statistically significant ( $r$  values reported in Figure 3,  $p < 0.05$ ).

**Spatial Variation between LPS07 and LPN07.** During the LP experiment during 2007, samples were collected concurrently at two sites on the beach approximately 50 m apart. The measurements at these sites are well correlated to each other (Spearman's  $\rho = 0.71$ ,  $p < 0.05$ ); however the two data series are significantly different ( $p < 0.05$ ) with LPS07 having higher ENT concentrations than LPN07. The same concentration was measured simultaneously at the two sites 18 out of 144 (12.5%) times. The mean difference between measurements at LPS07 and LPN07 collected at the same time is 56 MPN/100 mL and the maximum is 379 MPN/100 mL. Importantly, 59/144 (41%) measurements at LPS07 are over the California single-sample standard of 104 MPN/100 mL while only 27/144 (19%) are over the standard at LPN07.



**FIGURE 3.** Log<sub>10</sub>-transformed spectral densities plotted as a function of log<sub>10</sub>-transformed frequency. Linear regressions are shown as lines, with slopes (equal to  $\beta$  in  $E(f) \sim f^{-\beta}$ ) Pearson's  $r$ , and  $p$  value for regression. "Symb" in legend is the symbol used for each experiment.

Thus, the probability of measuring an exceedance of water quality standards changes depending on where one is sampling on the beach, within a short distance of 50 m. One explanation for the higher concentrations at LPS07 is that the site is located closer to a storm drain on the beach than LPN07. However, 28% of the simultaneous samples were actually higher at LPN07, the site further from the storm drain, indicating that proximity to the storm drain is not the only factor that impacts ENT concentrations.

**One Minute Time Series.** I measured ENT every minute for one hour during the LPS05 experiment (LPmin, Figure 1). ENT were extremely variable (average change of 34 MPN/100 mL per minute, maximum change of 140 MPN/100 mL per min). As with the 10 min time series, the variation between samples cannot be explained by measurement uncertainty based on the fact that measurements do not fall within the 95% confidence bounds of one-another (gray shading in Figure 1). The changes in ENT concentrations and  $\delta$  between consecutive samples during this hour are not significantly different from those observed during the LPS05 10-min experiment ( $p > 0.05$ ). If an estimate of ENT concentration with a coefficient of variation of 20% and 50% relative to the 1 h experiment mean were desired, then 10 and 2 samples would be required, respectively.

Spectral density decays as a power law with frequency with  $\beta = 0.5 \pm 0.4$  (Figure 3,  $r = 0.8$ ,  $p < 0.05$ ). The exponent is not different ( $p > 0.05$ ) from that measured for LPS05 where  $\beta = 0.6 \pm 0.3$ , suggesting that the scaling observed with the lower frequency LPS05 data set applies to a greater range of frequencies.

## Discussion

ENT concentrations collected at 10 and 1 min intervals along the shoreline of marine beaches illustrate that temporal variability is extreme. Changes in ENT concentrations between consecutive samples greater than the California single-sample standard of 104 MPN/100 mL are not unusual. Extreme variability is present in experiments conducted in a turbulent, well-mixed surf zone (HSB02), in waters transitioning from quiescent/tide-dominated to wave-dominated (LPS05 and LPmin), and in a quiescent tide-dominated

environment (LPN07 and LPS07). Variability, measured as the change in consecutive ENT measurements normalized by the experiment average ENT concentration, is not different between sites, thus does not appear to be a function of the degree of wave exposure.

It should be noted that the extreme variability documented here is not a result of the method used to enumerate ENT. In another study, we used membrane filtration in conjunction with mEI media to measure ENT concentrations at LP every 20 min (26). We saw similar ENT variability. It is likely that any ENT analysis method will give similar results regarding variability. However, experiments need to be conducted to document variability with methods that measure nucleic-acid targets for ENT quantification.

Although results are not reported here, *E. coli* were also measured using Colilert-24 and Colilert-18 (IDEXX) during the experiments described in Table 1. Colilert has been shown to perform well in California marine waters for *E. coli* enumeration (23, 28). Conclusions regarding variability in ENT apply to these bacteria as well. It is likely that variability over similar time scales will apply to other microbial targets including source-specific markers like those in *Bacteroidales* (29), but this should be confirmed.

Low frequency patterns associated with sunlight and tides are apparent in each time series that lasted for longer than 1 h. It is interesting that neither diurnal nor semidiurnal peaks are evident in the spectra (Figure 3). This is likely due to the relatively short duration of the time series relative to diurnal and semidiurnal periods.

Despite the lack of spectral peaks, coastal ENT concentrations are structured because time series can be described mathematically as decaying power-laws in the frequency domain. Even though the physical environments studied are different with regard to wave exposure, ENT concentrations are structured similarly with power-law exponents close to 1 (Figure 3). The fact that the power-law exponents are not equal to zero implies that the variability is not random, or white noise, as this would have produced a flat spectra. ENT time series share properties of  $1/f$  noise (30) and have a fractal dimension  $D \sim 2$ . Seuront and Lagadeuc (31) report  $D$  between 1.367 and 1.626 for temperature, salinity, and fluorescence in tidally mixed waters in the English Channel.  $E(f)$  of their data series declined more rapidly with increasing  $f$ , compared to those in Figure 3. Relative to my data series, low-frequency oscillations were more dominant than high-frequency oscillations in their data series.

The fact that the ENT data share characteristics with  $1/f$  noise indicates ENT are "patchy" and that there were ENT patches or filaments of all durations or sizes transported by the fixed sampling site during the experiments. Patchiness in time and space is expected to develop in coastal environments where intermittent sources, nonuniform currents, turbulent diffusion, and changing chemical or biological characteristics influence persistence and transport of ENT (15, 32).

How knowledge of the fractal dimension of the ENT series might be harnessed to provide recommendations for sampling plans to protect human health is not clear. By definition, a fractal time series has no characteristic time scale associated with it, so sampling at a particular time interval cannot be recommended. An important point is that ENT concentrations are not random white noise even though there are no spectral peaks. More work on understanding fate and transport of ENT in coastal waters is needed so that researchers can fully understand how patchiness develops.

The result reported here regarding extreme variability presents a challenge to policy makers and the protection of human health. Assuming ENT are from an urban runoff or municipal wastewater source and the epidemiological models (1-3) are correct, ENT concentrations correlate to health risk.

This suggests that not only are ENT patchy in time and space behaving as  $1/f$  noise, but so are human pathogens and human health risks. An inability to estimate the true concentration of ENT in coastal waters limits our ability to protect human health. A way of sampling the coastal ocean for ENT to uncover a true estimate of human health risk is needed. If a health-protective estimate is desired, then sampling should be conducted at night during ebbing (at HSB) or flooding (at LP) tides. The high frequency variability indicates that regardless of sampling time, a single sample of water tells one little about the true water quality, so multiple samples need to be collected. If it is not feasible to collect multiple samples, then a spatially or temporally composited sample will improve the estimate of the true water quality. At minimum, consecutive samples collected at 1 min intervals could be composited to obtain a better estimate of water quality. Policy makers, as well as scientists designing field campaigns for microbial source tracking and epidemiology studies, are cautioned that a single sample of water reveals little about the true water quality at a beach.

Predictive models (22, 33-35) may help to estimate average water quality given high frequency variability of measurements. These models use physical, chemical, and biological factors to predict concentrations of ENT. If enough high quality data are used to train models, they may be able to provide better estimates of the central tendency of daily ENT concentrations than single grab-sample measurements. Future work should examine this possibility by comparing model predictions to high frequency data measurements.

## Acknowledgments

R. Martone, M. Caldwell, D. Sivas, K. Yamahara, M. Pappas, L. Katz, and H. Gates assisted in organizing and implementing the field experiments. The students of "The California Coast: Science, Policy, and Law" class at Stanford University are acknowledged for their assistance in data collection. This work was supported by Stanford University's Law School Innovation in Teaching Fund and NOAA Oceans and Human Health Grant NA04OAR4600195. Some laboratory supplies were donated by S. Grant (UC Irvine). The author acknowledges K. Willis, N. Nidziko, M. Stacey, G. Shellenbarger, T. Julian, J. Hansel, D. Keymer, and two anonymous reviewers for comments that improved the manuscript.

## Supporting Information Available

Figures S1 and S2. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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ES071807V