Cowell Beach Microbial Source Tracking Study

Conducted by Stanford University
as part of the Source Identification Protocol Project (SIPP)

May 2014

Background

Cowell Beach was chosen as a microbial source tracking study site as part of SIPP due to its history of chronically poor microbial water quality. Cowell Beach is located in Santa Cruz, California (36°57.7' N, 122°1.5' W). The beach receives approximately 1,000,000 visitors per year, and has the worst summertime water quality among monitored California beaches, according to Heal the Bay's Beach Report Card. It was posted with water quality advisories 73 of 91 days during the summer of 2011. Cowell Beach experiences a Mediterranean climate with dry summers (May-Sept) and wet winters (Oct-Apr).

Cowell Beach is situated adjacent to a wharf, the San Lorenzo River outlet, and the Santa Cruz Harbor outlet. There is a large amount of wrack (dried kelp or other seaweed) that deposits on the beach that has been found to harbor high concentrations of fecal bacteria. Wrack has been suspected by many locals to be a major source of fecal contamination at Cowell beach. There are drainage pipes that discharge to the beach that contain runoff from Neary Lagoon and its watershed. In the summer, the pipes are buried in the sand. In the winter, they are above the sand. Homeless vagrants are believed to live in the vegetation surrounding Neary Lagoon and practice open defecation.

Several characteristics of Cowell Beach are similar to other California beaches, and thus it is hoped that the approaches used in the present study may inform microbial source tracking efforts in other locales. These characteristics include: popularity among tourists and surfers; potentially reduced surf zone circulation near a wharf; large amounts of wrack deposited on the beach; large resident population of avian wildlife; transient homeless populations; and aging infrastructure.

Potential Sources

There are a number of potential dry-weather sources of microbial pollution to the beach (Figure 1).

1. Wrack and sand at Cowell Beach harbor *Escherichia coli* and enterococci based on previous work by the county (Steve Peters, unpublished) and Imamura et al. [1].

2. Two pipes emanating from nearby Neary Lagoon drain lagoon discharge water to the beach (Figure 2) via overland flow in the winter and early spring. The pipe outlets are buried in the sand at the beach in dry weather when discharge from the pipes may potentially contaminate groundwater.

3. The nearby wharf attracts numerous birds and marine mammals that represent potential sources.

4. A flowing storm drain located to the west of the wharf. The storm drain discharges fresh water continuously.

5. The San Lorenzo River discharges a mixture of fresh and salt water depending on tidal condition.

6. The Santa Cruz Harbor discharges salt water during ebb tides.

7. A homeless population is present in the Cowell Beach watershed that practices open defecation.

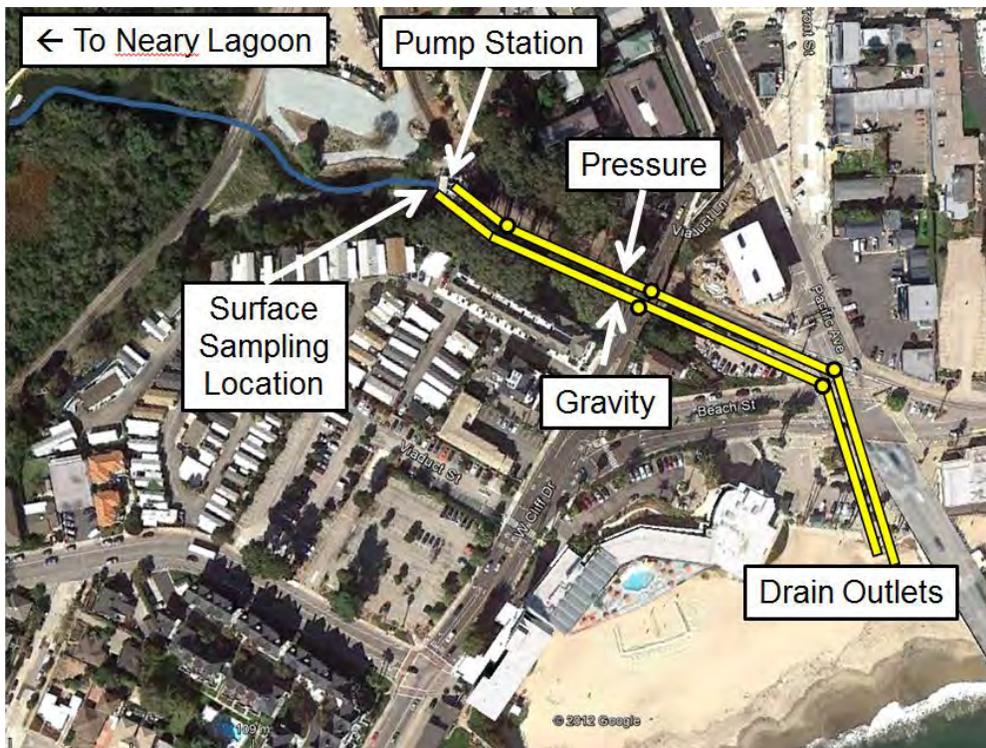**Figure 1. Possible dry weather sources of FIB to Cowell Beach.**

Figure 2. Schematic of the buried pipes in the beach adjacent to Cowell Beach. There is a pressurized and gravity pipe.

Stakeholder Involvement

Numerous stakeholders contributed to this project. We worked directly with the City of Santa Cruz, the County of Santa Cruz, the State Water Board, the Regional Water Board, Monterey Bay National Marine Sanctuary (NOAA), the California Coastal Commission, as well as members of the Clean Beach Task Force. The stakeholder meetings were organized and led by Stanford University. They were held on a quarterly to bi-yearly basis to jointly generate hypotheses, identify and discuss potential sources, exchange information on infrastructure, and discuss project findings and conclusions. Meetings were held in Santa Cruz at the City wastewater treatment plant. A final public meeting to present the findings of the study and discuss future actions by the city was held with the mayor and council members at the Santa Cruz City Council.

The City of Santa Cruz assisted with sampling by providing boat time to sample offshore of the beach. They also assisted by providing access to storm lines and Neary lagoon. Local citizens (surfers) assisted with the sampling during a dye study.

Our experience was that some agencies were not immediately forthcoming with relevant information. For example, it took nearly one year to learn exactly how the pipes connecting Neary Lagoon to the coastal ocean functioned and what potential there was for them to be a source of beach contamination. It is crucial that MST study teams be provided with the most recent sanitary infrastructure assessments, including information on known problems or postponed maintenance, early on in the study design process. After the MST study was completed, we continued to consult with the City and County regarding their next steps forward to address infrastructure concerns.

Hypotheses

The following four hypotheses were developed for the source tracking study. The initial focus was on ruling in or ruling out wrack as a source of contamination, while not ignoring the possibility of other sources. This focus was adopted because stakeholders, including city and county staff, felt strongly that wrack was an important FIB source based on their professional judgment.

H1.   Loading of fecal indicator bacteria (FIB) from wrack along the shore to the coastal ocean at Cowell Beach is significantly greater than loading of FIB from other sources to the coastal ocean at Cowell Beach.

H2.   FIB at Cowell beach are of human origin.

H3.   FIB at Cowell beach are of bird origin.

H4.   Removal of wrack from Cowell Beach by grooming reduces FIB concentrations at the beach.

Project Approach

H1 was tested by sampling potential sources of contamination to Cowell Beach, documenting spatiotemporal contamination patterns using off shore spatial, cross shore transect, weekly, and 24 h sampling, and then developing a process-based, mass-balance model of FIB at the beach (Figure 3, Tables 1 and 2). A process-based, mass-balance model was used to estimate FIB and marker fluxes from sand, wrack, and groundwater and assess their relative importance in controlling concentrations in the water column. A dye study was conducted to test connectivity between buried pipes and the ocean.

To test H2 and H3, we used molecular host-associated markers to investigate microbial pollution sources to Cowell Beach. We used human- and bird-associated molecular fecal markers to assess the presence of these fecal sources, respectively. Nearly all the weekly samples collected from the San Lorenzo River, the wharf, Cowell Beach, Neary Lagoon, the flowing storm drain, and the harbor were tested for human and gull markers as we wanted to capture any temporal variation in potential sources of contamination in these suspected sources. Every other water sample collected during the 24 h study (hourly samples) were tested for the human and gull marker. In addition, wrack and sand from Cowell beach with extraordinarily high concentrations of FIB were tested for the source tracking markers.

Figure 3 shows sampling locations used to test H1, H2, and H3. Table 1 provides the latitude and longitude of the sampling locations. The locations for the offshore spatial and transect sampling are provided in Table 2.

Figure 3. Map of Cowell Beach showing sampling locations used for testing H1, H2, and H3. Sampling locations for the weekly survey are highlighted in red, green and blue circles representing respectively, source samples, wrack samples and surf zone water samples. The blue line shows the location of the cross-shore transect and the two yellow lines show the locations of the gravity and force mains draining Neary Lagoon (also shown in Figure 2). Inset 'A' shows the location of the 24 h study transect in gray and the location of the groundwater samples with yellow circles (GW1-GW3). More detailed information on these sites and measurements are in Appendix 1 (Russell et al. 2013)[2]. Satellite photo provided by Google Earth (© 2013 Google and Terrametrics).

Table 1. Sites sampled during the weekly, 24 h, 48 h, and dye sampling studies.

Site ID	Site Type	Study Phase(s)	Latitude	Longitude	Site Description	Notes
H	Harbor	Weekly	36° 57.822'N	122° 0.106'W	Harbor	
SLR	River	Weekly	36° 57.912'N	122° 0.738'W	San Lorenzo River	
MB1	Ocean	Weekly	36° 57.800'N	122° 1.255'W	Main Beach, East	Also monitored by the Santa Cruz County
MB2	Ocean	Weekly	36° 57.831'N	122° 0.928'W	Main Beach, West	
W	Ocean	Weekly	36° 57.638'N	122° 1.292'W	Wharf	
CL1	Ocean	Weekly, 24 h, 48 h, dye	36° 57.721'N	122° 1.430'W	Cowell Beach, East	Also monitored by the Santa Cruz County
CL2	Ocean	Weekly	36° 57.670'N	122° 1.501'W	Cowell Beach, West	
SDM	Drain	Weekly	36° 57.576'N	122° 1.526'W	Storm Drain at Stairs	
O	Ocean	Weekly	36° 57.570'N	122° 1.493'W	Ocean adjacent to stairs	Also monitored by the Santa Cruz County
NS	Creek	Weekly	36° 57.798'N	122° 1.591'W	Neary Lagoon, collected at inlet to underground drain	
GW1	Groundwater	24 h	36° 57.732'N	122° 1.405'W		
GW2	Groundwater	24 h	36° 57.728'N	122° 1.397'W		
GW3	Groundwater	24 h	36° 57.724'N	122° 1.391'W		

Table 2. Sampling locations for offshore spatial grid and cross shore transect studies. Note: Various depths sampled at each transect site (Table continued on next page).

Site ID	Site Type	Study Phase(s)	Latitude	Longitude	Notes
OS1	Ocean	Offshore grid	36° 57.158'N	122° 1.306'W	
OS2	Ocean	Offshore grid	36° 57.307'N	122° 1.333'W	
OS3	Ocean	Offshore grid	36° 57.203'N	122° 1.238'W	
OS4	Ocean	Offshore grid	36° 57.265'N	122° 1.178'W	
OS5	Ocean	Offshore grid	36° 57.343'N	122° 1.213'W	
OS6	Ocean	Offshore grid	36° 57.432'N	122° 1.282'W	
OS7	Ocean	Offshore grid	36° 57.502'N	122° 1.387'W	
OS8	Ocean	Offshore grid	36° 57.557'N	122° 1.353'W	
OS9	Ocean	Offshore grid	36° 57.522'N	122° 1.268'W	
OS10	Ocean	Offshore grid	36° 57.425'N	122° 1.08'W	
OS11	Ocean	Offshore grid	36° 57.293'N	122° 0.982'W	
OS12	Ocean	Offshore grid	36° 57.392'N	122° 0.743'W	
OS13	Ocean	Offshore grid	36° 57.512'N	122° 0.827'W	
OS14	Ocean	Offshore grid	36° 57.595'N	122° 0.982'W	
OS15	Ocean	Offshore grid	36° 57.678'N	122° 1.18'W	
OS16	Ocean	Offshore grid	36° 57.708'N	122° 0.928'W	
OS17	Ocean	Offshore grid	36° 57.483'N	122° 0.775'W	
OS18	Ocean	Offshore grid	36° 57.532'N	122° 0.633'W	
OS19	Ocean	Offshore grid	36° 57.633'N	122° 0.52'W	
OS20	Ocean	Offshore grid	36° 57.713'N	122° 0.713'W	
OS21	Ocean	Offshore grid	36° 57.873'N	122° 0.132'W	
OS22	Ocean	Offshore grid	36° 57.592'N	122° 1.523'W	
OS23	Ocean	Offshore grid	36° 57.657'N	122° 1.448'W	
OS24	Ocean	Offshore grid	36° 57.747'N	122° 1.282'W	
OS25	Ocean	Offshore grid	36° 57.787'N	122° 1.018'W	
OS26	Ocean	Offshore grid	36° 57.783'N	122° 0.775'W	
OS27	Ocean	Offshore grid	36° 57.775'N	122° 0.62'W	
OS28	Ocean	Offshore grid	36° 57.15'N	122° 1.357'W	
OS29	Ocean	Offshore grid	36° 57.305'N	122° 1.347'W	
OS30	Ocean	Offshore grid	36° 57.202'N	122° 1.247'W	
OS31	Ocean	Offshore grid	36° 57.257'N	122° 1.173'W	
OS32	Ocean	Offshore grid	36° 57.328'N	122° 1.227'W	
OS33	Ocean	Offshore grid	36° 57.44'N	122° 1.283'W	
OS34	Ocean	Offshore grid	36° 57.483'N	122° 1.395'W	
OS35	Ocean	Offshore grid	36° 57.555'N	122° 1.353'W	
OS36	Ocean	Offshore grid	36° 57.525'N	122° 1.253'W	
OS37	Ocean	Offshore grid	36° 57.43'N	122° 1.098'W	
OS38	Ocean	Offshore grid	36° 57.292'N	122° 0.995'W	
OS39	Ocean	Offshore grid	36° 57.383'N	122° 0.842'W	
OS40	Ocean	Offshore grid	36° 57.512'N	122° 0.848'W	

Table 2. Continued

Site ID	Site Type	Study Phase(s)	Latitude	Longitude	Notes
OS38	Ocean	Offshore grid	36° 57.292'N	122° 0.995'W	
OS39	Ocean	Offshore grid	36° 57.383'N	122° 0.842'W	
OS40	Ocean	Offshore grid	36° 57.512'N	122° 0.848'W	
OS41	Ocean	Offshore grid	36° 57.6'N	122° 0.98'W	
OS42	Ocean	Offshore grid	36° 57.675'N	122° 1.168'W	
OS43	Ocean	Offshore grid	36° 57.702'N	122° 1.047'W	
OS44	Ocean	Offshore grid	36° 57.58'N	122° 0.847'W	
OS45	Ocean	Offshore grid	36° 57.533'N	122° 0.663'W	
OS46	Ocean	Offshore grid	36° 57.613'N	122° 0.508'W	
OS47	Ocean	Offshore grid	36° 57.72'N	122° 0.718'W	
OS48	Ocean	Offshore grid	36° 57.892'N	122° 0.133'W	
OS49	Ocean	Offshore grid	36° 57.592'N	122° 1.523'W	
OS50	Ocean	Offshore grid	36° 57.657'N	122° 1.448'W	
OS51	Ocean	Offshore grid	36° 57.747'N	122° 1.282'W	
OS52	Ocean	Offshore grid	36° 57.787'N	122° 1.018'W	
OS53	Ocean	Offshore grid	36° 57.783'N	122° 0.775'W	
OS54	Ocean	Offshore grid	36° 57.775'N	122° 0.62'W	
T1	Ocean, sand, kelp	Cross shore transect	36° 57.341'N	122° 1.379'W	Depth 7.62 m
T2	Ocean, sand, kelp	Cross shore transect	36° 57.424'N	122° 1.373'W	Depth 5.18 m
T3	Ocean, sand, kelp	Cross shore transect	36° 57.524'N	122° 1.405'W	Depth 4.57 m
T4	Ocean, sand, kelp	Cross shore transect	36° 57.616'N	122° 1.409'W	Depth 3.35 m

To investigate the impacts of intensive grooming on coastal water quality (H4) we performed two studies at Cowell Beach (Figure 4). The long-term impacts of beach grooming on water quality were assessed by comparing FIB concentrations during two summers, one with (2012) and one without (2011) intensive grooming (sites shown in blue and green in Figure 4). The immediate impacts of beach grooming were also assessed over a 48 h period in which intensive grooming first occurred at Cowell Beach (early summer 2012, inset A in Figure 4).

Figure 4. Map of Cowell and Main Beaches in Santa Cruz, CA with important locations investigated in the grooming studies highlighted. The location of the 48 h study is shown in Inset A. The sampling locations of the long-term study are shown with circles indicating water samples and squares indicating the wrack samples (CL1, CL2, MB1, MB2, Table 2). During every visit, wrack density measurements were taken at the two westerly locations. Satellite photo provided by Google Earth (© 2013 Google and Terrametics). Latitudes and longitudes of these sites are provided in Table 1.

Revised Hypotheses
Discussions with stakeholders revealed that the pipes draining Neary Lagoon (Figure 2) had not been adequately tested for leaks and cross connections by the City or County. Therefore, we hypothesized that these pipes could be important sources of contamination to the beach. Additional efforts were made to sample these pipes once this information was uncovered. The water in the pipes was tested for FIB. When high levels of FIB were found, the water was also tested for human and gull markers. The sampling locations for these buried pipes and associated sampling are given in Table 3.

Table 3. Location of samples collected during investigation into buried pipe system shown in Figure 2.

Site ID	Site Type	Study Phase(s)	Latitude	Longitude	Site Description
MH_OSUB513	Urban water	Manhole sampling	36° 57.828'N	122° 1.562'W	Upstream stormdrain
MH-CL	Urban water	Manhole sampling	36° 57.719'N	122° 1.447'W	At Cowell Beach grate
MH-Gutter	Urban water	Manhole sampling	36° 57.828'N	122° 1.562'W	Upstream gutter with water
MH-OSCA512	Urban water	Manhole sampling	36° 57.828'N	122° 1.562'W	Upstreadm stormdrain
MHG2	Urban water	Manhole sampling	36° 57.783'N	122° 1.537'W	Gravity 2
MHG3	Urban water	Manhole sampling	36° 57.781'N	122° 1.489'W	Gravity 3
MHNDR	Urban water	Manhole sampling	36° 57.792'N	122° 1.64'W	Diversion to WWTP
MHNSR	Lagoon	Manhole sampling	36° 57.798'N	122° 1.591'W	Neary surface
MHP1	Urban water	Manhole sampling	36° 57.785'N	122° 1.583'W	Pressure MH1
MHP2	Urban water	Manhole sampling	36° 57.783'N	122° 1.537'W	Pressure MH2
MHP3	Urban water	Manhole sampling	36° 57.781'N	122° 1.489'W	Pressure MH3
MHSD2	Urban water	Manhole sampling	36° 57.783'N	122° 1.537'W	Gravity (SD)
MHU2	Urban water	Manhole sampling	36° 57.783'N	122° 1.537'W	Gravity (U)

Project Outcomes

We analyzed the spatial-temporal patterns in FIB contamination at Cowell Beach to gain insights into potential sources (H1). Using a combination of spatial bay-wide and cross shore transect surveys using a city boat and volunteer Stanford scientific divers, we confirmed that there was a hot spot of pollution just west of the pier at Cowell Beach (Figure 5, see Appendix 1 for additional figures). The hot spot was at the shoreline and there was no hot spot offshore indicating there was a shoreline contamination source. Further, we determined during a 24 h sampling study that the contamination was greatest during low-sunlight hours, and that enterococci concentrations were highest at high tide, while *E. coli* concentrations were highest at low tide (Figure 6). These spatial and temporal patterns provide clues as to the pollution source. The decoupling of enterococci and *E. coli* concentrations during different tidal conditions suggests they may come from different sources.
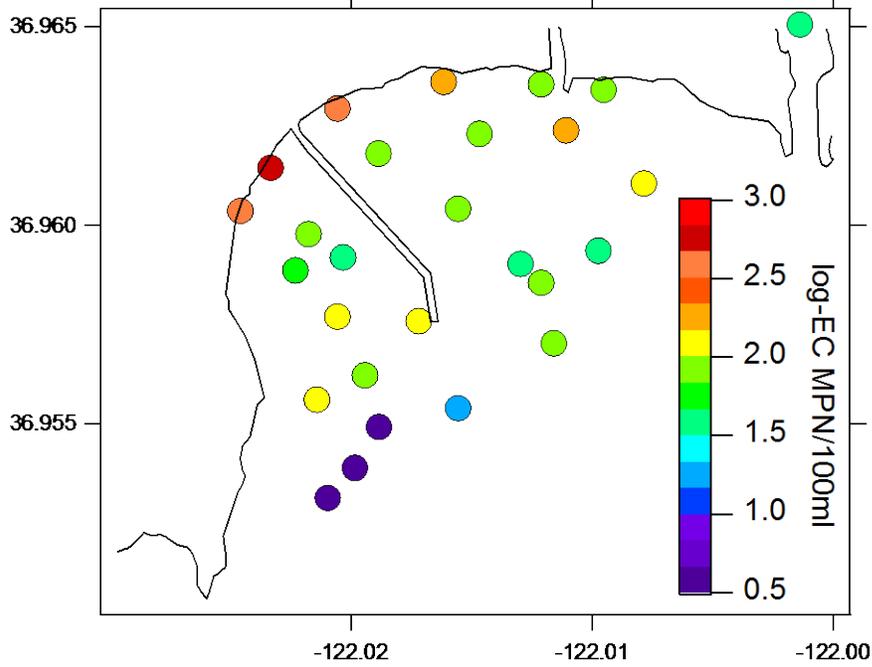
Figure 5. Concentrations of *E. coli* (log-10 transformed) at surface stations within the bay adjacent to Cowell beach, and the wharf. The highest concentrations were observed adjacent to the shoreline and next to the wharf suggesting a localized, shoreline source of contamination. Similar results were seen for enterococci (Appendix 1, Russell et al. 2013 [ii]).
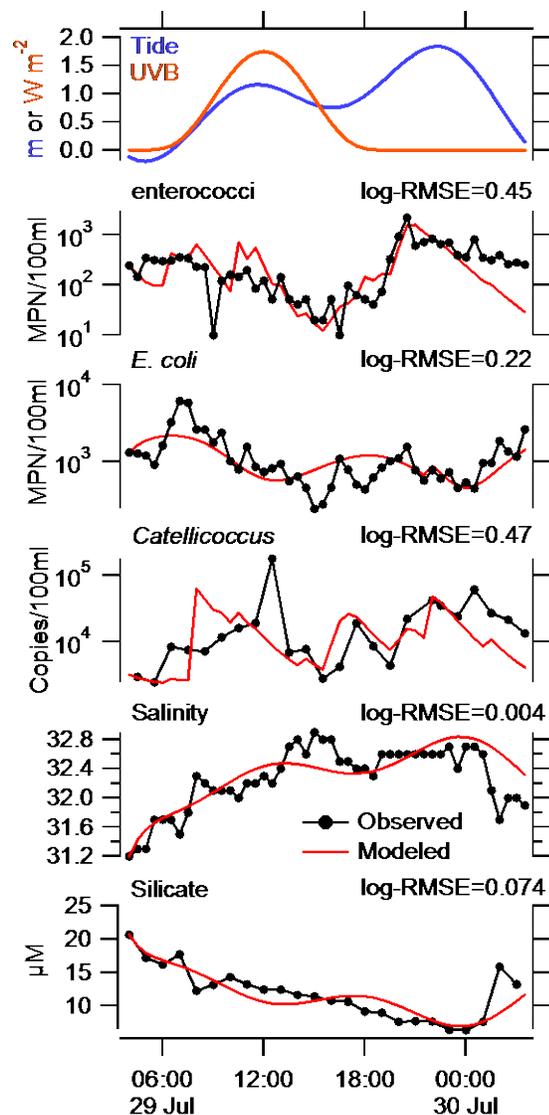
Figure 6. Observed and modeled data for tide, UVB intensity, enterococci, *E. coli*, *Catellicoccus*, salinity, and silicate. Black lines in the bottom five panels show measured data, red lines show calculated model best fits. Log-RMSE values represent the best fit root mean square error between the log$_{10}$-transformed modeled and observed data. Units for log-RMSE vary by panel and are the log of the unit specified on the left axis.

Sampling of potential sources ruled out the San Lorenzo River, the Santa Cruz Harbor, the wharf, and the flowing storm drain near the stairs at Cowell Beach as major contributors to shoreline FIB (Figure 7). This is because the concentrations of FIB in these sources were lower than, or similar to, the concentrations at Cowell Beach. Wrack and sand contained elevated concentrations of FIB on a per mass basis, but it is difficult to assess from just these measurements their potential to be important sources. A pipe buried in the sand (gravity main in Figure 7) just adjacent to the 'hot spot' of contamination at Cowell beach had extraordinarily high concentrations of FIB in its water (~1000 MPN/100 mL enterococci and ~10,000 MPN/100 mL *E. coli*) suggesting it could be an important FIB source to the coastal ocean via submarine groundwater discharge.
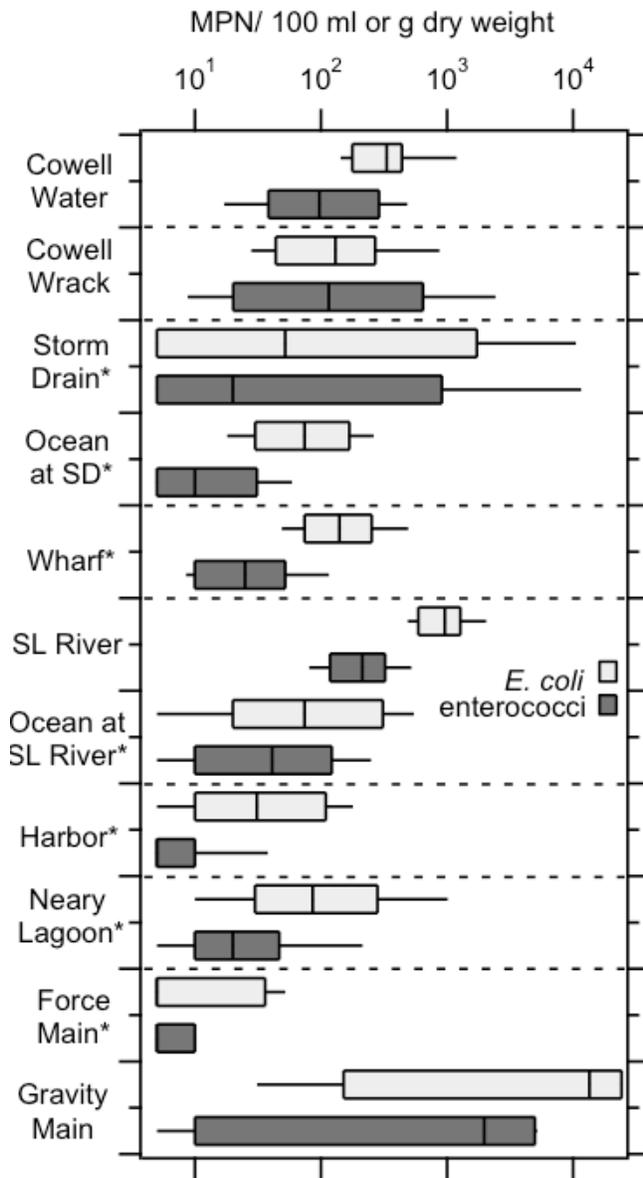
Figure 7. Box and whisker plots of FIB concentrations at Cowell Beach (both sample locations, water and wrack), storm drain (SD), wharf, and San Lorenzo (SL) River, harbor, Neary Lagoon, force main and gravity main. All data collected during the long-term survey and drainage pipe survey are shown. Water samples with significantly lower (p<0.1) log-mean concentrations of FIB than the Cowell Beach samples are highlighted with an '*'. Box represents 25th, 50th and 75th percentiles; whiskers represent 10th and 90th percentiles.

A mass balance model of the surf zone was developed to compare the fluxes of FIB to the ocean from the three sources found to have the highest potential for causing contamination at Cowell Beach: groundwater, kelp, and sand. The mass balance model used data from the 24 h study as well as various input parameters developed from first principles (Appendix 1 and Russell et al. 2013[ii]). As indicated in Figure 6, the model fit the data well. The model indicated that wrack could not be an important source of FIB to the surf zone. This is because even though the concentrations of FIB on wrack are very high (~100 MPN/g dry weight), the total number of bacteria present on the wrack within the reach of the tide is low relative to the numbers present in the surf zone. The model indicated that groundwater was an important source of *E. coli*, but not enterococci to the coastal ocean. Because the buried pipe (gravity main) was discharging extremely contaminated water to the beach aquifer, the pipe represents a potential source of these *E. coli*. The lack of groundwater-sourced enterococci to the surf zone may be a result of the differential removal of these bacteria as they are transported through the beach aquifer. A follow up dye study confirmed that water from the pipe is transported through the beach and discharges to the surf zone (Figure 8). The model indicated that sand was the major source of enterococci to the ocean. Even though sand had lower levels of enterococci per mass (~10 MPN/g dry weight) than the wrack, there is more sand on the beach and hence more enterococci from the sand to enter the surf zone, elevating concentrations there. Flux estimates from the model are presented in Table 4.

Table 4. Model calculated daily fluxes from groundwater, sand, and wrack.

Model	Daily Groundwater Flux [MPN or copies/ d/m] (% of total daily flux)	Daily Sand Flux [MPN or copies/ d/m] (% of total daily flux)	Daily Wrack Flux [MPN or copies/ d/m] (% of total daily flux)
enterococci	--[1] (0%)	1.2×10^8 (99.3%)	8.4×10^5 (0.07%)
E. coli	1.9×10^8 (97.8%)	--[1] (0%)	4.3×10^6 (2.2%)
Catellicoccus	--[1] (0%)	1.7×10^9 (73.5%)	6.1×10^8 (26.4%)

[1] No flux was calculated because the best fit model did not include this source.
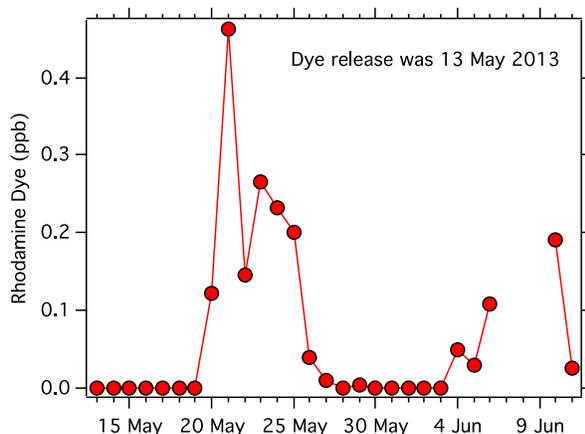

Figure 8. Concentrations of rhodamine dye at ankle depth of the surf zone in late spring of 2013.

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Human and gull-associated markers were used to further probe the extent to which humans and birds contributed to the contamination (H2 and H3). A low, persistent level of HF183Taqman (human marker) was found in surf zone samples, and a very high level in the buried gravity pipe (~10^6 copies / 100 mL) (Russell et al. 2013[ii]). This suggests the presence of raw sewage in the buried pipe that is passing through the beach and contaminating the surf zone. High levels of gull marker were found in the surf zone and in the sand (Figure 9, Appendix 1), and enterococci concentrations correlated to gull marker concentrations in the water. This suggests that bird feces on the sand may be an important source of enterococci to the beach. The gull marker detects both gulls and pigeons and the wharf attracts large numbers of these birds.

During the second year of the study (2012), intensive grooming was conducted at the beach, and we used this an opportunity to test H4. At the first intensive grooming event, the beach was sampled continuously for 48 hr. We found that this grooming event did not have an immediate impact of enterococci and *E. coli* in the surf zone (Appendix 2 and Russell et al. 2014[3]). Further, the water quality at Cowell Beach during the summer of intensive grooming was the same as the previous summer without intensive grooming (Figure 9, Appendix 2). The results indicate that intensive grooming did not improve water quality at the beach and thus suggest that wrack is not a major contributor to poor water quality, consistent with the modeling results discussed earlier.
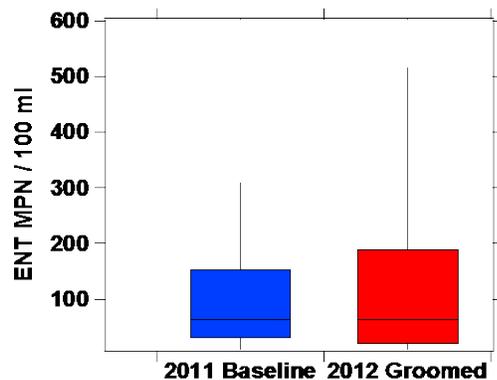

Figure 9. Comparison of enterococci (ENT) during the baseline year when there was no intensive grooming and the treatment year (2012) when there was intensive grooming. There was no difference in water quality. The plot for *E. coli* is very similar. The data are displayed as box and whisker plots with the box showing the 25th and 75th percentiles with the median line through the center. The tails show the 10th and 95th percentiles.

Lessons Learned

- Communication and cooperation is very important among stakeholders.

- It takes time for all the important information and knowledge to bubble up during stakeholder meetings. Hypotheses may need to be refined and work that was originally unplanned may need to be executed.

- Visible sources (wrack in this case) can mask infrastructure problems.

- A 'multiple line of evidence' approach is useful for reaching credible conclusions.

Next Steps

The city of Santa Cruz has applied for CBI grants to conduct infrastructure repairs. These grants include funds for working on their force main as well as a pump station and the construction of a flap for the pipe buried in the beach that contains raw sewage.

The city is doing follow up testing of human marker as they make repairs to sewer system.

The county has taken advantage of training on performing qPCR for MST markers provided by the Southern California Coastal Water Research Project as part of the SIPP and is now measuring the human marker using SIPP methods in house.

Note

The results of this study have been published in two peer-reviewed papers that are available by emailing Alexandria Boehm (aboehm@stanford.edu).

Russell, T.L., L.M. Sassoubre, D. Wang, S. Masuda, H. Chen, C. Soetjipto, A. Hassaballah and A.B. Boehm. 2013. A coupled modeling and molecular biology approach to microbial source tracking at Cowell Beach, Santa Cruz, CA, USA. *Environmental Science & Technology* 47:10231-10239.

Russell, T.L., L.M. Sassoubre, C. Zhou, D. French-Owen, A. Hassaballah and A.B. Boehm. 2014. Impacts of beach wrack removal on surf zone water quality at a marine beach. *Environmental Science & Technology* 48:2203-2211.

Imamura, G., R.M. Strickfaden, A.B. Boehm and J.A. Jay. 2011. Beach wrack is a reservoir for faecal indicator bacteria along the California coast. *FEMS Microbiology Ecology* 77:40-49.

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References

[1] G. Imamura, R. M. Strickfaden, A. B. Boehm, J. A. Jay. 2011. Beach wrack is a reservoir for faecal indicator bacteria along the California coast, *FEMS Microbiology Ecology*, 77, 40-49.

[2] T. L. Russell, L. M. Sassoubre, D. Wang, S. Masuda, H. Chen, C. Soetjipto, A. Hassaballah and A. B. Boehm. 2013. A coupled modeling and molecular biology approach to microbial source tracking at Cowell Beach, Santa Cruz, CA, USA. *Environmental Science & Technology* 47:10231-10239.

[3] T. L. Russell, L. M. Sassoubre, C. Zhou, D. French-Owen, A. Hassaballah and A. B. Boehm. 2014. Impacts of beach wrack removal on surf zone water quality at a marine beach. *Environmental Science & Technology* 48:2203-2211.

A coupled modeling and molecular biology approach to microbial source tracking at Cowell Beach, Santa Cruz, CA, USA

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Abstract

Consistently high levels of bacterial indicators of fecal pollution rank Cowell Beach as the most polluted beach in California. High levels of fecal indicator bacteria (FIB), *E. coli* and enterococci, are measured throughout the summer, resulting in beach advisories with social and economic consequences. The source of the fecal indicator bacteria (FIB), however, is unknown. Speculations have been made that the wrack accumulating on the beach is a major source of FIB to the surf zone. The present study uses spatial and temporal sampling coupled with process-modeling to investigate potential FIB sources and the relative contributions of those sources. Temporal sampling showed consistently high FIB concentrations in the surf zone, sand, and wrack at Cowell Beach, and ruled out storm drain, the river, the harbor, and the adjacent wharf as the sources of the high concentrations observed in the surf zone. Spatial sampling confirmed that the source of FIB to the beach is terrestrial rather than marine. Modeling results showed two dominant FIB sources to the surf zone, sand for enterococci and groundwater for *E. coli*. FIB from wrack represented a minor contribution to bacterial levels in the water. Molecular source tracking methods indicate the FIB at the beach is of human and bird origin. The microbial source tracking (MST) approach presented here provides a framework for future efforts.

Introduction

In much of the world fecal indicator bacteria (FIB), including enterococci (ENT) and *E. coli* (EC), are used to assess recreational water quality in an effort to protect the health of beachgoers. FIB are not pathogens, but their concentrations correlate with increased gastrointestinal illness in swimmers at beaches impacted by wastewater and urban runoff.[1-4] In the United States, there were over 23,400 beach advisories and closures in 2011 due to elevated FIB concentrations, representing a slight decrease (3%) from 2010 but a major increase (~325%) relative to 1998.[5] The majority of beach advisories and closures are caused by unknown sources.[5] This uncertainty represents a great challenge for beach remediation efforts.

There are numerous possible sources of FIB to coastal waters. FIB are often high in flowing sources from the land to the sea such as rivers, creeks, and storm drains, especially in urban areas.[6] In some cases, FIB in these sources have been attributed to failing sewage infrastructure.[7] Other possible FIB sources to coastal waters include wildlife feces[8], and sands and soils which can harbor persistent extraenteric FIB populations along the coast[9-15], wrack (decaying marine plants) which has been shown to harbor extraordinarily high FIB concentrations in some locations[16-22] and contaminated groundwater[11]. An additional possible source of FIB is treated wastewater discharge offshore via an outfall.

Microbial source tracking (MST) is the identification of fecal pollution sources in ambient waters. It typically consists of multiple phases including characterizing the temporal and spatial patterns of the FIB pollution, potential sources and associated fluxes, and molecular, host-specific markers to confirm the presence or absence of human and/or animal sources.[23] There are a number of host-specific markers that are both sensitive and specific to feces from their intended targets[24] that can be implemented in the final phase.

The use of process-based models (i.e., mass balance models that explicitly account for processes that control bacterial concentrations) can augment MST efforts as they can assist in understanding the relative importance of different sources and sinks in controlling local microbial concentrations. For example, a study in Avalon, California used a process-based model to show that a groundwater source was needed to explain the temporal pattern of microbial contamination at the beach.[11] A process-based finite element model was used in southern Lake Michigan, to show the relative importance of FIB physical transport and removal mechanisms including sedimentation and photoinactivation.[25] A mass-balance model in Buttermilk Bay, MA showed that bay sediments were the dominant source of FIB while birds, surface runoff, groundwater and streams played a minor role.[18] A process-based mass balance model developed for enclosed beaches evaluated the relative impacts of various shore sources on beach microbial water quality.[26]

The present study uses process-based, mass-balance modeling coupled with molecular host-specific markers to investigate microbial pollution sources to an urban marine beach: Cowell Beach, Santa Cruz, California (36°57.7' N, 122°1.5' W, Fig. 1). Cowell Beach, which receives approximately 1,000,000 visitors per year[27], has the worst summertime water quality among monitored California[28, 29] beaches. It was posted with water quality advisories 73 of 91 days during the summer of 2011.[30] The specific objectives of the study were to define the spatial and temporal patterns of summertime microbial pollution at the beach utilizing intensive sampling, assess contamination levels in potential sources and the associated fluxes to Cowell Beach, and use human- and bird-specific molecular fecal markers to assess the presence of these fecal sources. A process-based, mass-balance model was used to estimate FIB and marker fluxes from sand, wrack and groundwater and assess their relative importance in controlling concentrations in the water column. The methodology illustrated here can serve as a guide for implementing microbial source tracking (MST) at other beaches experiencing beach advisories and closures.

Materials and Methods

Field Site. Cowell Beach experiences a Mediterranean climate with dry summers (May – Sept) and wet winters (Oct-Apr). The study took place during the summers of 2011 and 2012, with little-to-no rainfall (0.83 and 0.38 cm, respectively[31]). There are a number of potential dry-weather sources of microbial pollution to the beach (Fig. 1). Wrack and sand at Cowell Beach harbor high concentrations of EC and ENT.[9, 16] Two pipes emanating from nearby Neary Lagoon drain lagoon water to the beach. The pipe outlets are buried in the sand at the beach; discharge from the pipes may potentially contaminate groundwater. The nearby wharf attracts numerous birds and marine mammals which represent potential sources. A flowing storm drain, the San Lorenzo River, and the Santa Cruz Harbor represent additional potential sources of FIB to the beach.

Spatial and Cross-shore Surveys. To isolate the spatial extent of the contamination problem, a boat was used to sample surface waters in the coastal ocean adjacent to the beach along several cross-bay transects (Fig. S1) on 26 July (flood tide) and 31 August (ebb tide) 2011 before sunrise during spatial surveys. Concurrently, six shoreline samples were collected in knee deep water. More details are in the supporting information (SI).

Scientific divers collected water and kelp (*Macrocystis pyrifera*) throughout the water column and sand at the sea floor at a series of locations spanning the cross-shore (Figs. 1 and S2) to assess levels of microbial contamination in a cross-shore survey. More details are in the SI.

Long-term Survey. In the long-term survey, we collected samples at least weekly between 24 June and 11 August 2011 and 22 May and 28 August 2012 to investigate differences between contamination in sources and beach water. Marine water samples were collected from Cowell Beach (west and east ends)

(2011 only), near the San Lorenzo River mouth, near the storm drain and near the wharf. Two wrack samples were collected from Cowell Beach (2011 only) if wrack was present. Wrack samples were collected from the high-high tide line as described below (Fig. 1). 'Source' water samples were collected from: San Lorenzo River, the storm drain, the Santa Cruz Harbor (2012 only), and Neary Lagoon (2012 only) (Fig. 1). Storm drain flow rates were measured onsite by timing the filling of a fixed volume container. San Lorenzo River flow rates were estimated from a USGS flow gauge.[32]

Drainage Pipe Survey. Samples were collected from manholes located along the two 66" drainage pipes connected to Neary Lagoon (Fig. 1) on three visits 23 July 2012, 12 September 2012 and 18 October 2012. The pipes were not sampled earlier in the study because their existence was not discovered by the authors until summer 2012. The two pipes are referred to henceforth as "force main" for the pipe connected to a large pump station (though flowing under gravity flow during summer) and "gravity main" for the other pipe. The "gravity main" was not sampled on the July sampling visit. The two pipes terminate in the subsurface at Cowell Beach.

Twenty-four Hour Study. A 24 h study was performed from 0400 h 29 July 2011 to 0330 h 30 July 2011 during a spring tide at Cowell Beach adjacent to the wharf (Fig. 1). Sampling occurred every 30 min. At each time point, five samples were collected: *exposed wrack*, *exposed sand*, *surf zone water*, *submerged wrack*, and *submerged sand*. One minute prior to the sampling time wave run-up was observed and the highest run-up point was defined as the *water line*. *Exposed wrack* and *exposed sand* were collected, respectively, at the location between 1-2 m above the *water line* that had the most wrack and 1.5 m above the *water line*. *Submerged wrack* and *submerged sand* were collected, respectively, from either the wave run-up or knee depth water and from the submerged sands under knee depth water. Groundwater was sampled once (GW2, GW3) and twice (GW1) from locations spanning the transect

(Fig. 1). Wrack spatial density was measured in the transect at the conclusion of the 24 h study so as not to disturb the wrack during the study following Dugan et al. [33]

General Field and Laboratory Methods. Three types of samples were collected in this study using sterile techniques: water, sand, and wrack. Detailed field and laboratory methods can be found in the SI. Sand and wrack represented composites from a 5 m stretch of beach in the alongshore direction. After collection, samples were stored on ice until and processed in the laboratory within 6 h for FIB, EC and ENT, using defined substrate assays. Concentrations are expressed as per dry weight for the sand and wrack. For data analysis, samples below the lower detection limit were assigned a value of half the detection limit; and samples above the upper detection limit were assigned that value.

Water and wrack and sand eluants were membrane filtered through 0.4 µm polycarbonate (PC) filters (EMD Millipore, Billerica MA) to preserve bacterial DNA for molecular analysis. Filters were flash frozen with liquid nitrogen and stored at -80°C until analysis. Within 15 months, they were extracted and processed in triplicate by qPCR to enumerate human-associated *Bacteroidales* HF183Taqman[34] and gull-associated *Catellicoccus*[35]. Samples yielding two or more positive PCRs and with a concentration within the range of standards were classified as positive in the range of quantification (ROQ). Samples with two or more positive PCR but having an averaged concentration below the lowest consistently detectable standard concentration were classified as below limit of quantification (BLOQ). Samples with zero or one positive PCR were classified as non-detect (ND).

Water temperature and salinity was measured in the field using a YSI 30 (YSI, Yellowsprings, OH). Silicate, dissolved inorganic nitrogen (DIN), and phosphate concentrations were measured in samples collected during the 24 h study using a nutrient autoanalyzer (see SI).

Statistical Analyses. Statistical analyses were performed in PASW Statistics 18 (IBM, Armonk, NY). Analyses performed included t-tests, one-way ANOVAs and Spearman's rank correlations. For t-test and one-way ANOVAs, the FIB and marker data were $\log_{10}$ transformed as they were found to be approximately log-normally distributed as determined by a Lilliefors test. Results are presented if $\alpha < 0.1$.

Model Formulation. An unsteady, one-dimensional mass-balance model was developed in Matlab (Natick, MA) to predict waterborne concentrations of EC, ENT, and *Catellicoccus* during the 24 h study, and assess the relative importance of sand, wrack and groundwater as bacterial sources (Fig. 2). The model assumes a well-mixed, constant-volume surf zone with constituent concentration C_{sz} [MPN or copies/100 ml]. The location of the surf zone, relative to a fixed datum, changes as the tide rises and falls maintaining a constant volume. At each 0.5 h time step, a fraction of the surf zone volume (α_{open} [h^{-1}]) is exchanged with offshore water with constituent concentration C_{open} [MPN or copies/100 ml]. This is a simplification of the complex mixing and transport processes that occur in the surf zone [36-38] and assumes that there is limited variation in modeled parameters in the alongshore direction. The width of the surf zone (L_{SZ} [m]) and slope of the beach (θ [mm^{-1}]) were set to 7 m and 0.05, respectively, based on observations during the study.

Bacterial sources to the surf zone include sand, wrack, submarine groundwater discharge, and offshore waters. The San Lorenzo River, the harbor, the storm drain, and marine mammals were not included because they were eliminated as sources as discussed in the discussion section. Bacteria associated with exposed (i.e., subaerial) sand and wrack enter the water column when they are inundated by rising flood tide water in a process referred to as 'washing' (parameterization in the SI). Values of C_{open} were set to the lowest measured value of the modeled bacteria in the surf zone over the course of the study. Groundwater was assumed to flow with constant concentrations of bacteria (C_{gw} [MPN or copies/100 ml]). The

groundwater flow rate was estimated using a model similar to Boehm et al. [11] along with measurements of silicate and salinity. Further details of the groundwater model are provided in the SI.

Bacterial sinks include exchange with offshore water, photoinactivation described by decay constant k_{sun} [$h^{-1}I^{-1}$] where I is the intensity of UVB in W/m^2 obtained from the Simple Model of the Atmospheric Radiative Transfer of Sunshine(SMARTS)[39], and first order decay via "dark" mechanisms (k_{dark} [h^{-1}]). Dark inactivation represents inactivation by all processes unrelated to sunlight.

The full mass balance for a given microorganism M is given by

$$M_{SZ}^t = M_{SZ}^{t-1} + \Delta t \begin{bmatrix} C_{open}V_{SZ}\alpha_{open} - C_{SZ}^{t-1}V_{SZ}\alpha_{open} + C_{gw}Q_{gw}^{t-1} - C_{SZ}^{t-1}Q_{gw}^{t-1} \\ + C_{sand}\dot{m}_{sand}\alpha_{sand}\delta_{x,flood} + C_{wrack}\dot{m}_{wrack}\alpha_{wrack}\delta_{x,flood} \\ - (k_{sun}I^{t-1} + k_{dark})M_{SZ}^{t-1} \end{bmatrix} \quad (1)$$

where the superscripts t and t-1 denote the current and previous time steps respectively, Δt is the time between model time steps (0.5 h), M_{sz} is the number of bacteria in the surf zone, V_{SZ} is the volume of the surf zone, $\dot{m}_{sand}$ is the mass of sand washed per time and is equal to the product of the area of beach washed between t-1 and t, the depth of sand washed (d$_{sand}$), and the sand bulk density [g/h], $\dot{m}_{wrack}$ is the mass of wrack washed per time and is equal to the mass of wrack covering the area of the beach washed between t-1 and t [g/h], C_{sand} and C_{wrack} are bacterial concentrations on sand and wrack [MPN or copies/ g], $\delta_{x,flood}$ is a Kronecker delta where x is "flood or ebb" describing the tidal conditions between the current and previous time steps, and α_{open}, α_{wrack}, and α_{sand} are exchange parameters defining respectively as the fraction of volume exchanged with the offshore/ time [h^{-1}], and the fraction FIB washed off wrack and sand [-]. With the groundwater flow rate (Q_{gw} [L/min/h]) and offshore exchange fraction (α_{open}) constrained using the silicate model, the following parameters were calculated for each modeled bacterium by minimizing the log-RMSE between observed surf zone bacterial concentrations those produced by the model: C_{gw}, α_{sand}, α_{wrack}, d_{sand}, k_{sun}, and k_{dark}. C_{gw}, α_{sand}, α_{wrack}, k_{sun} and k_{dark} were set to 0

unless they improved the model fit. It is acknowledged that the computer model developed here is not verified against an independent data set and is not suitable for forecasting.

Results

Spatial Survey. FIB in the bay adjacent to Cowell Beach were consistently higher in shoreline samples compared to near-shore samples (Fig. S1) (both EC and ENT, $p<0.01$). EC and ENT in the ocean near the mouth of the San Lorenzo River, in the Santa Cruz Harbor, and adjacent to the storm drain were lower ($p<0.01$) or not different than, respectively, those observed along the Cowell Beach shoreline. ENT and EC were significantly, negatively correlated with distance from shore (data from flood and ebb survey combined, respectively: $r_s= -0.42$, $p<0.01$; $r_s= -0.45$, $p<0.01$). Salinity (Fig. S3) ranged from 28.7 to 33.1 (median = 32.8) and EC was significantly negatively correlated with salinity ($r_s=-0.37$, $p<0.01$).

Cross-shore Survey. Offshore kelp had nearly 2 orders of magnitude lower EC and ENT than shore wrack (mean log difference [log-MPN/g dry] respectively: 1.9, $p<0.01$; 1.7, $p<0.01$) (Fig. S4). Offshore submerged sand had 2 to 3 orders of magnitude lower EC and ENT than subaerial, exposed sand on the beach (mean log difference [log-MPN/g dry] respectively: 2.8, $p<0.01$; 2.0, $p<0.01$) (Fig. S5). Offshore water had an order of magnitude lower EC and ENT than surf zone water (mean log difference [log-MPN/100 ml] respectively: 1.0, $p=0.01$; 1.3, $p<0.01$) (Fig. S6).

Long-term and Drainage Pipe Surveys: The median ENT and EC concentration in Cowell Beach water were respectively: 97 and 332 MPN/100 ml, and on wrack: 115 and 130 MPN/g dry weight. All Cowell Beach water samples were processed for both HF183 and *Catellicoccus*. HF183 was detected in 75% of samples at levels below the limit of quantification (which was 500 copies/100 ml). *Catellicoccus* was detected in 100% of water samples at concentrations between 510 and 2.2×10^5 copies/100 ml (Table 1).

The storm drain, wharf, San Lorenzo River, and harbor had significantly lower or comparable levels of FIB as Cowell Beach (Fig. 3). Accordingly, sample locations in the ocean near the discharge of the storm drain and San Lorenzo River have FIB concentrations significantly lower ($p<0.05$) than those observed at Cowell Beach. The median FIB fluxes from storm drain and the San Lorenzo River are respectively on the order of 10^7 and 10^{11} MPN/day for both ENT and EC. Molecular methods detected HF183 in the storm drain, wharf, San Lorenzo River, harbor (Table 1). Concentrations were ND or BLOQ in the storm drain, as high as 19,000 copies /100 mL in the river, as high as 890 copies/100 ml in the harbor and once detected at the wharf at 1,800 copies/100 ml. *Catellicoccus* was detected at the wharf, the San Lorenzo River and the harbor, but not the storm drain (Table 1). *Catellicoccus* in these sources varied from ND to 11,000 copies / 100 mL, and were significantly lower than at Cowell Beach ($p<0.05$).

Neary Lagoon and the pipes leading from it to the beach contained ENT, EC, and HF183 marker but low amounts of *Catellicoccus*. ENT and EC were as high as 5172 MPN/100 ml and >24192 MPN/100 mL, respectively, in the gravity main but low (<53 MPN/100 mL) in the force main (Fig. 3). Greater than 10^6 copies / 100 mL HF183 were present in the gravity main, but concentrations BLOQ were present in the force main. The gravity main consistently had higher FIB and HF183 concentrations than Neary Lagoon, suggesting additional bacterial sources along the main (mean log difference between lagoon and gravity main ENT, EC, and HF183 [log MPN or copies/100ml]: 1.25, $p=0.03$; 1.15, $p=0.06$; 1.23, $p=0.06$). Half of the gravity main samples ($n=4$) were above the EC upper detection limit so actual concentrations are higher than those reported. *Catellicoccus* was ND or BLOQ in all samples from the force main and found 50% of the time in the gravity main, and had a median concentration of 1020 copies/100 ml when detected.

Twenty-four Hour Study. A total of 48 *surf zone water* samples, 48 *exposed sand* and *submerged sand* samples, 47 *exposed wrack* samples and 44 *submerged wrack* samples were collected and processed for

FIB during the 24 h study. FIB concentrations in the *surf zone water* ranged from 10-2224 ENT MPN/100ml and 243-6131 EC MPN/100ml. 69% of the samples were above the single sample standard for recreational water quality standard of 104 ENT MPN/100ml and 96% were above the standard of 400 fecal coliforms MPN/100 ml.[40] ENT in *exposed sand, submerged sand, exposed wrack, submerged wrack* range from respectively the lower detection limit (DL, ~0.5 MPN/g) to a max of 32, 11, 8579, 132 MPN/g. EC in *exposed sand, submerged sand, exposed wrack, submerged wrack* range from respectively DL(~0.5 MPN/g) to 85, DL to 11, 9.7 to 6547, and DL to 16,853 MPN/g. FIB in sand and wrack were highly spatially variable but were consistently lower in submerged than exposed samples ($p < 0.01$ and 0.08 for sand ENT and EC respectively and $p < 0.01$ for both wrack ENT and EC). Waterborne ENT were significantly positively correlated with tide height ($r_s = 0.34$, $p = 0.02$) and significantly negatively correlated with UVB intensity ($r_s = -0.7$, $p < 0.01$). EC in water were significantly negatively correlated with tide height ($r_s = -0.50$, $p < 0.01$), but not associated with UVB intensity. ENT and EC were not correlated in the water samples ($r_s = 0.27$, $p = 0.20$).

A total of 24 surf zone water samples, 6 *exposed sand* samples, 5 *exposed wrack* samples and 4 groundwater samples were assayed for HF183. The marker was not detected in any of these samples, except in 2 water samples, where it was detected at a low level (BLOQ). The same samples plus an additional 9 *exposed sand* and 10 *exposed wrack* samples were analyzed for *Catellicoccus*. It was detected in all samples except for one groundwater at levels of up 10^5 copies / 100 ml or g dry weight (Table 1, Fig. S7). Note that *submerged sand* and *submerged wrack* samples were not tested for MST markers due to their low ENT and EC concentrations.

In wrack, ENT and EC were positively correlated ($r_s = 0.28$, $p = 0.06$), ENT and *Catellicoccus* were positively correlated ($r_s = 0.57$, $p = 0.03$), and EC and *Catellicoccus* were positively correlated ($r_s = 0.49$, $p = 0.06$). In sand, ENT and EC concentrations were significantly positively correlated ($r_s = 0.68$, $p < 0.01$).

Correlations between surf zone measurements were tested. There was a correlation between ENT and *Catellicoccus* (r_s=0.37,p=0.08). EC was positively correlated with silicate and DIN, and negatively correlated with salinity (respectively r_s=0.66, 0.58, -0.80; p≤0.01). There were also significant, positive correlations between silicate and DIN (r_s=0.68, p<0.01) and significant negative correlations between salinity, and DIN and silicate (respectively r_s=-0.56, -0.84; p≤0.01) (see SI for details).

Twenty-Four Hour Study: Model Fit and Parameters

The groundwater model, based on silicate, gives $Q_{qw,max}$ = 90 L/h/m of shoreline and α_{open} = 0.6/h (log-RMSE= 0.074 µM) (see SI for details). The parameters were verified using a salinity model (see SI for details). The best-fit parameters for ENT, EC, and *Catellicoccus* (Table 2) were selected based on minimization of log-RMSE between measured and modeled concentrations (Fig. 4) as described in the methods and the SI. The models provide a good fit to the measured data. The best-fit models include (1) ENT: inputs from wrack and sand and losses due to photoinactivation (2) EC: inputs from groundwater and wrack and losses due to photoinactivation (3) *Catellicoccus*: inputs from wrack and sand. The inclusion of other input and loss terms did not improve the models' fits.

The model sensitivity analysis is presented in its entirety in the SI. The ENT model was most sensitive to α_{sand}, k_{sun} and d_{sand}. The EC model was most sensitive to C_{gw}. Both models were sensitive to the physical parameters constrained by the fitting of the silicate model. The *Catellicoccus* model was most sensitive to α_{sand}.

Using the best-fit models, daily fluxes (per meter of shoreline) were calculated from sand, wrack and groundwater (Table 2). Based on the model results, during the 24 h study, wrack had a flux approximately

two orders of magnitude lower than sand (for ENT) and groundwater (for EC). Wrack flux of *Catellicoccus* was 0.4 log units lower than the sand flux (Table 2).

Discussion

ENT and EC at Cowell Beach were consistently high for the duration of the study, with typical concentrations above the state guidelines for coastal waters. Spatial and cross-shore surveys strongly suggested a shoreline source of bacteria rather than a marine source like marine mammals. Water quality data collected over two summers ruled out the nearby flowing storm drain, San Lorenzo River, marine mammals and other activities at the wharf, and the harbor as important FIB sources to Cowell Beach. FIB in and adjacent to these potential sources were usually at similar or lower levels than those at Cowell Beach. Additionally, the remote location of the San Lorenzo River and the storm drain relative to the beach would allow for substantial dilution before reaching Cowell Beach. FIB measured in the ocean near the storm drain outlet and the river mouth were low relative to FIB at Cowell. Having ruled out these potential sources, groundwater, wrack, and sand remained possible causes of the poor water quality at the beach during the study.

Wrack consistently had higher FIB concentrations than sand, reaching on the order of 10^3 MPN/g for EC and ENT. However, the FIB models for the Cowell surf zone during the 24 h study showed that wrack contributes only 0.7% and 2% for ENT and EC, respectively, of the total daily shoreline FIB flux. This finding is driven by the substantially smaller mass of wrack compared to the mass of sand that is washed on the beach.

The FIB models provided further insight into the role of sand and groundwater as FIB sources. Surf zone ENT was best modeled by including 'sand washing' as the major ENT source and 'wrack washing' as a minor ENT source. In contrast, surf zone EC was best modeled by including contaminated groundwater

as the major EC source and 'wrack washing' as the minor EC source. Thus sand and groundwater were the main ENT and EC sources, respectively. 'Sand washing' is mainly a high tide source[9, 14] while groundwater a low tide source.[41-43] Therefore, in the surf zone, the two FIB have distinct relationships with the tide, and are uncorrelated with each other. Although EC is also present on sand, model findings suggests that sand washing elutes ENT and EC differently under identical environmental conditions; the sand washing parameter is 0.4 for ENT and 0 for EC. Previous research has suggested that the different surface properties of ENT and EC impact their attachment and detachment in beach sands,[44] but future research will be needed to investigate the reason for the inferred divergent behavior.

The model indicated that the main source of ENT was beach sands. Both ENT and the molecular marker for *Catellicoccus* were elevated in the beach sands. The latter reached concentrations on the order of 10^5 copies / g. Based on previous measurements of ENT and EC in gull feces[45], the highest observed FIB concentrations on sand could be caused by the presence of 0.01 µg feces/ g sand, which is not unreasonable given the large bird population that feeds at and nests on the wharf. *Catellicoccus* was also elevated in the surf zone where its concentration correlated positively to ENT. Like ENT, the model of *Catellicoccus* indicated sand was its major source. However the lower sand washing parameter for *Catellicoccus* (0.002) relative to ENT (0.4) indicates that these two have different detachment properties during sand washing. This is the first study to our knowledge to document such high concentrations of *Catellicoccus* in sand, and there is presently no research on the ability of the bacterium to attach and detach to sand so it is difficult to speculate on why the parameter varies between the two bacteria. However, the findings suggest an avian source of ENT to the sand, and subsequently the water, at Cowell Beach. The nearby ~1 km long wharf attracts numerous birds including nesting pigeons which also carry *Catellicoccus*.[35] Avian defecation on the beach may be the ENT source to the sand, and potentially the wrack as well. An avian source was recently documented by Converse et al. [46] as a source of FIB to the beach in Racine, WI, using a combination of MST techniques including a gull associated marker targeting

Catellicoccus marimammalium. As previous work has shown that ENT can grow in beach sands,[10] it is possible ENT growth is also a cause for the high sand ENT concentrations.

The model indicated that tidally modulated submarine groundwater discharge was the major source of EC to the Cowell surf zone. This is further corroborated by the positive correlations between EC and silicate and negative correlation with salinity, expected given a groundwater source.[41, 42, 47, 48] The groundwater EC concentration was used as a fitting parameter in the model and was required to be 20,000 MPN/100 ml. The gravity main which terminates in the sands at the beach, contained water with EC concentrations above the upper detection limit (24,196 MPN/ 100 ml), so the gravity main could be the EC source to the groundwater. The gravity main also had high ENT, but the model indicates a groundwater ENT source is not needed to explain surf zone concentrations. It is possible that ENT is less efficiently transported through the subsurface than EC owing to the different surface characteristics of the bacteria.[44] During the study, we preformed limited sampling at the top of the groundwater table (4 total) and FIB levels were low relative to the groundwater concentrations determined by the model. At Avalon Beach where contaminated groundwater is known to be the cause of poor water quality, beach groundwater was frequently found to be free of fecal bacteria[23] owing to the complex spatiotemporal dynamics of beach groundwater. Follow up groundwater sampling at Cowell Beach has found concentrations as high as >24,192 MPN/100 mL (data not shown). Thus, the fitted groundwater EC concentration is not unrealistic. The model has a relatively simple parameterization of the bacterial flux from groundwater - it uses a fixed bacterial concentration. In reality, the concentration is likely variable in space and time,[23] but information on the variability at Cowell is presently lacking. As more information on the groundwater contamination becomes available, it may be possible to incorporate dynamic groundwater concentrations into the model. Although the measured concentrations of EC at Cowell Beach are consistent with a groundwater source (highest at low tide, correlated with silicate and salinity), an alternative explanation is that there is another low salinity, high silicate water delivering EC during low tide to the site that has yet to be identified.

The results of the 24 h study illustrate that photoinactivation plays an important role in attenuating FIB. This finding aligns well with previous research on the role of photoinactivation in controlling waterborne FIB [11, 49-51]. The best-fit values of k_{sun} for ENT and EC were 0.30 [h^{-1} I^{-1}] which are similar to those at Avalon Beach in California[11]. Conversely, the best-fit model for *Catellicoccus* did not require inclusion of photoinactivation. Previous studies have shown that qPCR-detected fecal organisms show reduced sensitivity to sunlight relative to organisms detected using culture-dependent methods as the short segments of DNA that the assays target are unlikely to be destroyed during photoinactivation. [11, 52, 53]

The HF183 human marker was frequently detected at Cowell Beach in the surf zone (47% of all samples from the 24 h and long term studies), although it was often at concentrations BLOQ (<500 copies/ 100 ml). Even though the concentrations were low, this finding suggests that there is a consistent human source to this beach. A similar finding was observed previously at a beach with a known sewage contaminated groundwater source. [11] The gravity main, which drains into the beach groundwater table, was found have high concentration of the HF183 human marker (on the order of 10^6 copies/100 ml) and could represent a dominant source of the human fecal pollution to Cowell Beach.

Multiple lines of evidence from a sanitary survey to identify potential sources of contamination, long-term and short-term FIB surveys, modeling, and source-specific molecular assays were used to successfully identify important pollution sources at Cowell Beach. Results from independent data sets and analyses each provided evidence regarding the importance of groundwater and sand as vehicles for the delivery of FIB to the beach, and humans and gulls for contributing FIB. This approach represents a framework for source tracking that can be used for future projects. There is potential for future refinement of the model. In addition to accounting for dynamic bacterial concentrations in groundwater that has already been discussed, how the model deals with transport could be improved to consider a surf zone that

is not well mixed, and alongshore and cross shore transport explicitly rather than lumping all transport into a single dilution term.

Associated Content

Supporting Information

Some methods, results, Figures S1-S16 and Tables S1-S2 are presented in the SI. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgements

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Tables

Table 1: Results from the molecular assays. Numbers of samples processed and percentages falling in the designations of Range of Quantification (ROQ), Below Limit of Quantification (BLOQ) and Non-Detect (ND) are shown for the both the HF183 assay and the *Catellicoccus* assay. Median and range values are given for the samples in the ROQ. " −" indicates no samples were in the ROQ or only one sample was in ROQ and no range is provided.

Description	n	HF183					*Catellicoccus*				
	#	% ND	% BLOQ	% ROQ	Median (ROQ) [copies/ 100ml or g dry]	Range (ROQ) [copies/ 100ml or g dry]	% ND	% BLOQ	% ROQ	Median (ROQ) [copies/ 100m or g dry]	Range (ROQ) [copies/ 100ml or g dry]
Cowell Beach (Long-term)	16	25	75	0	–	–	0	0	100	2480	510 – 218,000
Cowell Beach (24 h)	24	92	8	0	–	–	0	0	100	12,500	2,450 – 178,000
Storm Drain	20	75	25	0	–	–	95	5	0	–	–
Wharf	19	47.3	47.3	5.2	1,790	–	0	5.2	94.8	1,830	694 – 5,440
San Lorenzo River	20	5	45	50	2,030	668- 19,100	0	5	95	2,650	437- 11,000
Harbor	12	33	42	25	842	683- 889	0	83	17	1,270	527-2000
Neary Lagoon	15	7	33	60	7,030	893-17,600	40	60	0	–	–
Force Main	9	67	33	0	--	--	78	22	0	–	–
Gravity Main	8	0	13	87	718,000	3040-2,050,000	50	0	50	1,020	862-1,150
Exposed Wrack (24 h)	15	100[1]	0	0	--	--	0	6.7	97.3	5,080	651-1,590,000
Exposed Sand (24 h)	15	100[1]	0	0	--	--	0	0	100	1880	257-186,000

[1] Five wrack and six sand samples were tested for HF183

Table 2: Best fit model parameters and model calculated daily fluxes.

Model	C_{gw} [MPN or copies/ 100ml]	α_{sand} [-]	α_{wrack} [-]	d_{sand} [m]	k_{sun} [$I_{UVB}^{-1}h^{-1}$]	k_{dark} [h^{-1}]	Daily Groundwater Flux [MPN or copies/ d/m] (% of total daily flux)	Daily Sand Flux [MPN or copies/ d/m] (% of total daily flux)	Daily Wrack Flux [MPN or copies/ d/m] (% of total daily flux)
enterococci	0	0.4	1	0.25	0.30	0	--[1] (0%)	1.2×10^8 (99.3%)	8.4×10^5 (0.07%)
E. coli	2.0×10^4	0	1	0.25	0.30	0	1.9×10^8 (97.8%)	--[1] (0%)	4.3×10^6 (2.2%)
Catellicoccus	0	0.002	1	0.25	0	0	--[1] (0%)	1.7×10^9 (73.5%)	6.1×10^8 (26.4%)

[1] No flux was calculated because the best fit model did not include this source.

Figures

Figure 1: Map of Cowell Beach showing sampling locations and potential pollution sources. Important locations and potential sources are highlighted with text. Sampling locations for the long-term survey are highlighted in red, green and blue circles representing respectively source samples, wrack samples and surf zone water samples. The blue line shows the location of the cross-shore transect and the two yellow lines show the locations of the gravity and force mains draining Neary Lagoon. Inset 'A' shows the location of the 24 h study transect in gray and the location of the groundwater samples with yellow circles (GW1-GW3). Satellite photo provided by Google Earth (© 2013 Google and Terrametrics).

Figure 2: Conceptual model of FIB dynamics in a well-mixed surf zone (SZ). L_{SZ} is the horizontal width of the SZ, Z_{sz} is the depth of the SZ, I_{UVB} is the UVB solar intensity, C_{SZ} is the FIB concentration in the SZ, C_{open} is the offshore FIB concentration, C_{gw} is the groundwater FIB concentration, Q_{gw} is the groundwater flow rate, k_{sun} and k_{dark} are the FIB inactivation rates in the SZ, C_{wrack} and C_{sand} are the FIB concentrations on wrack and sand respectively, $\dot{m}_{wrack}$ and $\dot{m}_{sand}$ are the masses washed per a unit time of wrack and sand respectively and α_{wrack}, α_{sand} and α_{open} are exchange parameters defining respectively the fraction FIB washed off wrack and sand and the fraction of volume exchanged with the offshore. Units for each parameter are shown in brackets.
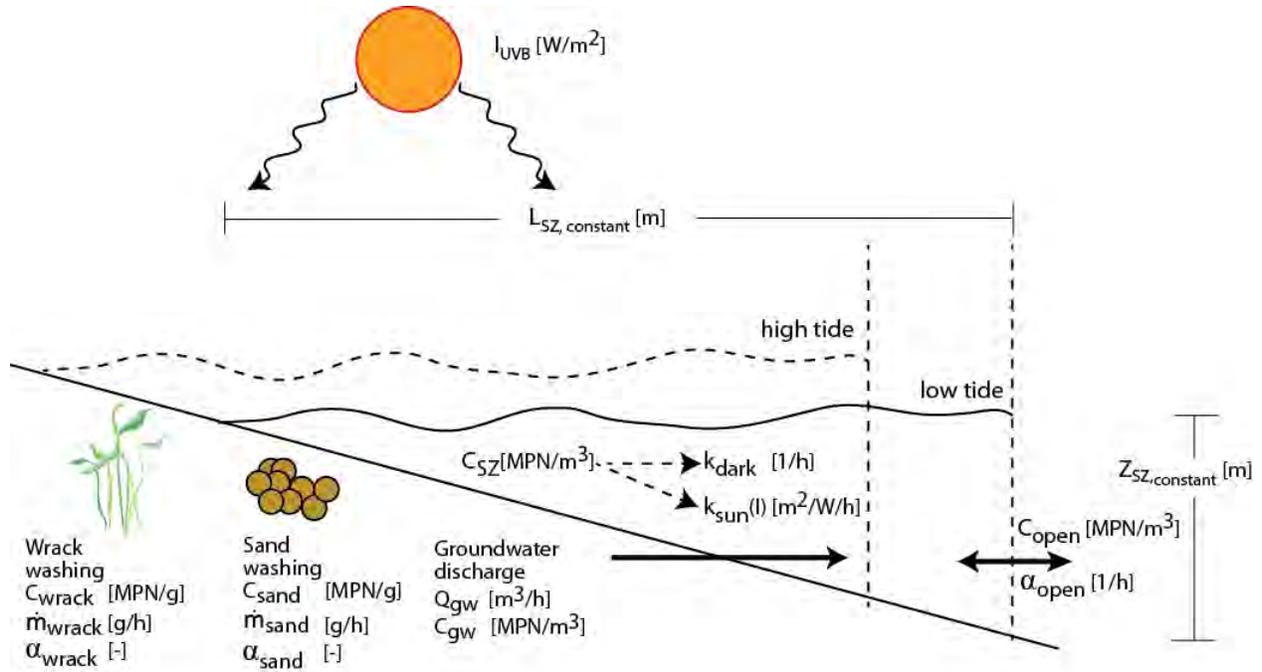
Figure 3: Box and whisker plots of FIB concentrations at Cowell Beach (both sample locations, water and wrack), storm drain (SD), wharf, and San Lorenzo (SL) River, harbor, Neary Lagoon, force main and gravity main. All data collected during the long-term survey and drainage pipe survey are shown. Water samples with significantly lower (p<0.1) log-mean concentrations of FIB than the Cowell Beach samples are highlighted with an '*'. Box represents 25th, 50th and 75th percentiles; whiskers represent 10th and 90th percentiles.
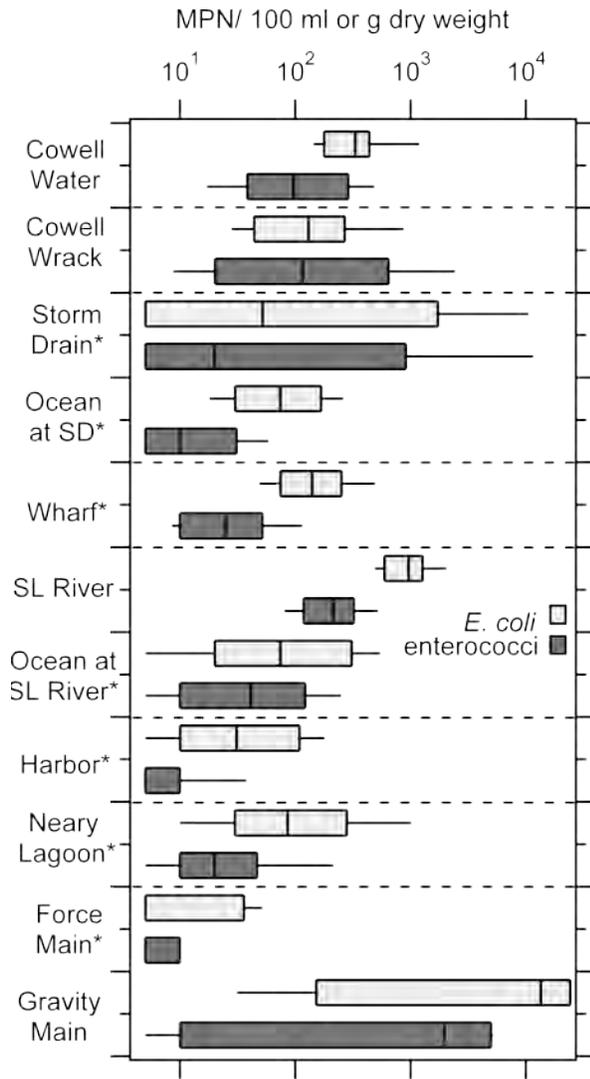
Figure 4: Observed and modeled data for tide, UVB intensity, enterococci, *E. coli*, *Catellicoccus*, salinity, and silicate. Black lines in the bottom five panels show measured data, red lines show calculated model best fits. Log-RMSE values represent the best fit root mean square error between the log_{10}-transformed modeled and observed data. Units for log-RMSE vary by panel and are the log of the unit specified on the left axis.
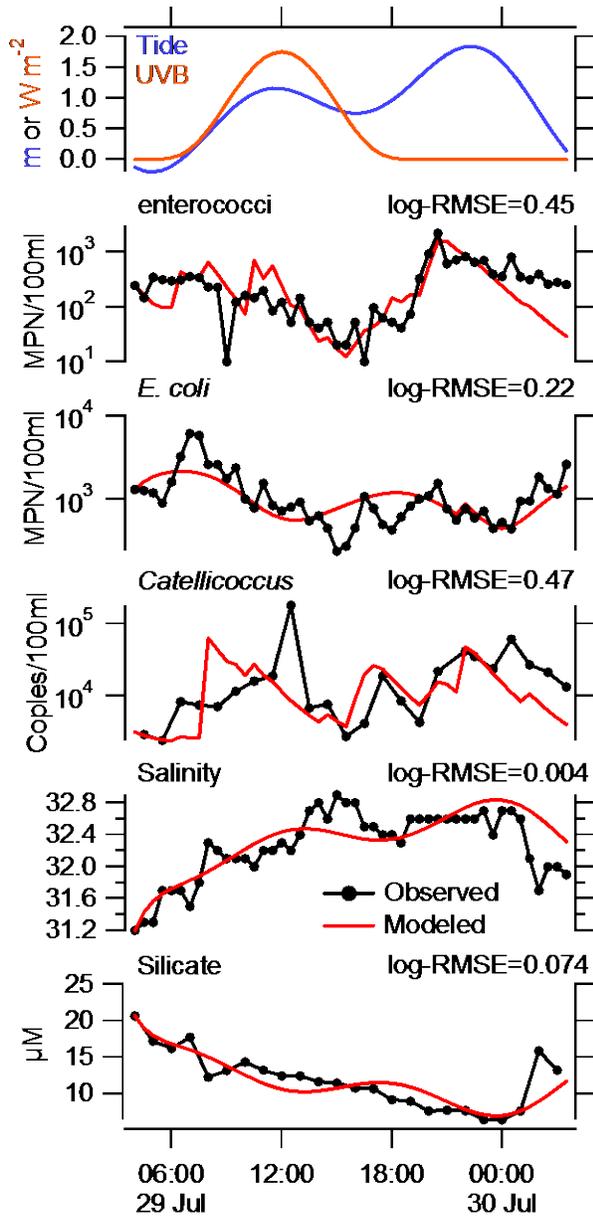
Supporting Information

Methods

Water Samples: Water samples were collected in high-density polyethylene (HDPE) bottles that had been soaked in 10% hydrochloric acid (HCl) and triple rinsed in deionized (DI) water.

Wrack Samples. Wrack samples were collected in sterile bags (Nasco, Fort Atkinson, WI). Wrack samples were composited from approximately 3-5 locations along a 5 m length of beach parallel to the waterline and preserving the relative ratios of different plant species present. A subset of each wrack sample was dried at 105 °C for 24 h to determine moisture content. Bacteria were eluted from the wrack samples following a modified version of the method described in Imamura et al. [1] Briefly, 20 or 30 g wet weight of wrack was added to 200 mL or 300 mL respectively of autoclaved phosphate buffered saline + magnesium chloride (0.085 g KH_2PO_4 + 0.19 g anhydrous $MgCl_2$ per a L of solution, henceforth referred to as PBS+) in a sterile bottle and hand shaken for 3 minutes. The mixture was allowed to settle for 30 s and half of the eluent was gently poured off into a new sterile, DNA free container for analysis.

Sand Samples. Submerged (submarine) and exposed (subaerial) sand samples were collected from the top 3 cm of the beach using a sterile scoop and stored in a sterile bag. Sand samples were composited from approximately 5-8 locations across an approximately 5 m length of beach parallel to the waterline. Exposed (i.e., subaerial) sand moisture content was estimated with an HH2 moisture probe (Delta-T Devices, Cambridge, England), submerged sands were assumed to be saturated. Bacteria associated with sand were eluted following the methods in Boehm et al. [2].

Conductivity measurements from the moisture probe were converted to moisture content (%) using a calibration curve. The calibration curve was based on Cowell Beach sand conductivity measurements and the change in weight after sands were dried at 105°C for 24 h. The calibration curve was used to assign

moisture contents to all exposed sands. The moisture content of saturated sands was based on the porosity estimated by measuring the volume of water required to saturate 50 mL of dried sand.

Defined Substrate Assays for FIB Concentrations. All water, and sand and wrack eluents were analyzed for enterococci (ENT) and *E. coli* (EC) using Enterolert and Colilert 18 (IDEXX, Fremont, CA), respectively. Ten milliliters of sample were added to each of two bottles containing 90 mL of Butterfield's solutions (Webber Scientific, Hamilton, NJ), mixed with media, added to Quanti-Tray/2000s and incubated according to manufacturer instructions. For data analysis, samples below the lower detection limit of 10 most probable number (MPN)/ 100 mL were assigned a value of 5 MPN/ 100 mL; and samples above the upper detection limit of 24,196 MPN/ 100 mL were assigned that value. Sand and wrack eluent samples were normalized to dry weights for statistical analyses. All samples were stored on ice until processing, which occurred within 6 h of collection.

Molecular Analyses. Samples were archived using membrane filtration on 0.4 μm, 47 mm diameter polycarbonate (PC) filters (EMD Millipore, Billerica, MA) to preserve bacterial DNA for molecular analysis. Filters were immediately placed in beaded CryoTubes (GeneRite, New Brunswick, NJ), flash frozen in liquid nitrogen, and stored at -80 °C until extraction. Volumes archived: (1) long-term survey: 200 mL of water and 50 mL of wrack eluent (2) drainage piping: 200 mL of water (3) 24 h study: 100 mL of water, 100 mL of sand eluent, and 12.5 mL of wrack eluent. No replicate filters were archived.

DNA was extracted from the PC filters using the DNA-EZ ST2 kit (GeneRite, North Brunswick, NJ) following manufacturer's instruction with slight modification where 500 μL lysis buffer was used and 400 μL crude supernatant was transferred for later purification steps. Two qPCR assays were tested on the DNA extract, the human-associated *Bacteroidales* HF183Taqman[3] and gull-associated *Catellicoccus* [4]. Two microliters of DNA extract were added to a 25 μL or 20 μL reaction mixture for the HF183 assay and the *Catellicoccus* assay respectively. Universal mastermix was used for both assays (Taqman®

Universal PCR Master Mix, Life Technologies, Foster City, CA). For the HF183Taqman assay, final concentrations of 1.2 µM of primers (F-primer: ATCATGAGTTCACATGTCCG and R-primer: CGTAGGAGTTTGGACCGTGT) and 0.1 µM of probe ([6FAM]-CTGAGAGGAAGGTCCCCCACATTGGA-[TAMRA]) were used. For the *Catellicoccus* assay, final concentrations of 0.25 µM of primers (F-primer: AGGTGCTAATACCGCATAATACAGAG and R-primer: GCCGTTACCTCACCGTCTA) and 0.125 µM of probe ([6FAM]-TTCTCTGTTGAAAGGCGCTT-[MGB]) were used. In addition, a final concentration of 0.2 mg/mL of BSA (Life Technologies, Grand Island, NY) was added to the qPCR reaction. qPCR was run on a StepOne Plus platform (Life Technologies, Grand Island, NY). The default thermal cycling program (anneal at 60°C) was used for both assays, with 40 and 45 cycles for HF183Taqman[®] assay and *Catellicoccus* assay, respectively. The lower limit of quantification (LLOQ) and the lowest detectable concentration varied depending on the volume of water filtered. For 200 mL water samples, the LLOQ was 500 copies/100 mL, the lowest detectable concentration (assuming 1 copy of the gene amplifies in a reaction) is 50 copies/100 mL for both assays.

Cross-shore Survey. The sampling locations were chosen along a transect extending from Cowell Beach (north end of the transect) to the offshore kelp forest (south end of the transect) (Figures 1 and S2). Kelp was only sampled when a plant was present at the sample location.

Water samples were collected in sterile, triple rinsed HDPE bottles initially filled with autoclaved deionized (DI) water and then purged with air underwater and refilled with surrounding water. Kelp blades were removed from plants and placed into sterile sealable bags. As much water as possible was removed from the bags before sealing, and at the surface any remaining water was drained. Sand samples were collected in sterile centrifuge tubes initially filled with DI water and then purged underwater with air, and subsequently filled with sand. Depth of sample collection was measured using a dive computer.

Drainage Pipe Survey. The drainage pipe survey was conducted during a period of dry weather. There was <1 cm of rain during the period of the Drainage Pipe Survey (1 July 2012 through 18 October 2012).[5]

Twenty-four Hour Study. Every hour during the 24 h study, water samples were archived for nutrient analysis by syringe filtering water through 0.2 μm pore size filter (VWR International, Radnor, PA) into an opaque 10% HCl-washed and triple rinsed bottle and were subsequently frozen upon return to the laboratory. Samples were analyzed for silicate, dissolved inorganic nitrogen and phosphate using flow injection analysis on a Lachat QuickChem 800 (Zellweger Analytics, Lincolnshire, IL).

A total of four groundwater samples were collected from temporary wells installed at the top of the water table. A sterilized hand auger was used to drill to the top of the water table where a screened PVC pipe was inserted. An acid washed, screened Teflon tube was inserted into the pipe and water was extracted from the well using a battery operated peristaltic pump. A small length of silicon tubing was used inside of the peristaltic pump. 500 mL of groundwater was purged out of the well through the tubing before sampling. Groundwater was assayed for FIB, host specific markers and nutrients as described in the main body of the manuscript and above in the SI. Groundwater sampling locations (GW1-GW3) are shown on Inset A, Figure 1 in the main manuscript.

The wrack density measurement procedure followed that of Dugan et al. [6]. Measurements of wrack mass were taken in a series of one square meter increments along a 1 m wide transect from above the high-high tide line to the water using a portable electronic balance (Model 311, Salter Brecknell, Fairmont, MN).

For modeling purposes, the offshore value for salinity were assumed to be equal to the highest salinity observed in the surf zone measured during the 24 h study (32.9). This value falls within the range of offshore salinities measured during the spatial survey (32.8-33.1). For modeling purposes the offshore nutrient concentrations were assumed to be equal to the lowest concentration measured during the 24 h

study. For silicate this offshore value was set to 6.3 µM which is reasonable as a midrange value for the

area (1.5 -11µM for, respectively, a non-upwelling and upwelling event[7]).

Twenty-four Hour Study: Model Formulation. For the model, submarine groundwater discharge was

assumed to be driven primarily by tidal stage following the formulation utilized by _ENREF_2Boehm et

al. [8]. Although submarine groundwater discharge is driven by a combination of waves and tidal forcing,[9,]

[10] for simplicity the model does not adjust for unsteady submarine groundwater discharge rates introduced

by wave forcing. Groundwater flow rate varied between 0 L/h/m of shoreline at high tide and a fitted

parameter of the maximum flow rate $Q_{gw,max}$ [L/h/m] at low tide using the following

$$Q_{gw}^t = Q_{gw,max} \frac{(z_{max} - z^t)}{(z_{max} - z_{min})} \tag{S1}$$

where the superscripts t denotes the time step, Q_{gw} is the groundwater flow rate, z_{max} [m] is the maximum

tide height during the study, z_{min} [m] is the minimum tide height during the study and z^t [m] is the tide

height at the time step . Tide heights used in the analysis were provided by NOAA [11]. The water balance

is given as follows

$$V_{SZ}^t = \text{constant} \tag{S2a}$$

$$\frac{\Delta V_{SZ}^t}{\Delta t} = 0 = Q_{GW}^{t-1} + Q_{offshore,in}^{t-1} - Q_{surf\ zone,out}^{t-1} \tag{S2b}$$

$$Q_{surf\ zone,out}^{t-1} = Q_{GW}^{t-1} + Q_{offshore,in}^{t-1} \tag{S2c}$$

let α_{open} =constant such that $Q_{offshore,in}^{t-1} = V_{SZ}\alpha_{open}\Delta t$ (S2d)

$$Q_{surf\ zone,out}^{t-1} = Q_{GW}^{t-1} + V_{SZ}\alpha_{open}\Delta t \tag{S2e}$$

where the superscript t and t -1 denotes the current and previous time step, Vsz is the volume of the surf

zone, Δt is the model time step, the subscript GW represents groundwater, the subscript *offshore, in*

represents water flowing into the surf zone from the offshore, the subscript *surf zone, out* represents surf

zone water flowing out to the offshore.

Following the form of the microbial model (presented in the main manuscript), the mass balance for a given tracer N is given by

$$N_{SZ}^t = N_{sz}^{t-1} + \Delta t [C_{open} V_{SZ} \alpha_{open} - C_{sz}^{t-1} V_{SZ} \alpha_{open} - C_{sz}^{t-1} Q_{gw}^{t-1} + C_{gw} Q_{gw}^{t-1}] \qquad (S3)$$

where the superscripts t and t - 1 denote the new and old time steps respectively, N_{sz} is the mass of a given nutrient or tracer in the surf zone, C_{open} is the offshore concentration, C_{SZ} is the surf zone concentration and C_{GW} is the groundwater concentration. Unlike the microbial model, the tracer model assumes no decay. This model was used to constrain the maximum groundwater flow rate ($Q_{gw,max}$) and the offshore exchange constant (α_{open}) using silicate as a tracer. Silicate serves as a good tracer for submarine groundwater discharge as it tends to be high in groundwater and relatively low in the coastal ocean.[7, 12-14] The two parameters were constrained by minimizing the log-root mean square error (log-RMSE) between observed silicate concentrations and those estimated from the model described below. Silicate concentrations, measured for every other time point, were interpolated to provide values for the 30 min time steps used by the model. A model of salinity was run to verify these constrained parameters in which the salinity of the groundwater was used as a fitting parameter.

For the microbial model, bacteria densities on exposed sand and wrack (C_{sand} and C_{wrack} [MPN or copies/g], respectively) as a function of location across the beach were interpolated from values measured during the 24 h study. The mass of wrack washed per a unit time ($\dot{m}_{wrack}$ [g /h]) was calculated using the wrack density measured as previously described and the measured waterline. UVB intensity was estimated using the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS).[15] UVB is used as a proxy for sunlight intensity and its use is not meant to imply that photoinactivation with longer wavelengths is unimportant.

Results

Performance of Microbiological Assay Controls. All field blanks and method blanks were negative indicating no cross contamination during field work, wrack and sand elution, filtration, or DNA extraction.

Spatial Survey. The results from the spatial survey are shown in Figures S1 and S3.

Cross-shore Survey: The results of the cross-shore survey are shown for kelp/wrack, sand and water in Figures S4-S6. Note that the sample on the left of these plots is collected onshore from the exposed beach (sand and wrack) or from the surf zone (water).

Twenty-four Hour Study. Wrack spatial density was measured at 1 m intervals throughout the entire length of the transect as described in the methods section of the manuscript. Wrack was generally concentrated at two locations, one near the low-low tide line and the other just below the high-high tide line (Figure S8). The wrack was frequently observed to be buried in the sand and wrack which was substantially buried was not measured in this assessment. Locations with no data on the plot had less than 20 g of wrack in the 1 m^2 transect grid.

The results of the salinity and nutrient samples are shown in Figure 4.

Submerged wrack and sand had lower concentrations of fecal indicator bacteria than their exposed counter parts (Figure S9). This finding supports the concept that fecal indicator bacteria are washed from wrack and sand as they are inundated by the tide but does not explain why sand was not a dominant source of EC in the model. Sand, and particularly wrack, collected during the 24 h study had high concentrations of the molecular marker for *Catellicoccus* (Figure S7).

Groundwater salinity was used as fitting parameter to minimize the log-root mean square error (log-RMSE) between the model and the observed salinity data (minimum log-RMSE= 0.004). The resulting best fit groundwater salinity was 23 which may indicate that a brackish groundwater is discharged. The resulting salinity model was used to confirm the parameters generated with the silicate model ($Q_{gw,max}$ and α_{open}). This was done by confirming that the observed salinity trends matched those generated by the model (Figure 4) as we would expect for a groundwater source of freshwater to the surf zone.

Model sensitivity was tested for various model parameters by varying two parameters at a time while holding all other parameters constant. Model sensitivity was assessed graphically in the plots of the log-RMSEs generated for the varying parameters (Figures S10-S16). Figures S10-S16 show sensitivity of the modeled bacterial and silicate concentrations to α_{sand}, $\alpha_{wrack,}$ k_{sun}, k_{dark}, d_{sand}, C_{gw}, α_{open} and $Q_{max,gw}$. Where appropriate, analyses are only shown for one of the two fecal indicator bacteria as the parameters were only relevant for one of the models. Generally the best fit model parameters presented in the main body of the manuscript were chosen to give the minimum log-RMSE, and provide the most realistic values based on physical conditions at Cowell Beach.

The ENT model, driven by sand and wrack sources, was most sensitive to α_{sand}, k_{sun} and d_{sand}. A range of values can provide similar log-RMSEs for these parameters when all other model parameters are held constant. The d_{sand} value was selected to minimize the depth of sand utilized in the model, although the analysis shown in Figure S12 shows that there is a series of combinations of α_{sand} and d_{sand} values that can provide similarly good fits.

The EC model, driven by groundwater flow, was most sensitive to C_{gw} and α_{open}. As shown in Figure S14 a range of values for C_{gw} and α_{open} can provide similarly low log-RMSE. As the value for α_{open} was constrained by the silicate concentration model, the value presented in the main manuscript represents the best fit value for C_{gw} at that value of α_{open}.

The *Catellicoccus* model, driven by sand and wrack sources, was most sensitive to α_{sand}. The value for α_{sand} determined is much smaller than that for ENT.

The silicate model is sensitive to both α_{open} and $Q_{max,gw}$. The best fit values used by the model minimized the log-RMSE between the observed and model concentrations and minimized $Q_{max,gw}$. A lower value of $Q_{max,gw}$ was determined to be more realistic, even though similar fits could be achieved with larger values.

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Tables

Table S1: Nutrient and bacterial concentrations in groundwater samples.

Sample Location	Date	Phosphate µM	Silicate µM	DIN µM	ENT MPN/100mL	EC MPN/100mL
GW1	7/29/2011 06:30	17.6	94.7	146	<10	<10
GW1	7/29/2011 19:45	17.6	97.6	112	<10	<10
GW1	Average	17.6	96.1	129	<10	<10
GW2	7/29/2011 07:45	2.7	54.3	231	10	31
GW3	7/30/2011 03:45	2.1	53.9	196	31	20

Table S2: Spearman rank correlations for the surf zone water samples from the 24 h study. Values are shown for r_s and (p). Correlations with p values ≤ 0.1 are shown in bold.

r_s (p)	Enterococci	E. coli	Catellicoccus	Silicate	Salinity	DIN	Phosphate	Turbidity
Enterococci		0.27 (0.20)	**0.37 (0.08)**	-0.19 (0.37)	-0.14 (0.53)	-0.26 (0.22)	0.19 (0.39)	**0.55 (<0.01)**
E. coli	0.27 (0.20)		-0.17 (0.43)	**0.66 (<0.01)**	**-0.80 (<0.01)**	**0.58 (<0.01)**	**0.50 (<0.01)**	0.13 (0.55)
Catellicoccus	**0.37 (0.08)**	-0.17 (0.43)		**-0.43 (0.04)**	0.18 (0.41)	-0.34 (0.10)	0.21 (0.33)	0.22 (0.29)
Silicate	-0.19 (0.37)	**0.66 (<0.01)**	**-0.43 (0.04)**		**-0.84 (<0.01)**	**0.68 (<0.01)**	0.09 (0.69)	-0.27 (0.20)
Salinity	-0.14 (0.53)	**-0.80 (<0.01)**	0.18 (0.41)	**-0.84 (<0.01)**		**-0.56 (<0.01)**	-0.19 (0.37)	0.21 (0.33)
DIN	-0.26 (0.22)	**0.58 (<0.01)**	-0.34 (0.10)	**0.68 (<0.01)**	-0.56 (<0.01)		**0.47 (0.02)**	0.19 (0.38)
Phosphate	0.19 (0.39)	**0.50 (<0.01)**	0.21 (0.33)	0.09 (0.69)	-0.19 (0.37)	**0.47 (0.02)**		**0.43 (0.04)**
Turbidity	**0.55 (<0.01)**	0.13 (0.55)	0.22 (0.29)	-0.27 (0.20)	0.21 (0.33)	0.19 (0.38)	**0.43 (0.04)**	

Figures

Figure S1: Spatial study transects during ebb tide (top panel) and flood tide (bottom panel). The location of the circles shows where samples were collected. The color of the large and small circles represents the enterococci and *E. coli* concentrations, respectively. The outline of the shoreline is shown in black. Note the location of the wharf, the San Lorenzo River and the Santa Cruz Harbor (two breaks shown in upper right of shoreline outline).
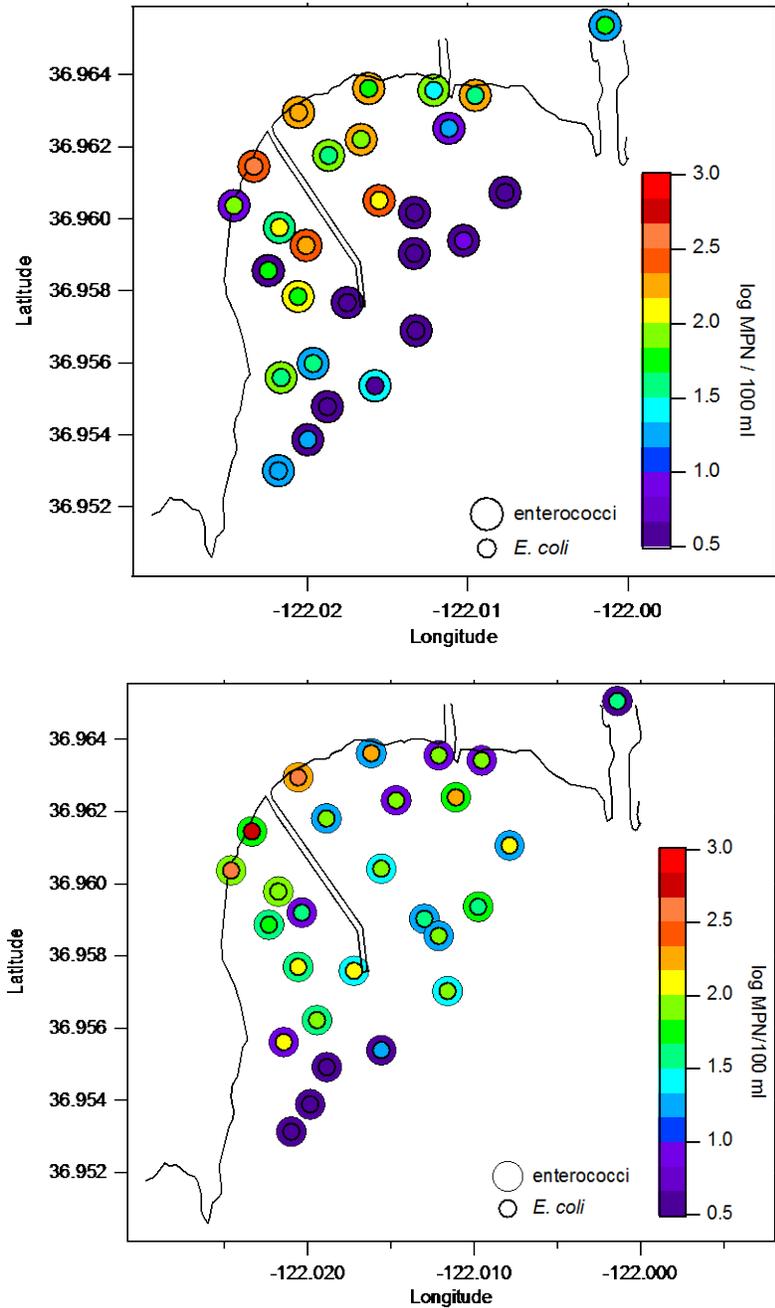
Figure S2: Sampling locations for the cross-shore survey. Circles represent the surface locations beneath which samples were collected in a depth profile.
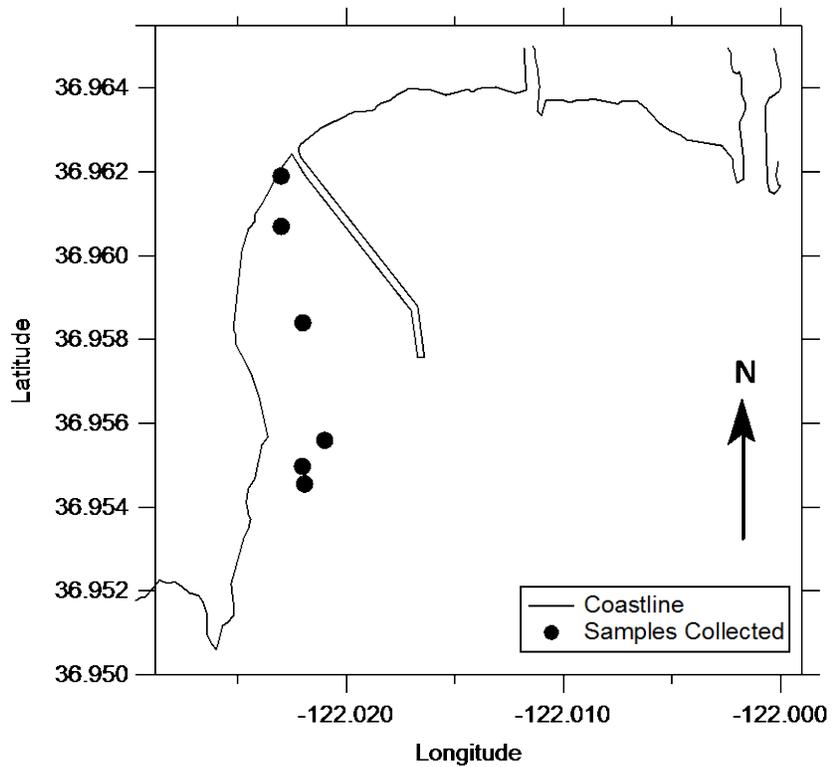
Figure S3: Spatial study sampling locations during ebb tide (top panel) and flood tide (bottom panel). The location of the circles shows where the samples were collected; the color represents the salinity. The shoreline is shown in black. Note the location of the wharf, the San Lorenzo River and the Santa Cruz Harbor (two breaks shown in upper right of shoreline outline). Note the different scales on the two plots.
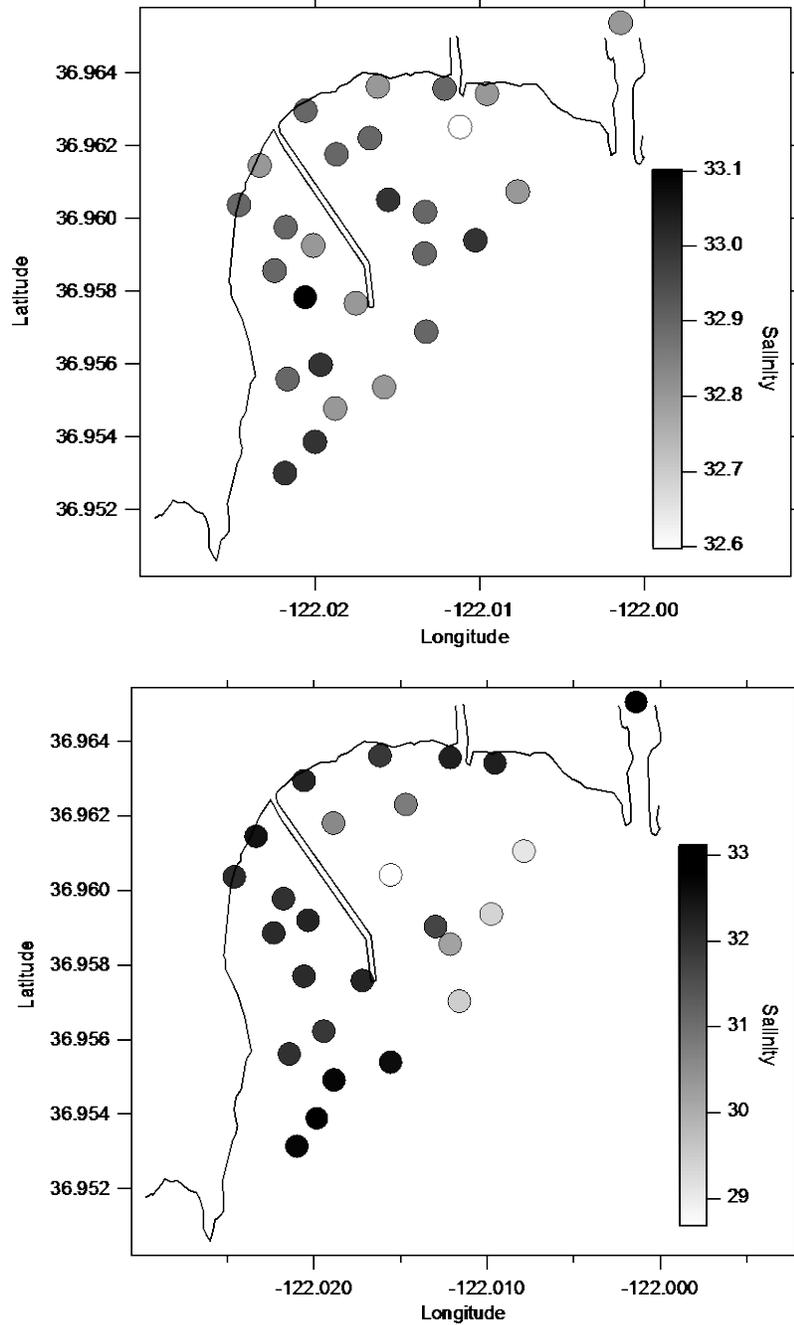
Figure S4: Concentration of *E. coli* (EC) and enterococci (ENT) measured on kelp and wrack collected during the cross-shore survey. The surface sampling locations are shown in Figure S2. The sampling depth and distance from shore sample are illustrated by location of the markers. The location of the seafloor is shown in yellow for reference. Concentration is indicated by the color of the marker. The top panel shows the EC concentrations while the bottom panel shows the ENT concentrations.
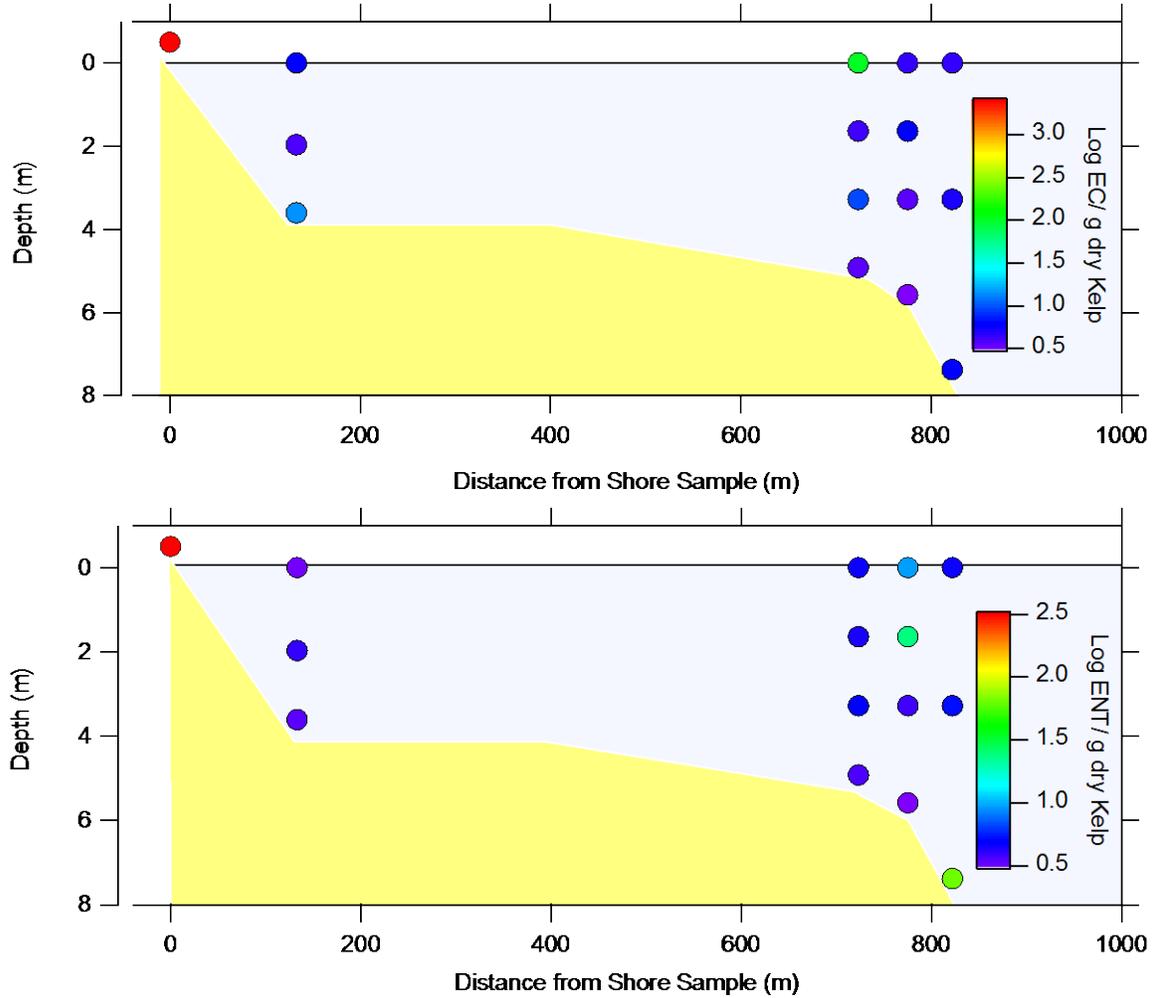
Figure S5: Concentration of *E. coli* (EC) and enterococci (ENT) measured on sand collected during the cross-shore survey. The surface sampling locations are shown in Figure S2. The sampling depth and distance from shore sample are illustrated by location of the markers. The location of the seafloor is shown in yellow for reference. Concentration is indicated by the color of the marker. The top panel shows the EC concentrations while the bottom panel shows the ENT concentrations.
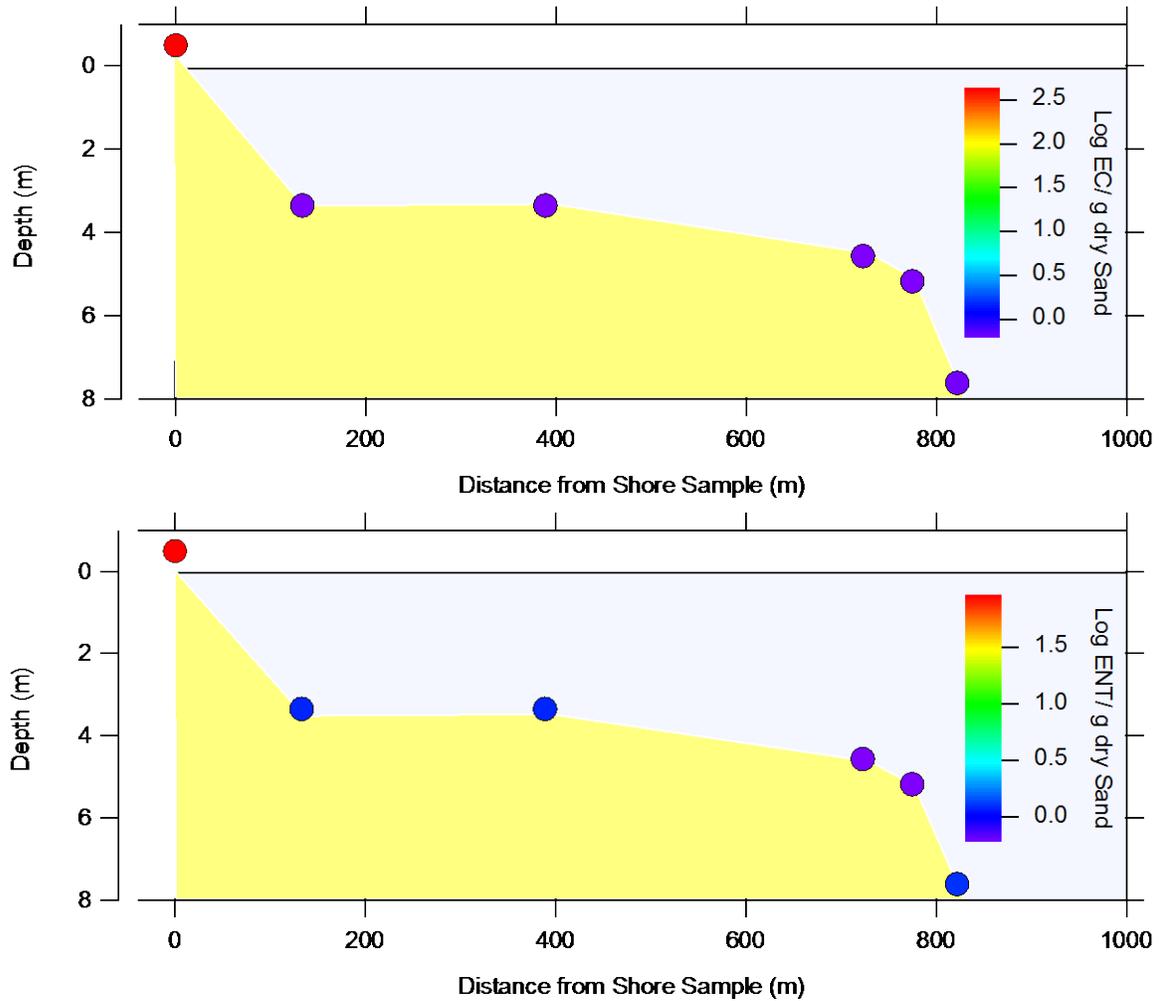
Figure S6: Concentration of *E. coli* (EC) and enterococci (ENT) measured in water collected during the cross-shore survey. The surface sampling locations are shown in Figure S2. The sampling depth and distance from shore sample are illustrated by location of the markers. The location of the seafloor is shown in yellow for reference. Concentration is indicated by the color of the marker. The top panel shows the EC concentrations while the bottom panel shows the ENT concentrations.
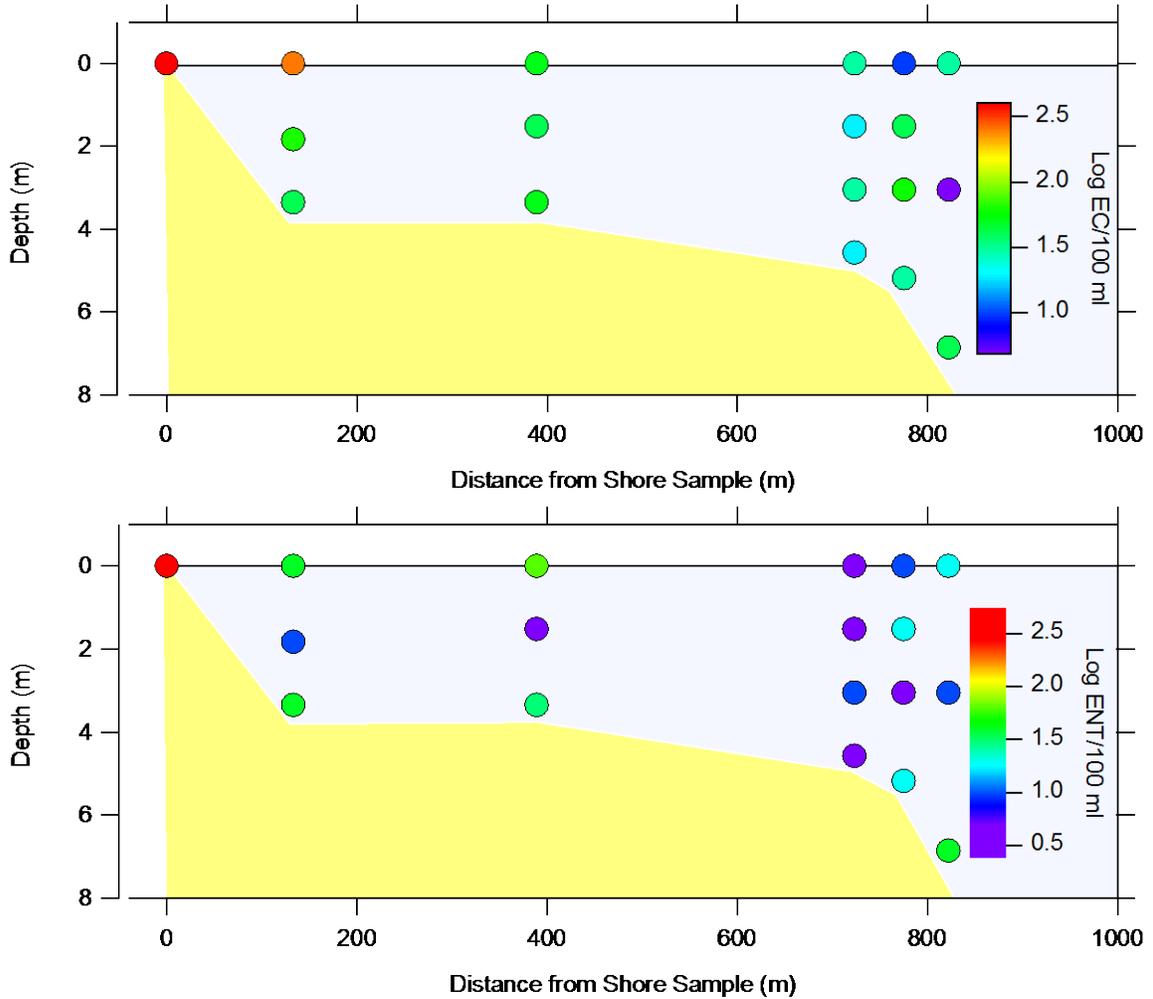
Figure S7: Box and whisker plot of *Catellicoccus* results from 24 h study. Plots are shown for exposed (Ex.) sand (n=15), exposed wrack (n=15) and water (n=24). Box represents 25th, 50th and 75th percentiles; whiskers represent 10th and 90th percentiles. Care must be taken when comparing concentration of FIB on the wrack and sand with water concentrations due to the different units.
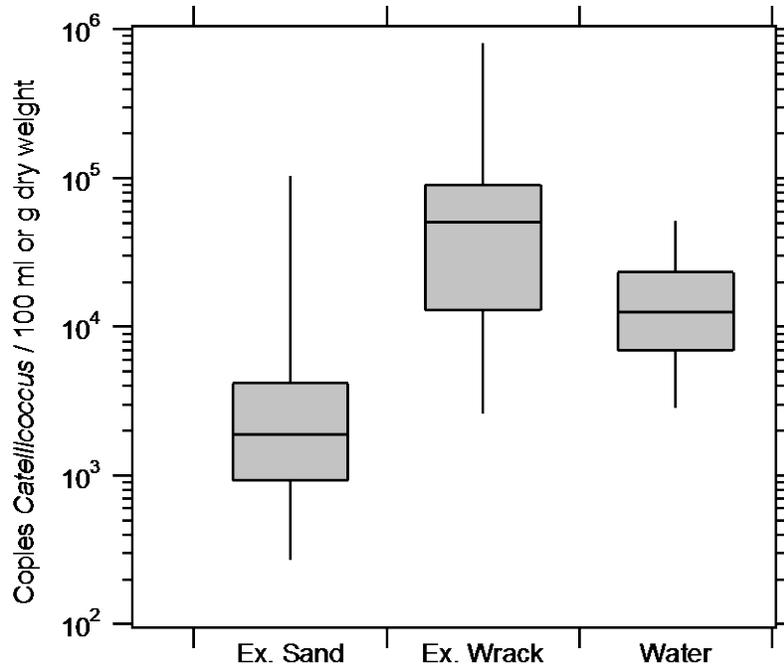
Figure S8: Measured wrack density at conclusion of 24h study. Distances are measured from a datum located well above the high-high tide line. At high tide the water was located at 1.6 m on this scale and at low tide the water was located at 55 m on this scale.
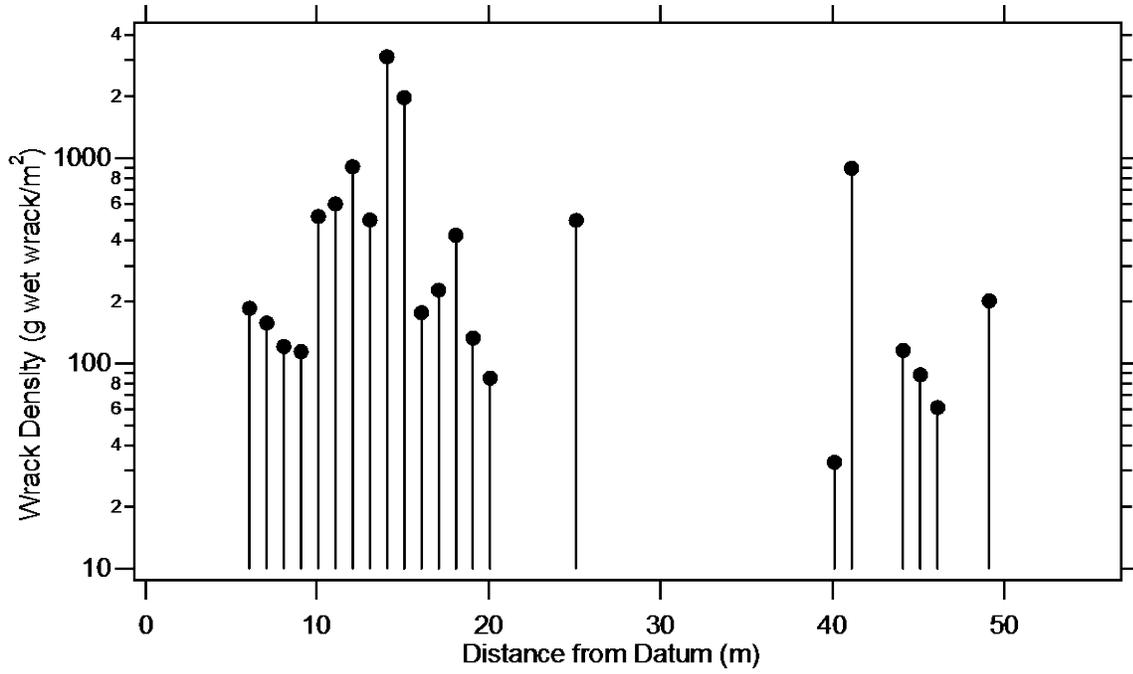
Figure S9: Box and whisker plots showing the concentration of enterococci (ENT) and *E. coli* (EC) in the samples collected during the 24 h study (panels 'A' and 'B' respectively). Box represents 25th, 50th and 75th percentiles; whiskers represent 10th and 90th percentiles. All exposed (Ex.) and submerged (Sub.) sand and wrack concentrations are shown in MPN/g dry weight and all water concentrations are shown in MPN/100mL. Care must be taken when comparing concentration of FIB in the wrack and sand with water concentrations due to the different units.
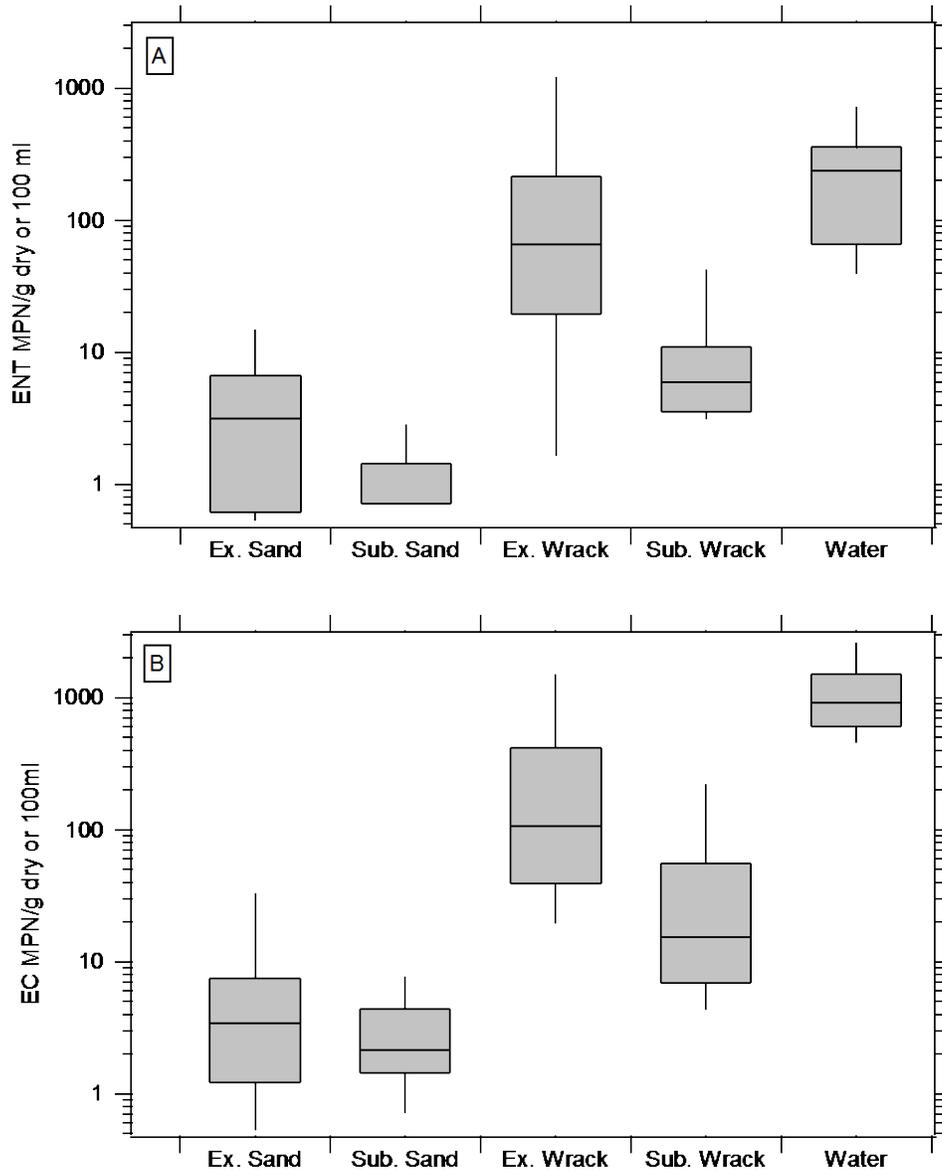
Figure S10: Sensitivity analysis for α_{sand} and α_{wrack} for both enterococci (left panel) and *E. coli* (right panel). Contour lines show the log-RMSE values. All other model variables held constant for the sensitivity analysis.
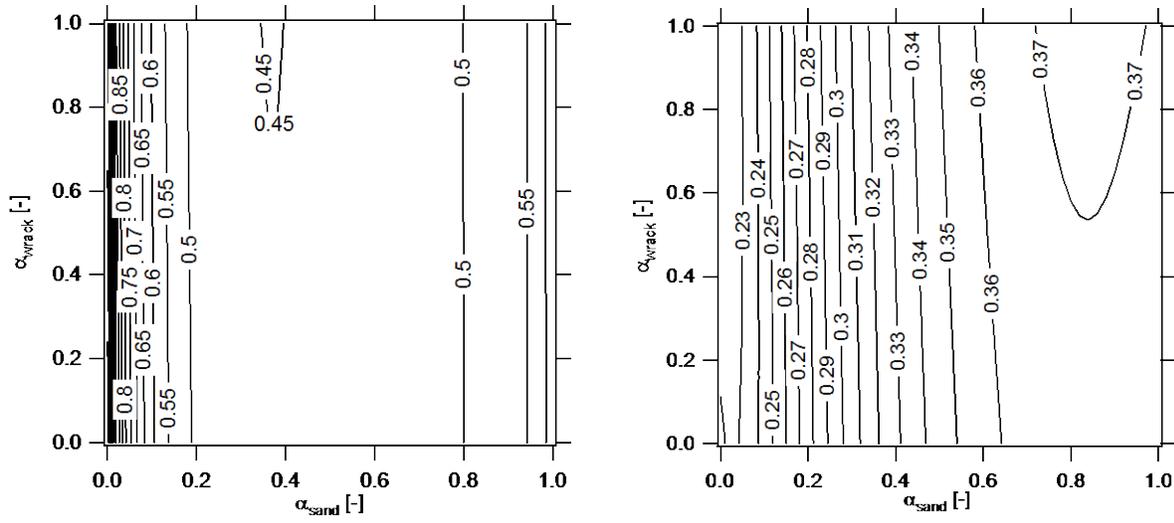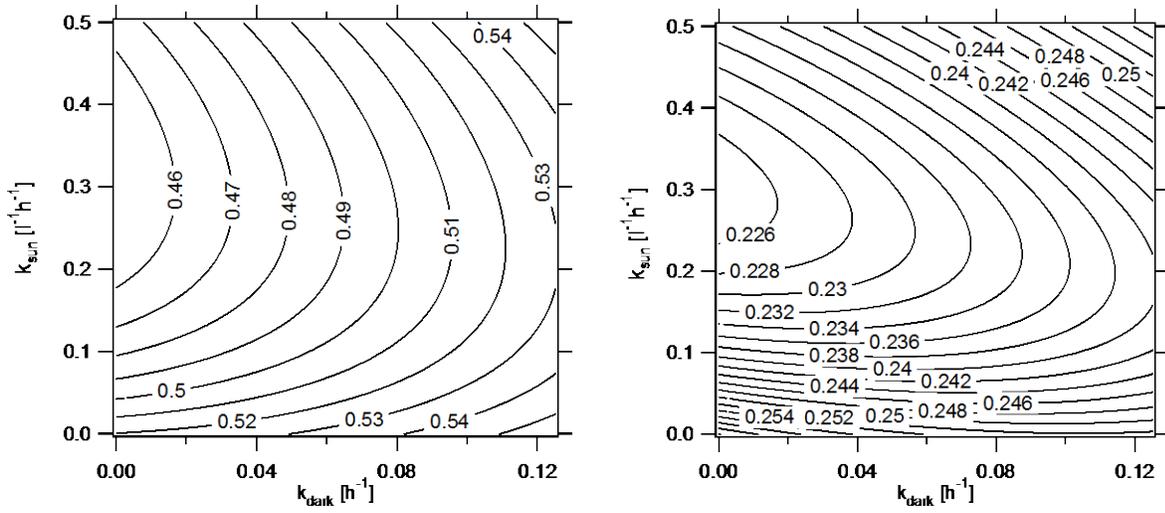
Figure S11: Sensitivity analysis for k_{sun} and k_{dark} for both enterococci (left panel) and *E. coli* (right panel). Contour lines show the log-RMSE values. All other model variables were held constant for the sensitivity analysis.

Figure S12: Sensitivity analysis for α_{sand} and d_{sand} for enterococci. Contour lines show the log-RMSE

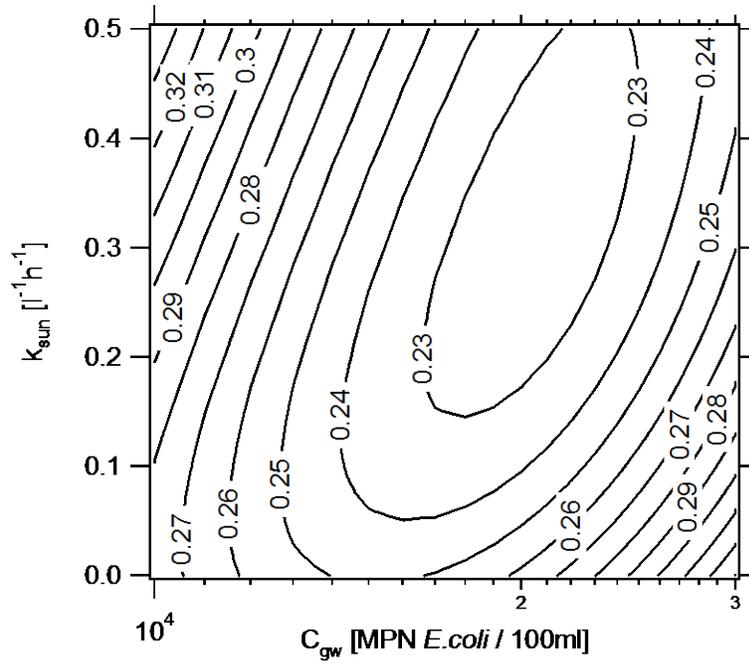values. All other model variables were held constant for the sensitivity analysis. Best fit values presented

in the manuscript were selected to minimize d_{sand} within the region with the lowest log-RMSE.
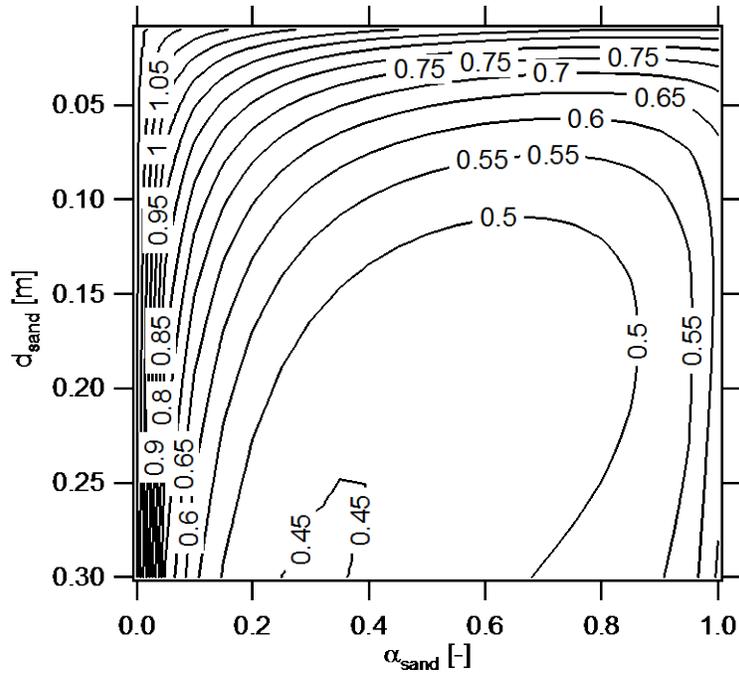
Figure S13: Sensitivity analysis for C_{gw} and k_{sun} for *E. coli*. Contour lines show the log-RMSE values. All other model variables were held constant for the sensitivity analysis.

Figure S14: Sensitivity analysis for α_{open} and C_{gw} for *E. coli*. Contour lines show the log-RMSE values.

All other model variables were held constant for the sensitivity analysis.
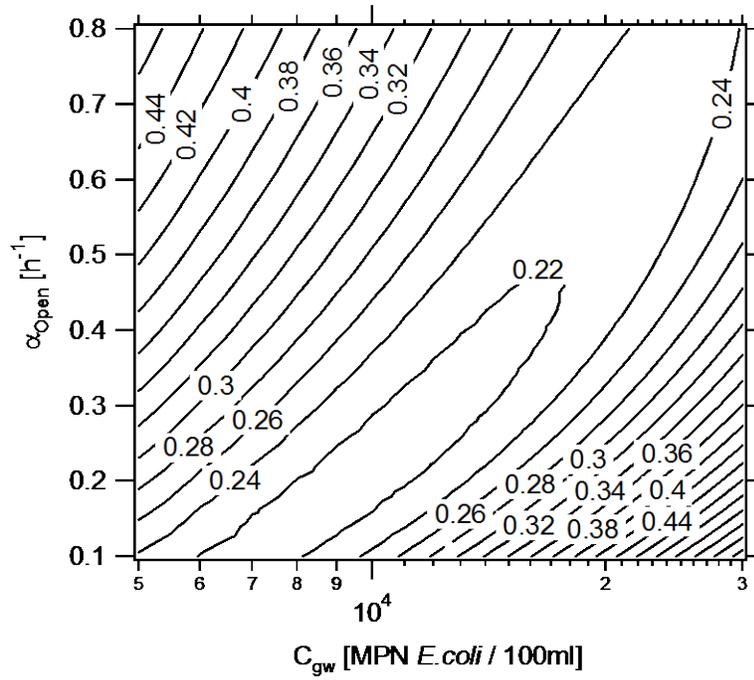
Figure S15: Sensitivity analysis for α_{open} and $Q_{max,gw}$ for silicate. Contour lines show the log-RMSE values. All other model variables were held constant for the sensitivity analysis. Best fit values for α_{open} and $Q_{max,gw}$ were selected to minimize the log-RMSE and $Q_{max,gw}$.

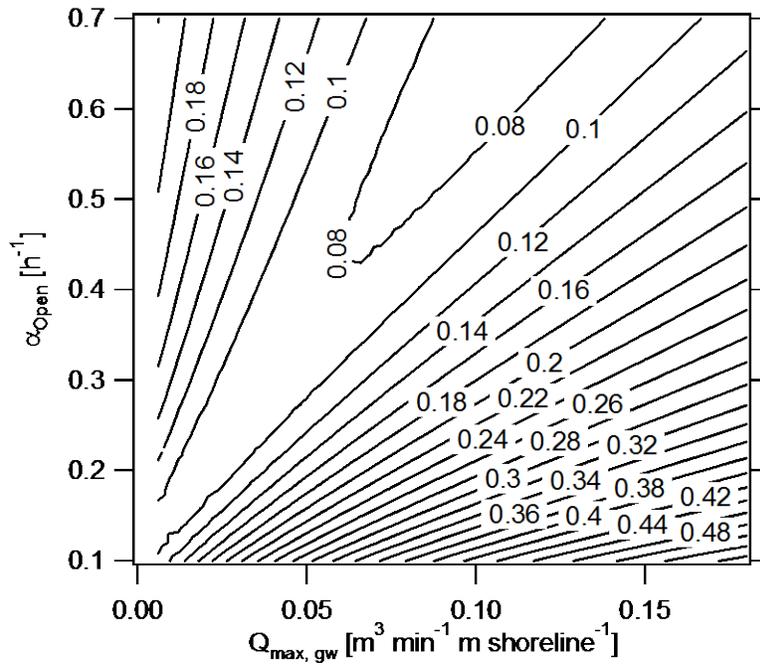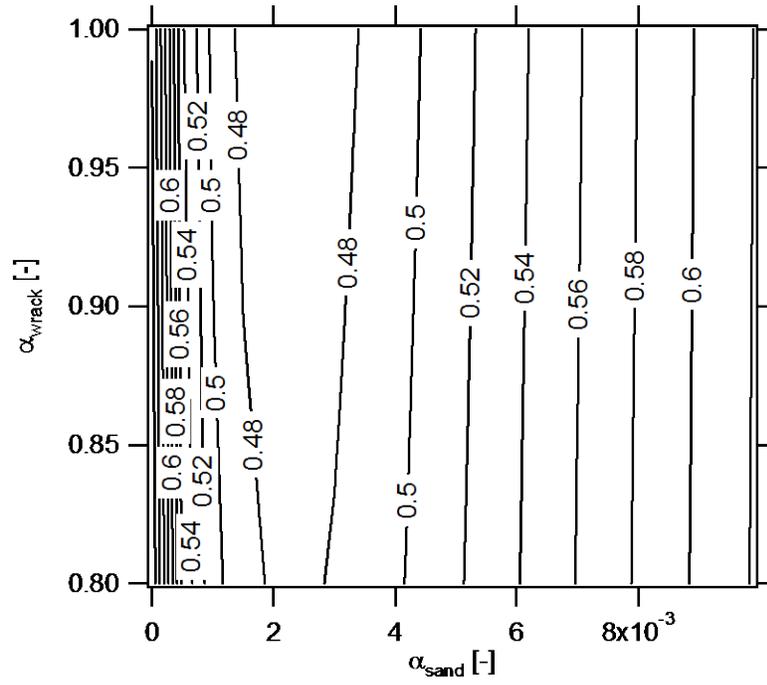Figure S16: Sensitivity analysis for α_{sand} and α_{wrack} for the *Catellicoccus* molecular marker. Contour lines show the log-RMSE values. All other model variables were held constant for the sensitivity analysis.

Impacts of beach wrack removal via grooming on surf zone water quality

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Abstract

Fecal indicator bacteria (FIB) are used to assess the microbial water quality of recreational waters. Increasingly, non-fecal sources of FIB have been implicated as causes of poor microbial water quality in the coastal environment. These sources are challenging to quantify and difficult to remediate. The present study investigates one non-fecal FIB source, beach wrack (decaying aquatic plants), and its impacts on water quality along the Central California coast. The prevalence of FIB on wrack was studied using a multi-beach survey, collecting wrack throughout Central California. The impacts of beach grooming, to remove wrack, were investigated at Cowell Beach in Santa Cruz, California using a long term survey (two summers, one with and one without grooming) and a 48 h survey during the first ever intensive grooming event. FIB were prevalent on wrack, but highly variable spatially and temporally along the nine beaches sampled in Central California. Beach grooming was generally associated with either no change or a slight increase in coastal FIB concentrations, and increases in surf zone turbidity, silicate, phosphate and dissolved inorganic nitrogen concentrations. The findings suggest that beach grooming for wrack removal is not justified as a microbial pollution remediation strategy.

Introduction

Concentrations of fecal indicator bacteria (FIB), enterococci (ENT) and *Escherichia coli* (EC), are used worldwide to assess recreational water quality. FIB are not pathogens, but are favored for water quality monitoring because they are present in high concentrations in feces, and epidemiological studies have shown that their concentrations correlate with risks of illness during recreational water quality contact.[1-3] The majority of epidemiological studies that have related FIB to human illness have been conducted at beaches where the source of contamination is treated wastewater[3-6] or urban runoff presumably contaminated with raw sewage.[7, 8] However, FIB are present in a number of non-fecal sources such as river sediments, [9-11] beach sands[12-14] and decaying lacustrine and marine plants (wrack).[15-20] There is growing concern that FIB at some beaches may emanate from non-fecal sources confounding the FIB-risk relationship.

The present study focuses on the effect of wrack on surface water quality at a marine beach. Previous studies have identified wrack at both marine and lacustrine beaches that can harbor FIB; FIB concentrations on wrack have been observed as high as 10^6 culture forming units (CFU)/g of wrack (Table S1).[15-27] EC has been shown to grow on wrack,[21] both ENT and EC were shown to grow in water with wrack added,[15, 21] and ENT was shown to grow in sand when wrack was present.[15] The source of the FIB found on wrack is generally not known. Wrack FIB may represent naturalized strains or they may be deposited onto wrack by animals present on the shore including gulls and pigeons. Wrack may provide a nutrient rich habitat that is favorable for the attachment and survival of FIB.[20] Although a study on wrack composed primarily of *Cladophora* at a Lake Michigan beach found a number of human bacterial pathogens including *Clostridium botulinum*, pathogenic *E. coli*, *Salmonella*, *Shigella* and *Campylobacter*[28] to be present, there is no study linking exposure to FIB-laden wrack to health risk. Thus FIB-laden wrack on a beach is generally perceived to be a nuisance possibly leading to beach water quality advisories that are not indicative of increased periods of health risk.

Beach grooming is used by beach managers to remove trash and debris from the sand, and generally improve beach aesthetics. Several studies, two in the Great Lakes[29, 30] and one in Southern California,[31] investigated the impacts of beach grooming on concentrations of FIB in beach sands. The studies found that grooming can both increase and decrease the concentrations of FIB in beach sands. Authors speculated that decreases in FIB in beach sands were caused by increased desiccation resulting from the overturn of sand during grooming[29] and that increases in concentration were due to mixing of surface bird droppings into moist subsurface sands that are protected from sunlight.[30] To date no study has examined the effect of beach grooming to remove wrack-laden FIB on beach water quality.

The primary goals of this study were to investigate the pervasiveness of FIB on wrack and to investigate how intensive beach grooming to remove wrack impacts water quality. To investigate the pervasiveness of FIB on wrack, we collected wrack samples from nine beaches in Central California three times over 14 months. To investigate the impacts of intensive grooming on coastal water quality we performed two studies at Cowell Beach in Santa Cruz, California, a site where FIB are found in high concentrations on wrack.[15, 25] The long-term impacts of beach grooming on water quality were assessed by comparing FIB concentrations during two summers, one with and one without grooming. The immediate impacts of beach grooming were assessed over a 48 h period in which intensive grooming first occurred at Cowell Beach. Although removal of contaminated wrack from a beach may improve water quality, wrack plays an important role in the delicate beach ecosystem by providing habitat, food and the primary flux of nutrients to the ecosystem.[32-36] Understanding the impacts of grooming is imperative for beach managers to be able to weigh the possible benefits of grooming against the known negative ecosystem impacts.

Materials and Methods

Multi-beach Survey. Wrack samples were collected from nine beaches in central California (Figure 1): Pacifica State Beach, Gray Whale Cove State Beach, Montara State Beach, San Gregorio State Beach, Four Mile Beach, Natural Bridges State Beach, Lighthouse Field State Beach, Cowell Beach, and New

Brighton State Beach. These beaches were selected because wrack deposits on them frequently. Sampling occurred during the mornings of 3 July 2012, 22 May 2013, and 4 September 2013. Approximately 250 g of wrack were composited from a 50 m alongshore stretch of beach with the distribution of plant species in the wrack preserved. Samples were processed for FIB and dry masses as described below.

Land use in the two km radius around the beach was assessed using the 2006 NLCD land use rasters.[37] Beaches where designated as 'urban' where more than 50% of the area in the circle were defined as developed open space, developed low intensity, developed medium intensity, and/or developed high intensity. All other beaches had the majority of their land use as mixed forests, shrubs and/or grasslands and were designated as 'undeveloped'. Generalized estimating equations (GEEs) were used to analyze if urban beaches had different concentrations of ENT and EC on wrack compared with undeveloped beaches. GEEs were necessary to account for the repeated measures.

Water quality data (EC and ENT concentrations) for the all beaches except for Gray Whale and Four Mile were obtained from local monitoring agencies.[38, 39] Gray Whale and Four Mile are not routinely monitored due to historically low concentrations[38, 39] and were assigned values of one half the detection limits (5 most probably number (MPN)/100 ml) for ENT and EC. Water quality measured at each beach closest in time to the three wrack sampling events was extracted, and then the relationship between log-transformed FIB in water and log-transformed FIB on wrack was investigated using GEEs. Waterborne FIB concentrations were measured within two days of wrack sampling for all beaches except Lighthouse State Beach. At Lighthouse State Beach, waterborne FIB concentrations were measured within two weeks of wrack sampling.

Long-term Grooming Study. Water and wrack from Cowell and Main Beach in Santa Cruz, CA (36°57.7' N, 122°1.5' W, Figure 2) were collected at least weekly, before sunrise, during the summer for two years (2011 and 2012). The beach to the east of the wharf (Figure 2) is known as Main Beach, but the

two beaches are collectively referred to as Cowell Beach henceforth. During the first year and the first week of the second year (24 June -4 August 2011 and 22 May -31 May 2012), the beaches were not regularly groomed and when they were, grooming was restricted to the stretch of beach above the high tide line; hereafter this period of time will be referred to as the 'ungroomed' period. During the second year (6 June -28 August 2012), the beach was intensively groomed meaning that three to five days a week, all plant material on the shore was removed down to the water's edge ('groomed' period). Grooming was performed with a tractor fitted with a rake and root grapple. Data from the ungroomed period were previously published as part of a microbial source tracking study.[25] The FIB concentrations of water and wrack during the 'ungroomed' versus 'groomed' periods were compared using t-tests. All FIB data were log-transformed prior to analysis.

Wrack spatial mass density measurements were taken in the cross-shore direction on a one-meter wide transect extending from above the high tide line to the ocean following Dugan et al. [32] during each visit at the two westerly locations indicated in Figure 2. Wrack wet mass was measured in a series of one square meter increments along the cross shore transect using a portable electronic balance (Model 311, Salter Brecknell, Fairmont, MN). During the groomed period, the wrack density measurements were taken in the early morning before any grooming activities took place on the beach. Log-transformed wrack density (log-kg/m^2 of beach) in the transects was compared between the groomed and ungroomed periods using a t-test.

High Frequency Study of Grooming Impacts. A 48 h study was performed at Cowell Beach (Figure 2, Inset A). The timing of the 48 h study corresponded with the first ever intensive grooming of Cowell Beach. Further, the beach had not been groomed in any way 21 days prior to the study. The study lasted from 0400 h 4 June 2012 to 0300 h 6 June 2012. Grooming began at 0530 on 5 June 2012 at the spring, low tide and concluded at 1230 on 5 June 2012. Grooming consisted of a combination of mechanical grooming with a backhoe and a tractor fitted with a rake and root grapple, as well as hand raking. There

was a small rain event midday on 4 June 2012. Rainfall and solar radiation data were obtained from a weather station located on the adjacent wharf.[40]

During the 48 h study, five types of samples were collected: water, exposed sand, exposed wrack, submerged sand, and submerged wrack. The samples were collected along a 5 m wide (alongshore direction) transect that extended in the cross shore direction from above the high tide line to below the low-tide line. Water samples were collected at 30 min intervals from surface water at knee depth in the surf zone. Exposed and submerged sand and wrack samples were collected at 60 min intervals in the locations described below. The location of the exposed (subaerial) sand and exposed wrack samples were set relative to the waterline. The waterline was defined as the highest point in the transect that was touched by wave run-up in the minute prior to the start of sampling. Exposed sand and wrack samples were collected at, respectively, 1.5 m above the waterline and 1-2 m above the waterline. Submerged sand samples were collected at the same location as the water samples, after the water was sampled. Submerged wrack samples were collected from either the wave run-up or the surf zone. If no wrack was present in the previously defined region for a particular time point, no sample was collected. The sand and wrack samples were composited over the 5-meter wide (alongshore) transect in the locations previously described.

Wrack, sand, and water samples were processed for FIB following the protocols provided below. Phosphate, dissolved inorganic nitrogen, and silicate were measured in water samples collected on 60 min intervals. Water samples for nutrient analysis were filter sterilizing through a 0.2 µm pore size filter (VWR International, Radnor, PA), placed in opaque, 10% HCl washed and rinsed HDPE bottles, and subsequently frozen at -20°C before analysis on a Lachat QuickChem 800 (Zellweger Analytics, Lincolnshire, IL).

Wrack spatial mass density was measured three times during the 48 h study, twice before grooming and once after grooming. Wrack density was measured along the middle of the study area in a one-meter wide transect following Dugan et al. [32]

The water quality data (FIB, nutrients, and turbidity) were used to test three hypotheses. (H1) Water quality was different during the rainfall event than during the matched time period the following day when no rain was falling, (H2) the water quality during active grooming was different from water quality during the matched time period the previous day with no grooming, and (H3) water quality after intensive grooming was completed was different from water quality during the matched time period the previous day before grooming occurred. FIB concentrations in the surf zone are influenced by tidal and diel cycles owing to the presence of shoreline FIB sources,[41] photoinactivation,[42, 43] and tidally forced transport and mixing of nearshore waters. Thus, each data point collected during a treatment time period was paired with a data point from the control time period collected at the exact same time of day. This pairing aimed to control for the effects of tides and sunlight during hypothesis testing. For example, for the rain event hypothesis testing (H1), water quality data from 1030 h June 4 [rain] were paired with data collected at 1030 h June 5 [no rain], and 1100 h June 4 with 1100 h June 5, and so forth (Table 1). Potential interday variability of these environmental cycles and potential lag effects of the rain event and beach grooming were not accounted for in the analysis. The hypotheses were tested using paired t-tests. FIB and turbidity data were log-transformed; nutrient data were not transformed.

We also tested for differences between FIB in exposed sand and wrack during ungroomed and groomed periods using t-tests. Sand and wrack FIB concentrations were log-transformed for statistical analysis. Wrack solids content (see below) and FIB concentrations were analyzed to investigate for possible correlations using wrack samples from both the ungroomed and groomed periods.

Wrack Community Composition and FIB. We tested whether the plant taxa present in the wrack from the long term study (n=100 samples) were associated with FIB concentrations of the bulk wrack. Identification to the lowest possible taxonomic level (genera or species) was performed on samples that had been dried at 105°C for 24 h.[44, 45] The mass of each taxon present in the sample was measured using a digital balance and then assigned a fraction of the total mass of the sample. Community data for each sample were then transformed to binary data (present/absent). Bray-Curtis dissimilarity coefficients were calculated pairwise for the samples to generate a 100 x 100 similarity matrix. The ENT and EC concentrations for each sample were designated as above or below the 75th percentile ('high' vs. 'not-high'). An analysis of similarity (ANOSIM) was used to test whether the community of plants present in wrack samples with 'high' FIB were more similar to each other than the community of plants present in the wrack samples with 'not-high' FIB. Additionally, log-mean FIB concentrations were calculated when each taxon group was present versus absent and a t-test was used to determine if those log-means were significantly different. Primer v.6 (Primer-E Ltd, Ivybridge, United Kingdom) was used for the multivariate analyses.

General Methods. All wrack samples were collected in sterile bags and reflected the ratios of various species present. Bacteria on wrack samples were eluted following a slightly modified version of Imamura et al. [15]. See Supporting Information (SI) for details on bacteria elution and determination of solids content.

All sand samples were collected using sterile scoops, placed in sterile bags, and represented a composite of 5-8 samples collected across a 5 meter alongshore stretch of beach. Bacteria from sand samples were eluted following Boehm et al. [46]. See SI for details on bacteria elution and determination of sand moisture content.

Water samples were collected in triple rinsed sterile plastic bottles. Water samples were assayed for salinity in the field using a YSI-30 (YSI, Yellow Springs, OH) and turbidity in the laboratory using a DRT-15 CE turbidimeter (HF Scientific, Fort Myers, FL).

All samples were stored on ice and processed for FIB within 6 h. Concentrations of ENT and EC were measured by the defined substrate methods of Enterolert and Colilert-18 (IDEXX Laboratories, Fremont, CA) (see SI for details). For data analysis, samples above the upper detection limit were assigned that value and samples below the lower detection limit were assigned half of the detection limit.

Field blanks and method blanks were taken during both the long-term study and the 48 h study. A detailed description of these blanks is provided in the SI.

Unless otherwise specified, statistical analyses were performed in PASW Statistics 18 (IBM, Armonk, NY). Results significant at the $\alpha < 0.1$ level are presented. Correlations were tested using a Spearman's rank analysis.

Results

Performance of Microbiological Assay Controls. All field blanks and method blanks were negative for FIB indicating no cross contamination during field work and sample processing.

Multi-beach Survey FIB. Concentrations of FIB on wrack were highly variable across the three sampling events ranging from below the lower detection limit (~0.5 MPN/g dry) to above the upper detection limit (approximately 4000-6000 MPN/ g dry, varies due to solids content). The median concentration for all beaches and sampling events was 1.66 log-MPN/g dry for ENT and 1.17 log-MPN/g dry for EC. Wrack at 2 of the 9 beaches sampled was found to have ENT and/or EC above the limit of detection on one of the sampling events (Figure 1). The highest wrack concentrations were found at

Montara and Four Mile Beach. Concentrations were significantly lower during the second sampling visit as compared to the other visits (log-mean difference, all beaches 1.3-1.7 log-MPN/g dry, p≤0.034). Wrack ENT and EC concentrations were positively correlated (r_s=0.67, p<0.001). Wrack solids content was significantly negatively correlated with EC concentration (r_s=-0.54, p=0.003) but was not correlated with ENT (p=0.73).

Gray Whale, Montara, San Gregorio and Four Mile beaches were primarily surrounded by undeveloped land cover while the other five beaches were surrounded by primarily developed land cover. Dominant land cover was not found to be associated with concentrations of wrack ENT (p=0.11) or EC (p=0.93). When wrack concentrations were compared to beach water quality, there was no association between water and wrack contamination for ENT (p=0.3) but there was an association with EC (GEE β=0.32, p=0.07).

Long-term Grooming Survey. Water and wrack FIB concentrations were broken into two periods for analysis: ungroomed and groomed. These two periods represent respectively a control period with minimal grooming activities (only above the high-high tide line) and a treatment period with intensive whole-beach grooming. There were approximately 25 tons of wrack removed from the beach during the ungroomed period compared to the >390 tons removed during the grooming period.[47]

Wrack did not have significantly different concentrations of ENT or EC during the two periods (Table 2). However, wrack did have significantly higher solids content (i.e., was drier) during the ungroomed period relative to the groomed period (Table 2) suggesting there was potentially older wrack on the beach during the ungroomed period. Waterborne ENT and EC were significantly higher during the grooming period relative to the ungroomed period (p≤0.023, log mean difference of 0.3 log-MPN/100 ml for both). Water was also significantly more turbid during the groomed versus ungroomed periods (p=0.037, log-mean difference of 0.17 log-NTU).

The mass of wrack on the beach during each sampling event was measured before sunrise and before any potential grooming activities took place. During the groomed period, the beach was intensively groomed 3-5 days a week but wrack consistently re-deposited throughout the day and night after grooming. Consequently, during the groomed period, wrack was present on the beach during most sampling events. Mean wrack mass normalized by the area of beach surveyed was not significantly different (p=0.11) during the groomed period relative to the ungroomed period (Figure S1).

Considering FIB concentrations measured on all wrack (groomed and ungroomed periods), ENT and EC concentrations on wrack were significantly positively correlated (r_s=0.35, p<0.01). EC concentrations were significantly negatively correlated with the solids content (r_s=-0.23, p=0.02) – the drier the wrack, the lower the EC.

High Frequency Study of Grooming Impacts. High concentrations of ENT and EC in the surf zone water were observed throughout the 48 h study (Figure 3). In total 47% and 74% of water samples were above the single sample standard for recreational waters[48] of 104 MPN/100 ml ENT and 400 MPN/100 ml EC respectively. Exposed wrack concentrations were as high as 18 and 1541 MPN/g dry for ENT and EC respectively. Additional results for the sand and wrack concentrations are presented in the SI.

Waterborne concentrations of ENT, EC, nutrients, and turbidity were used to test the following three hypotheses: (H1) water quality was different during the rainfall event than during the matched time period the following day when no rain was falling, (H2) the water quality during active grooming was different from water quality during the matched time period the previous day with no grooming, and (H3) water quality after intensive grooming was completed was different from water quality during the matched time period the previous day before grooming occurred (Table 1).

H1 could not be rejected for either FIB or silicate, but was rejected for phosphate, DIN and turbidity. The rainfall was found to not affect FIB concentrations ($p \geq 0.16$) or silicate ($p=0.97$). DIN and phosphate were lower during the rain event compared to its control time period (mean difference = 6.9 and 0.55 µM, $p \leq 0.007$). The control time period for the rain event coincides active grooming and thus may confound the results. However, a qualitative assessment of the time series indicates no obvious increases in FIB or nutrient concentrations or decreases in salinity coincident with the rain event. The decrease in salinity prior to the rain event may be due to submarine groundwater discharge.[25]

H2 was rejected for ENT and nutrients, but not for EC and turbidity. During active grooming, the water was found to have significantly lower ENT (mean difference = 0.66 log ENT MPN/100 ml, $p=0.001$); however EC was not significantly different ($p=0.31$). Turbidity was also not different between the treatment and control time periods ($p=0.63$). All three of the nutrients measured had significantly different concentrations during active grooming (mean differences between treatment and control periods = 8.1 µM DIN, 0.44 µM phosphate, -5.5 µM silicate, $p \leq 0.031$) (Table 1).

H3 was rejected for ENT, nutrients, and turbidity but not for EC. After grooming, ENT concentrations decreased by 0.18 log-ENT MPN/100 ml ($p=0.04$) compared with the control period, but EC concentrations were not significantly different. Turbidity was significantly higher after grooming by 12.9 NTU (mean difference = 12.9 NTU, $p<0.001$). Nutrients were also significantly higher after grooming compared to the control time period (mean differences = 9.0 µM DIN, 0.67 µM phosphate, 6.7 µM silicate, $p<0.001$ for all).

Exposed sand ENT and EC concentrations were not significantly different before and after the grooming event ($p=0.75$ and 0.60 respectively). Exposed wrack ENT and EC concentrations were not significantly different before and after the grooming event ($p=0.3$ and 0.4 respectively).

Wrack spatial mass density was measured three times during the study, twice before grooming and once after grooming. Before grooming there were approximately 50-90 kg of wrack (wet mass) in the 53 m^2 study area and after grooming there were approximately 2 kg.

Wrack Community Composition and FIB. The following taxonomic groups were frequently observed in wrack samples and were included in the analysis: *Macroystis pyrifera*, *Phyllospadix scouleri*, *Egregia menziesii*, *Cystoseira osmundacea*, *Pterygophora californica*, *Ulva stenophylla*, *Plocamium pacificum*, *Chondracanthus exasperatus*, *Dilsea californica*, *Cryptopleura* spp., and *Nereocystis luetkeana*. An 'other' category was also included for unidentifiable wrack including wrack that was aged and/or disintegrated.

We investigated whether the plant taxa present in the wrack collected as part of the long term study were significantly different when ENT and EC was 'high' versus 'not high' in the wrack where 'high' was defined as concentrations over the 75th percentile -- 363 and 659 MPN/g dry for ENT and EC respectively. Species composition tended to be more similar amongst samples with 'high' ENT compared to samples with 'not high' ENT (Global R=0.086, p=0.043), but a similar result was not observed for EC (p=0.71).

Log-mean ENT was significantly higher when *Macroystis pyrifera* and *Cryptopleura* spp. were present versus absent in samples (respectively log-mean increase=0.94 and 0.55 log-MPN/g dry, p= 0.04 and 0.07) (Figure S2). Log-mean EC was significantly higher when *Plocamium pacificum*, *Nereocystis luetkeana* and *other* were present versus absent in samples (respectively log-mean increase=0.46, 0.42 and 0.6 log-MPN/g dry, p= 0.07, 0.07 and 0.01) (Figure S2).

Discussion

Fecal Indicator Bacteria on Wrack. The multi-beach wrack survey found that FIB are ubiquitous on wrack at beaches in Central California. This finding is consistent with previous research showing that FIB are present on wrack at both freshwater and marine beaches. The highest concentrations of FIB measured on wrack herein are consistent with those previously measured at marine beaches (Table S1).[15, 17, 25] Some wrack samples collected in this study were above the upper detection limits (4000-6000 MPN/g dry), so the actual concentrations may exceed those previously observed at marine beaches. At lacustrine beaches, wrack concentrations have been observed that are two log units higher than those observed in this study.[20, 26] In laboratory microcosms, lacustrine wrack has been shown to have a FIB carrying capacity approximately 4.5 log units above the highest concentrations observed during this study demonstrating the potential for wrack to serve as a reservoir where FIB may potentially multiply.[21] The multi-beach wrack survey found that wrack FIB concentrations were highly variable both spatially and temporally. FIB concentrations on wrack were not associated with the dominant land cover surrounding the beach, although further investigation into the determinants of the concentrations is warranted. ENT concentrations in the water were not associated with wrack ENT concentrations, but higher EC concentrations in water were associated with higher concentrations on wrack at the beaches sampled suggesting there could be a connection between water quality and wrack contamination.

Wrack community composition and solids content were associated with wrack FIB concentrations. The wrack community composition analysis found that there are significant differences in wrack concentrations of ENT based on the taxonomic make-up of the wrack. This suggests that the species/genera of wrack present impact the ability of FIB to deposit and survive on wrack. The solids content analysis found a significant negative correlation between solids content and EC suggesting that EC may persist better when more moisture is present. EC in beach sands have demonstrated the same trend.[12, 14, 49] To our knowledge, this is the first study to investigate and identify a connection between FIB concentrations and wrack species. These finding suggest that further research on the presence, survival and possible growth of FIB on wrack and particularly different species of wrack is warranted.

Impacts of Grooming and Implications. A primary goal of this study was to investigate how intensive beach grooming to remove wrack impacts microbial water quality. On both the short time scale of the 48 h study and the long time scale of an entire season, there were either no changes, or only minor changes to the microbial water quality between periods with and without intensive grooming. When changes were observed in the long term survey, they were in the opposite direction as one might hope – FIB increased in water at the groomed beach. This research is consistent with Russell et al. [25] who used modeling in conjunction with field observations to show that wrack plays only a minor role in controlling FIB concentrations at Cowell Beach (1-2% of the total shoreline FIB flux). Wrack found at Cowell Beach can harbor high concentrations of FIB, however the total mass of wrack is not large enough for it to be a major contributor of FIB to the water column.

During the long term study, water quality during a treatment time period (summer 2012) was compared to water quality during a control time period (summer 2011 and two weeks of late spring 2012) when the treatment was not in place. The ideal control against which to compare the treatment would be a replicate control beach sampled during the same period of time as the treatment beach. This would control for potential interannual variations in water quality that might obscure treatment effects. However, there is no 'replicate' Cowell Beach available to serve this purpose and use of a different beach with potentially different FIB sources and transport characteristics as a control would introduce sources of unknown bias. We compared water quality during the treatment period (summer 2012) with water quality during a control period of the same season (two weeks in late spring 2012) and the results were unchanged (results not shown). This suggests that there was minimal interannual variation in beach water quality.

There are two ways in which we hypothesized intensive grooming could impact the microbial water quality. Grooming could remove wrack, a potential source of FIB, and thus decrease FIB flux into the ocean. Alternatively, grooming could increase FIB concentrations in beach sands. We found that intensive

grooming was associated with at most minor changes in waterborne FIB concentrations, and no changes in FIB in sands. Removing wrack and disturbing the sands during grooming does not substantially change the FIB fluxes to the coastal ocean. However, grooming was associated with an increase in turbidity and a two fold increase in nutrient concentrations in the surf zone suggesting that intensive grooming affects coastal water clarity and water quality at this beach. Together these findings suggest that beach grooming for wrack removal is not justified as a strategy to reduce coastal FIB concentrations. Further work on the effects of wrack removal on coastal FIB concentrations at diverse beaches is warranted.

This is the first study to document the impacts of wrack removal of surf zone water quality, so it is not possible to directly compare our results with those of others. Other studies have examined how grooming affects FIB levels in sands, and those studies demonstrated that grooming can both increase[29, 30] and decrease[29] concentrations of FIB in sands. Kinzelman et al. [29] showed that dry weather beach closures could be reduced with optimized grooming techniques. However, it has also been shown that grooming can increase concentrations of FIB in sands,[29, 30] which would likely have the opposite impact on coastal water quality. Considering both this research and previous studies, beach mangers should carefully consider the potential impacts to water quality that can result from beach grooming.

Associated Content

Supporting Information (SI)

Additional methods, results, acknowledgements, Table S1 and Figures S1-S3 are presented in the SI. This material is available free of charge via the Internet at http://pubs.acs.org.

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Tables

Table 1: Results for the paired time periods analysis of the high frequency study of grooming impacts (48 h study). Numbers in the event column correspond to numbered hypotheses. Details and time and date columns define the periods compared. The mean differences represent the results of paired t-test and show the mean difference (a-b, as specified in the details column) and the statistical significance (p). Bold indicates the mean difference is statistically significant at the α=0.1 level.

Event	Details	Time and Date	Mean Difference [a-b], (p)					
			Log-ENT [Log-MPN/100 ml]	Log-EC [Log-MPN/ 100 ml]	DIN [µM]	Phosphate [µM]	Silicate [µM]	Turbidity [NTU]
(1) Rain	(a) Raining	1030-1400h 4 June	0.32 (0.16)	0.12 (0.17)	**-6.9 (0.004)**	**-0.55 (0.007)**	-0.17 (0.97)	**-3.7 (0.02)**
	(b) No Rain	1030-1400h 5 June						
(2) Active Grooming	(a) Active Grooming	0530-1230h 5 June	-0.66 (0.001)	-0.10 (0.31)	**8.1 (<0.001)**	**0.44 (<0.001)**	**-5.5 (0.031)**	-0.47 (0.63)
	(b) No Grooming	0530-1230h 4 June						
(3) Groomed Beach	(a) Groomed	1300h 5 June – 0300 h 6 June	**-0.18 (0.039)**	-0.03 (0.73)	**9.0 (<0.001)**	**0.67 (<0.001)**	**6.7 (<0.001)**	**12.9 (<0.001)**
	(b) Ungroomed	1300h 4 June – 0300 h 5 June						

Table 2: Long-term grooming study results for wrack and water before and after grooming. Mean differences are provided only for statistically significant differences (p<0.1) as determined by a t-test.

	Mean Ungroomed	Mean Groomed	Mean Difference [Ungroomed – Groomed]	Significance of mean difference
Wrack ENT [log-MPN/g dry]	1.91	1.65		
Wrack EC [log-MPN/g dry]	2.28	2.13		
Wrack Solids Content [-]	0.46	0.41	0.058	0.036
Water ENT [log-MPN/100 ml]	1.63	1.93	-0.30	0.015
Water EC [log-MPN/100 ml]	2.12	2.39	-0.27	0.023
Water Turbidity [log-NTU]	0.29	0.46	-0.17	0.037

Figures

Figure 1: Map of the nine beaches sampled for wrack FIB along the Central California coast. The location of the marker indicates the spatial location of the beach and the color of marker represents that concentration of EC (upper) and ENT (lower) measured. Each site has three markers enclosed in a dark box indicating the concentration measured on the three sampling trips. The sampling trips are arranged chronologically in the gray boxes organized with the first sampling even located closest to the coastline. Boxed numbers indicate the beach: (1) Pacifica State Beach (2) Gray Whale Cove State Beach (3) Montara State Beach (4) San Gregorio State Beach (5) Four Mile Beach (6) Natural Bridges State Beach (7) Lighthouse Field Sate Beach (8) Cowell Beach (9) New Brighton State Beach.
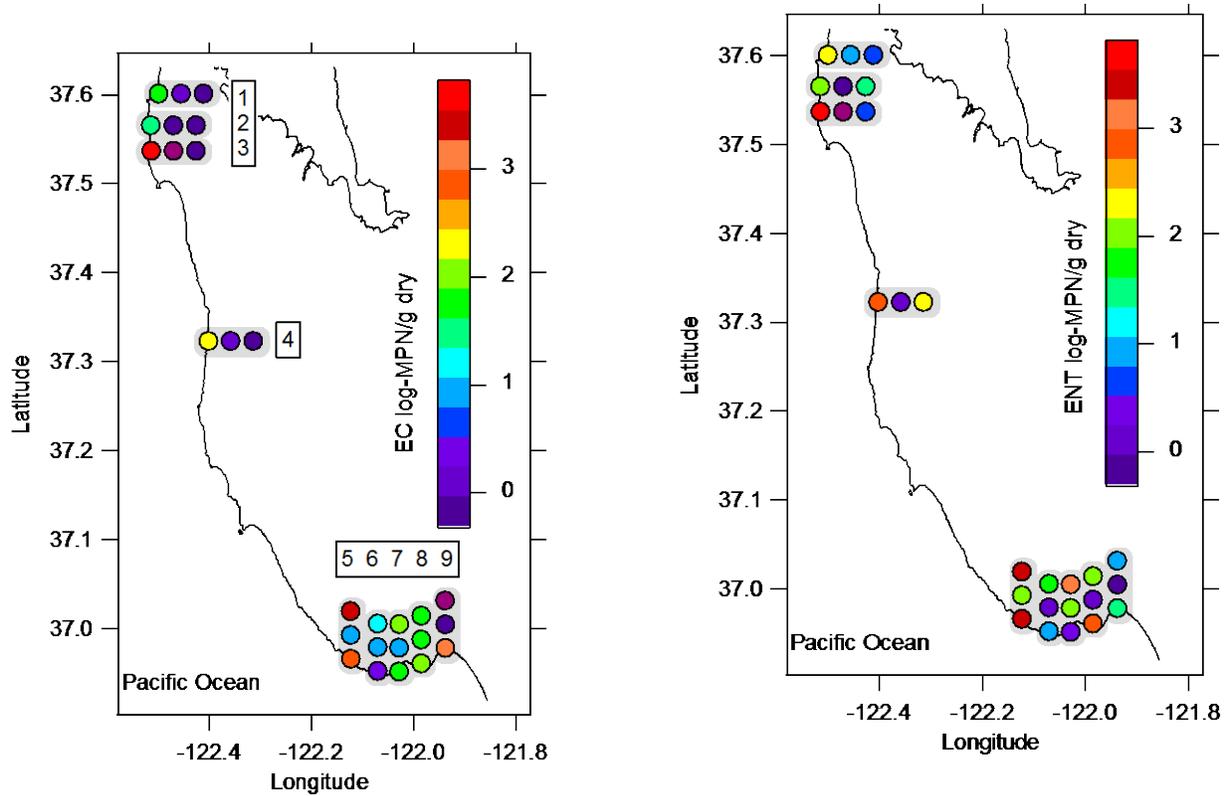
Figure 2: Map of Cowell and Main Beach in Santa Cruz, CA with important locations highlighted. The location of the 48 h study is shown in Inset A. The sampling locations of the long-term study are shown with circles indicating water samples and squares indicating the wrack samples. During every visit, wrack density measurements were taken at the two westerly locations. Satellite photo provided by Google Earth (© 2013 Google and Terrametrics).

Figure 3: Results of the 48-hour study. The gray bars show the periods with rain and active grooming activity. In the left figure, panels from top to bottom show: rainfall, solar radiation, tide level, ENT concentrations in the surf zone and EC concentrations in the surf zone. In the right figure, panels from top to bottom show: turbidity, salinity, silicate concentrations, phosphate concentrations and dissolved inorganic nitrogen (DIN).
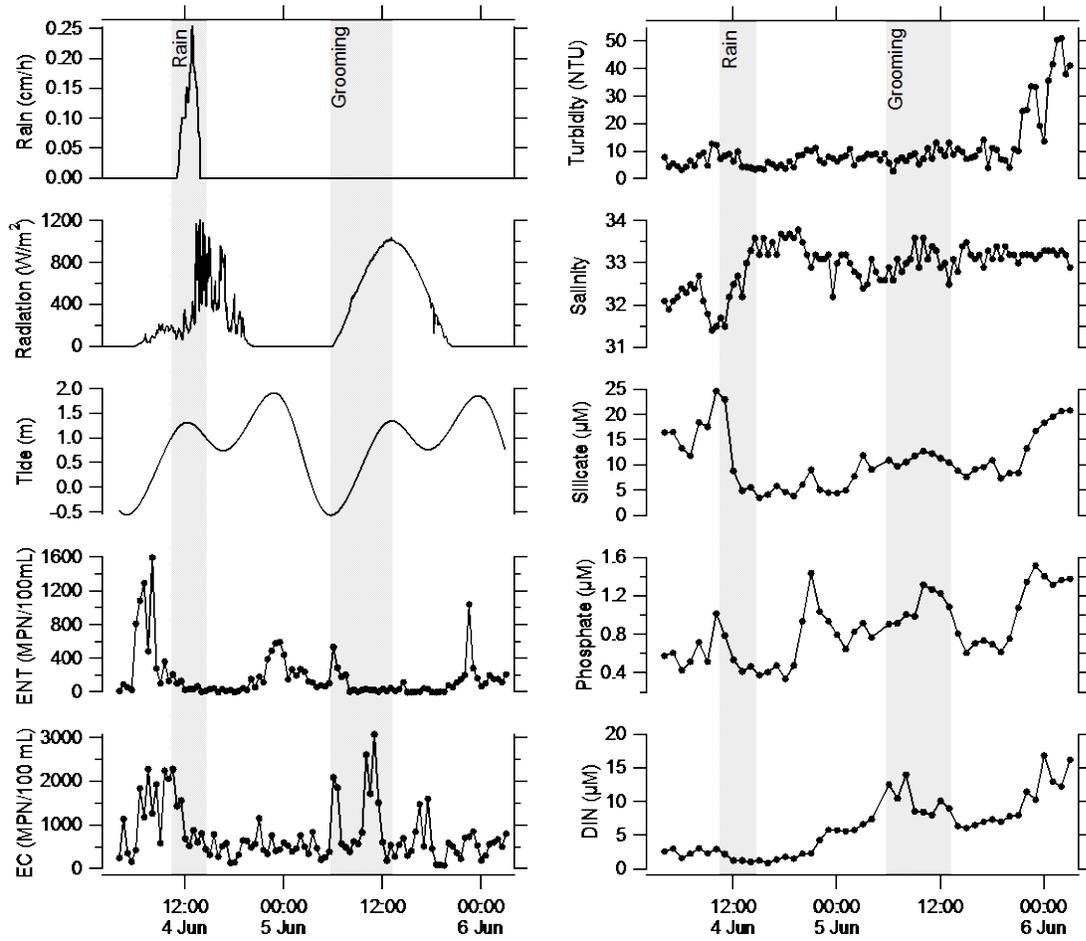
Methods

Wrack Samples. Wrack samples were eluted for bacteria following a slightly modified version of Imamura et al. [1] Briefly 20 g of wet mass wrack were added to 200 mL of autoclaved phosphate buffered saline + magnesium chloride (0.085 g KH_2PO_4 + 0.19 g anhydrous $MgCl_2$ per a L of solution) in a sterile bottle. The mixture was hand shaken for 3 minutes, allowed to settle for 30 s and half of the eluent was gently poured off into a new sterile contained and used for FIB analysis. Solids content of the wrack samples was determined by drying a subset of each sample at 105°C for 24 h.

Sand Samples. Sand samples were eluted for bacteria following Boehm et al. [2] Briefly, 20 g wet mass sand were added to 200 mL autoclaved deionized water and hand shaken for 3 min. The mixture was allowed to settle for 30 s and the eluent was used for FIB analysis. Moisture content of the exposed (subaerial) sand samples was measured with an HH2 moisture probe (Delta-T Devices, Cambridge, England). The instrument was calibrated to site sands by generating a calibration curve with field sand samples dried at 105°C for 24 h. Submerged sand samples were assumed to be saturated. Dry masses of submerged sands were calculated based on the measured porosity and the dry bulk density.

FIB Enumeration. Samples were processed for ENT and EC using the defined substrate methods of Enterolert and Colilert-18 (IDEXX Laboratories, Fremont, CA). Ten milliliters of sample or eluent were diluted in 90 mL of Butterfield's solution (Webber Scientific, Hamilton, NJ), mixed with appropriate media and added to Quanti-Tray/2000s. The sealed trays were incubated and read according to manufacturer's instructions. For data analysis, samples above the upper detection limit of 24,196 MPN/100 mL were assigned that value and samples below the lower detection limit of 10 MPN/100 mL were assigned a value of 5 MPN/100 mL. Wrack and sand FIB concentrations were normalized to MPN/g dry weight

Field Blanks and Method Blanks. Field blanks were taken during both the long-term study and the 48 h study (n=3). Field blanks consisted of a sterile bottle or bag filled with sterile DI water that was taken in the field, open and closed during sampling, placed in the sampling cooler and processed along with other samples for FIB as previously described. Method blanks for the wrack and sand elutions (n=4) were performed by testing elution solutions for FIB as previously described.

Results

High Frequency of Grooming Study. Wrack and sand concentrations measured during the 48 h study are shown in Figure S3. Concentrations are shown for the exposed and submerged samples. The submerged wrack and sand samples include many samples that were below the lower detection limit.

Acknowledgements

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Tables

Table S1: The **highest** concentrations of fecal indicator bacteria (FIB) previously measured in wrack at marine and lacustrine locations. Information is summarized for the wrack species tested and media used for FIB enumeration as provided by the authors.

Study	Location	Marine or lacustrine beach	Plant species or description	FIB enumeration media	Unit	enterococci	*E. coli* or fecal coliforms (FC)
Badgley et al. [3]	Florida	Both	Mixed	mEI	CFU/g	$10^{1.9\ a}$	--
Byappanahalli et al. [4]	Great Lakes	Lacustrine	*Cladophora*	mTEC	CFU/g dry	--	$\sim10^{8.5\ b}$
Byappanahalli et al. [5]	Great Lakes	Lacustrine	*Cladophora*	mTEC	CFU/g	--	$10^{4.3\ c}$
Byappanahalli and Whitman [6]	Great Lakes	Lacustrine	*Cladophora*	Membrane filtration/ Defined Substrate	CFU/g MPN/g	$10^{5.6}$	$10^{4.8}$
Imamura et al. [1]	California	Marine	Mixed	mEI/ mTEC	CFU/g dry	$\sim10^{3.7\ c}$	$\sim10^{4.1\ c}$
Olapade et al. [7]	Great Lakes	Lacustrine	*Cladophora*	mTEC	CFU/g	--	$10^{2.8}$
Russell et al. [8]	California	Marine	Mixed	Enterolert /Colilert	MPN/ g dry	$10^{3.9}$	$10^{3.8}$
Valiela et al. [9]	Massachusetts	Marine	Eelgrass and seaweeds	mFC	CFU/g	--	$10^{2.5}$ (FC)[d]
Whitman et al. [10]	Great Lakes	Lacustrine	*Cladophora*	mE/ mTEC	CFU/g	$10^{6.0}$	$10^{6.2}$

[a]: Calculated by combining enterococci concentrations and vegetation density.

[b]: Measured in incubated algal mats.

[c]: Highest log-mean values

[d]: Typical wrack concentrations estimated based on Heufelder [11] and unpublished data.

Figures

Figure S1: Total wrack mass measured in two 1 m wide transects normalized by the area of beach assessed at Cowell Beach during each sampling event for the long-term survey. Ungroomed and groomed periods are shown in circles and squares respectively. The one sample visit with no wrack present is plotted as 5 g of wrack/ m^2.
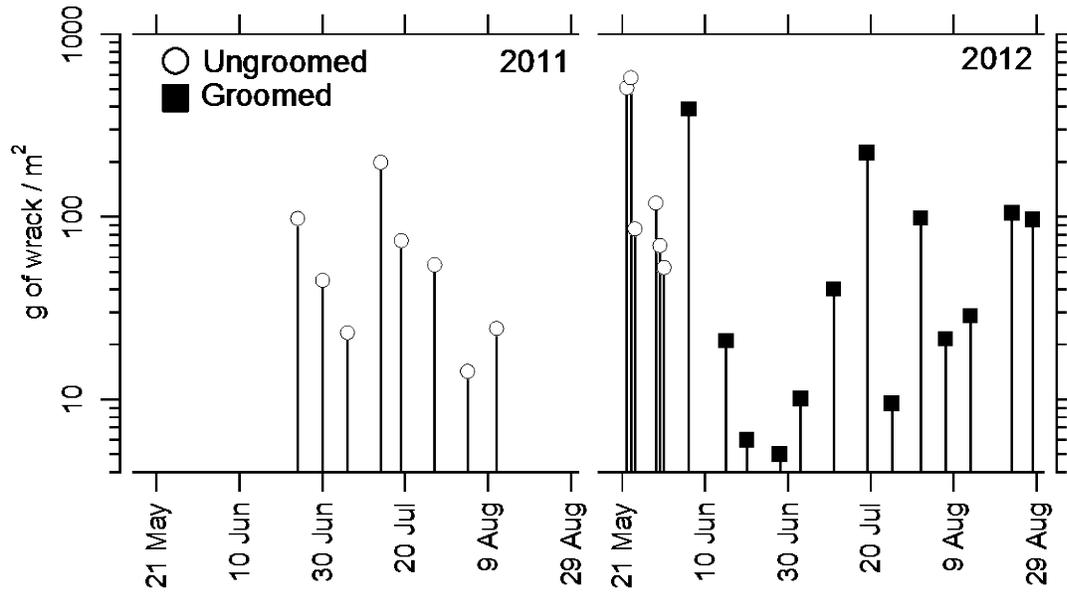
Figure S2: Log-mean ENT and EC concentrations on wrack (top and middle panels respectively) collected throughout the long-term survey divided by presence/absence in each taxonomic group. White bars represent the log-mean when a taxonomic group was absent, black bars represent the log mean when a given taxonomic group was present. Significantly different log-means as indicated by a t-test are denoted with a '*'. The percentage of samples with each taxonomic groups present/absent are shown in the bottom panel. Taxonomic groups are: M= *Macroystis pyrifera*, PS= *Phyllospadix scouleri*, E= *Egregia menziesii*, CO= *Cystoseira osmundacea*, PC= *Pterygophora californica*, U= *Ulva stenophylla*, PP= *Plocamium pacificum*, CE= *Chondracanthus exasperatus*, D= *Dilsea californica*, CS= *Cryptopleura* spp., NL= *Nereocystis luetkeana*, O= other (disintegrated or unidentifiable).
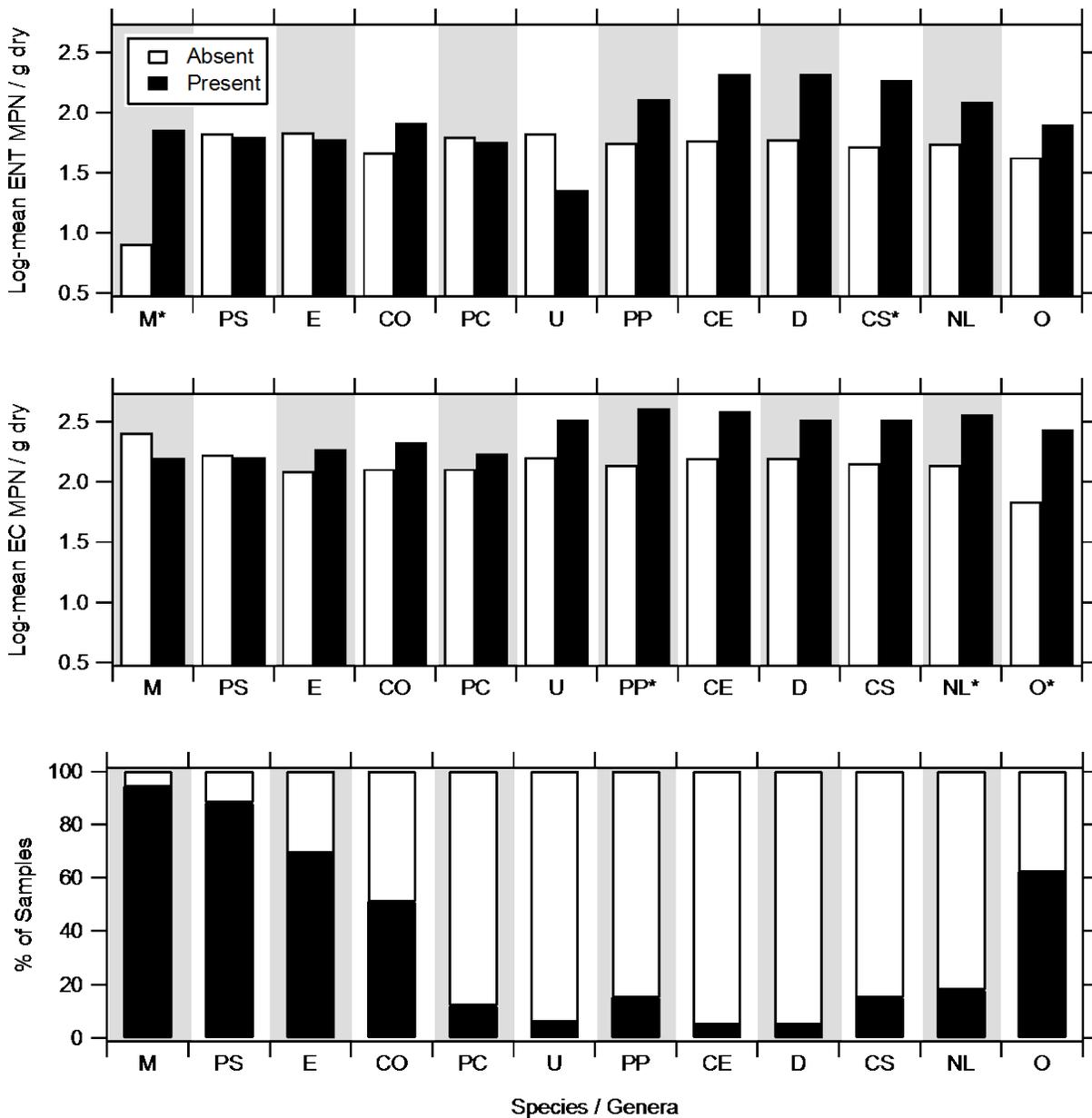
Figure S3: Wrack (top panel) and sand (bottom panel) concentrations measured during the 48 h study. Box represents 25th, 50th and 75th percentiles; whiskers represent 10th and 90th percentiles. Circles show statistical outliers. All samples shown including before and after grooming.