RESEARCH ARTICLE

Wrack promotes the persistence of fecal indicator bacteria in marine sands and seawater

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Abstract

Algae on freshwater beaches can serve as reservoirs for fecal indicator bacteria (FIB). Wrack (especially kelp) at marine beaches might sustain FIB as well. This study examines the relationship between beach wrack, FIB, and surrounding water and sediment at marine beaches along the California coast. Surveys of southern and central California beaches were conducted to observe environmental wrack-associated FIB concentrations. FIB concentrations normalized to dry weight were the highest in stranded dry wrack, followed by stranded wet and suspended 'surf' wrack. Laboratory microcosms were conducted to examine the effect of wrack on FIB persistence in seawater and sediment. Indigenous enterococci and *Escherichia coli* incubated in a seawater microcosm containing wrack showed increased persistence relative to those incubated in a microcosm without wrack. FIB concentrations in microcosms containing wrack-covered sand were significantly higher than those in uncovered sand after several days. These findings implicate beach wrack as an important FIB reservoir. The presence of wrack may increase water and sediment FIB levels, altering the relationship between FIB levels and actual health risk while possibly leading to beach closures. Further work will need to investigate the possibility of FIB growth on wrack and the potential for pathogen presence.

Introduction

At freshwater beaches, macrophytes such as the green alga *Cladophora* have been shown to be an important reservoir for *Escherichia coli* and enterococci (Byappanahalli *et al*., 2003, 2007; Whitman *et al*., 2003; Englebert *et al*., 2008; Vanden Heuvel *et al*., 2009; Badgley *et al*., 2010), which are often referred to as fecal indicator bacteria (FIB). Weiskel *et al*. (1996) found that marine vegetation (wrack) serves in the same fashion for fecal coliforms at a bay in Massachusetts; however, the body of work thus far has focused on freshwater locations. This study aims to investigate the potential of marine wrack to serve as a reservoir for *E. coli* and enterococci and to impact surrounding seawater and sediment quality.

FIB concentrations in beach water are monitored routinely throughout the world in an effort to protect public health. Exceedances of local water quality standards are often due to storm-related runoff (Boehm *et al*., 2002a, b; Schiff *et al*., 2003; Reeves *et al*., 2004; Ahn *et al*., 2005; Jeong *et al*., 2005; Pednekar *et al*., 2005; Lee *et al*., 2006); however, dry weather exceedances are of special concern in many areas due to the increased beach usage during these times. Dry weather FIB contamination of beach water can have a number of sources, including leaking sewer lines (Boehm *et al*., 2003), groundwater discharge (Boehm *et al*., 2004), human bathers (Elmir *et al*., 2007), and resuspension from sediment with high FIB concentrations (An *et al*., 2002; Craig *et al*., 2004), although often the source of dry weather contamination is unknown.

Sediment at both freshwater (Laliberte & Grimes, 1982; Bolton *et al*., 1987; Burton *et al*., 1987; Davies *et al*., 1995; Fujioka *et al*., 1999; Solo-Gabriele *et al*., 2000; An *et al*., 2002; Desmarais *et al*., 2002; Alm *et al*., 2003, 2006; Whitman & Nevers, 2003; Whitman *et al*., 2003, 2006; Ishii *et al*., 2006, 2007) and marine (Gerba & McLeod, 1976; Martinez-Manzanares *et al*., 1992; Davies *et al*., 1995; Oshiro & Fujioka, 1995; Craig *et al*., 2002, 2004; Desmarais *et al*.,

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2002; Boehm & Weisberg, 2005; Ferguson *et al.*, 2005; Jeong *et al.*, 2005; Boehm *et al.*, 2006; Lee *et al.*, 2006; Beversdorf *et al.*, 2007; Yamahara *et al.*, 2007; Mika *et al.*, 2009) beaches has been recognized as a potential reservoir for FIB. Sediment can provide a more favorable environment for FIB survival than the water column due to reduced sunlight inactivation (Sinton *et al.*, 1999) and predation (Brettar & Hofle, 1992; Davies & Bavor, 2000), nutrient and organic carbon availability (Gerba & McLeod, 1976; Laliberte & Grimes, 1982; Blumenroth & Wagner-Dobler, 1998), and the presence of a surface for biofilm formation (Brettar & Hofle, 1992; Davies *et al.*, 1995; Decho, 2000). Contaminated sediment may serve as a nonpoint source for FIB contamination in water. Additionally, direct exposure has been linked to human health risk (Heaney *et al.*, 2009).

Freshwater macrophytes such as *Cladophora* are another important FIB reservoir (Byappanahalli *et al.*, 2003, 2007; Whitman *et al.*, 2003; Englebert *et al.*, 2008; Vanden Heuvel *et al.*, 2009). Leachate from *Cladophora* may provide nutrients that serve to enhance the survival of FIB (Byappanahalli *et al.*, 2003). Dried *Cladophora* is capable of harboring *E. coli* for over 6 months, and allows for regrowth after rehydration (Byappanahalli *et al.*, 2003). Additionally, studies have found that macrophytes can promote the survival of *E. coli* in fresh water (Kleinheinz *et al.*, 2009; Vanden Heuvel *et al.*, 2009).

This study quantifies FIB concentrations on wrack at marine beaches along the California coast, and also examines how the presence of wrack affects the concentrations of FIB in sediments and seawater. Environmental samples were taken in order to test the hypothesis that marine wrack harbors FIB. Laboratory microcosms were conducted to test the hypothesis that the presence of wrack enhances the survival of FIB in seawater and sediment. This is one of the first studies to look specifically at the potential role of wrack in affecting FIB concentrations and occurrence at marine beaches.

Materials and methods

Cowell Beach environmental survey

Sample collection

Nine wrack samples were collected from three separate regions at Cowell Beach, Santa Cruz, CA (36° 57.666′N, 122° 1.502′W), on 1 August 2007, at 06:30. Samples were a mixture of *Nereocystis luetkeana, Macrocystis pyrifera, Chondracanthus exasperatus, Egregia menziesii, Phyllospadix torreyi*, and *Laminaria saccharina* (brown bull, giant perennial, turkish towel, feather boa, surf grass, and sugar wrack kelp, respectively). Triplicate samples were collected from three distinct areas: dry, wet, and surf. Wrack samples collected from the 'dry' region of beach were stranded on the sand, away from the water line. Wrack samples collected from the 'wet' region of beach were stranded near the upper reaches of the swash zone, and wrack samples from the 'surf' zone were continuously suspended in seawater. All samples were stored on ice between sampling and analysis, and were analyzed for FIB within 10 h of collection.

Analysis

Approximately 50 g wet weight of each sample was placed into 1-L bottles with 500 mL of a sterile phosphate-buffered saline (PBS) + $MgCl_2$ solution (working solution, EPA 1604, henceforth referred to as PBS+), shaken for 3 min, and sampled immediately. PBS+ was withdrawn from the bottle and analyzed in triplicate using membrane filtration (see Membrane filtration). The wrack was withdrawn from the bottles and dried at 105 °C for 24 h to determine its dry mass. This method was adapted from Whitman *et al.* (2003).

Seawater microcosms

Experimental setup

Seawater containing indigenous FIB was collected from Cowell Beach in Santa Cruz, CA, on 7 August 2007, at 06:00 and transported on ice to the lab. The seawater was passed through a 250-μm sieve to remove large particles. 3.5 L of sieved seawater was placed into each of four sterile containers; 142 g wet weight of wrack was placed into two of the containers, while the other two containers were used as controls. The amount of wrack relative to water was chosen to approximate the conditions observed in the surf zone at Cowell Beach (data not shown). All four microcosms were incubated in the dark at 15 °C for 15 days.

Analysis

A total of 12 time points were taken over 15 days; 60 mL water samples were taken from each container at each time point and 10 mL was analyzed in triplicate using membrane filtration for *E. coli* and enterococci. *Escherichia coli* and enterococci concentrations were analyzed using MI and mENT agar, respectively, following EPA method 1604 and APHA standard method 9230 C. The lower limit of detection was 10 CFU 100 mL^{-1}. The concentrations obtained from replicate samples from replicate microcosms were log transformed and averaged for creating time series. Over the course of the experiment, 0.75 L of water was removed from the 3.5 L microcosms.

Los Angeles County (LAC) environmental surveys

Sample collection

Samples were collected from nine LAC beaches between 07:00 and 09:00 over 12 days (24 July to 4 August 2008).

One sample of wrack was collected from each zone (dry, wet, and surf) when available; samples included *N. luetkeana, M. pyrifera*, and *E. menziesii* (brown bull, giant perennial, and feather boa kelp, respectively). Sand samples were also taken from the dry and wet zones; one sand sample was collected immediately beneath the wrack sample, and another was taken at least 3.3 m away from this sample (and at least 3.3 m away from any pile of wrack), parallel to the shore. Only the top layer (between 1 and 2 cm) of sand was collected. All samples were held on ice for no longer than 6 h before beginning analysis.

Wrack analysis

Between 5 and 10 g wet weight of wrack was placed into 120-mL bottles with 90 mL of PBS+. The bottle was shaken for 3 min and sampled immediately. PBS+ was withdrawn and analyzed in triplicate using membrane filtration. Wrack was extracted from the bottles and dried between 90 and 105 °C for at least 24 h to determine dry mass.

Sediment analysis

Between 45 and 46 g wet weight of sediment (homogenized by stirring with a sterile spatula) was placed into 120-mL bottles with 60 mL of PBS+. The bottle was shaken for 2 min and then allowed to rest for 1 min. The supernatant was poured into another 120-mL bottle, 60 mL PBS+ was added to the original bottle, and the process was repeated. The supernatant was then analyzed in triplicate using membrane filtration. An additional 5–10 g wet weight of sediment from each sample was dried between 90 and 105 °C for at least 2 days in order to determine the wet to dry ratio (mass of sediment sample divided by sediment mass after drying).

Sediment microcosms

Sample collection

Sediment was collected between 07:00 and 08:00 on 2 January 2009, from Puerco Beach in Malibu, CA, for the first experiment. Sediment was collected from Will Rogers State Beach in Santa Monica, CA, between 07:00 and 8:00 on 15 May 2009, and 1 July 2009, for the second and third experiments, respectively. The sand was chosen from locations that often have high FIB concentrations based on previous data (Heal the Bay, http://www.healthebay.org/about-bay/beach-report-card); the location at Puerco was near a storm drain, and the location at Will Rogers was from a stagnant pool comprised primarily of dry-season runoff.

Wrack was collected between 07:00 and 08:00 from Cabrillo Beach in San Pedro, CA, on the same day for all three experiments. The wrack was collected from the surf zone in order to minimize FIB contributions from the wrack (the typical wrack concentrations observed during the surveys as described in this study were very low for surf wrack).

Experimental setup

Microcosms were set up in 25 × 36 × 15 cm (length, width, height) sterile bins on the roof of Boelter Hall at UCLA. Boelter is an eight-story building with a flat roof; the microcosms were placed in such a way as to maximize sun exposure. The bins were filled to about 10 cm with sand containing indigenous FIB populations. The first experiment (average high of 18 °C over the course of the experiment) was composed of two microcosms: one with just the sand-filled bin and another with wrack placed on top of the sand in the bin. The second and third experiments (each with average highs of 25 °C) had these same two microcosms and an additional two microcosms that were composed in the same manner, except loosely covered by cardboard boxes to minimize the effect of solar disinfection.

Sample analysis

For all the experiments, five sediment samples and five wrack samples were analyzed for FIB and moisture content at the initial time point. Five days later, five sediment samples per microcosm were taken from random locations within the bin and analyzed for FIB and moisture content. Wrack and sediment samples were analyzed in the same manner as the LAC environmental surveys.

Membrane filtration

Cowell Beach survey, water microcosms

Samples were filtered through 47-mm, 0.45-μm membranes (HA type, Millipore, Billerica, MA) and transferred to 15 × 60 mm Petri dishes containing specific agar. In accordance with EPA method 1604, *E. coli* were enumerated by transferring membranes to dishes containing MI agar, incubating at 35 °C for 24 h, and counting blue colonies. In accordance with APHA standard method 9230 C, membranes were transferred to mENT agar and incubated at 35 °C for 48 h. Red colonies were counted as enterococci.

LAC surveys, sediment microcosms

In accordance with EPA methods 1603 and 1600, samples were filtered through 47-mm, 0.45-μm membranes (Fisher, Pittsburgh, PA) and transferred to 15 × 60 mm Petri dishes containing specific agar. In order to enumerate *E. coli*, membranes were transferred to dishes containing modified membrane-thermotolerant *E. coli* (mTEC) agar and incubated at 35 °C for 2 h and then at 45 °C for 22 h. Colonies with magenta halos were counted as *E. coli*. Enterococci were enumerated using membrane-*Enterococcus* indoxyl-β-D-glucoside (mEI) agar with incubation at 41 °C for 24 h. Colonies with blue halos were counted as enterococci.

Statistical analyses

Means and 95% confidence intervals were calculated from log-transformed data across replicates. One-way ANOVA and one-way repeated measures ANOVA were used for the Cowell Beach and LAC surveys, respectively, to establish statistical significance for differences in FIB concentrations. One-tailed Student's *t*-tests and Satterthwaite's approximate *t*-tests were used for statistical comparisons between two groups. Two-way ANOVA was used for statistical comparison for the second and third sediment microcosms. Half-detection limits were used for nondetects; plates with too many colonies to count were assigned a value (400 CFU) near the countable limit. Exponential decay curves were fitted to the seawater microcosms using linear least squares methods in order to determine the time at which 90% of the original FIB concentration had been removed.

Results

Environmental surveys

FIB were measured in wrack collected from dry, wet, and surf zones at Cowell Beach (Fig. 1). Dry wrack had the highest concentrations (normalized to dry weight), followed by wet and then surf wrack. Enterococci levels in dry wrack were approximately two orders of magnitude greater than those in wet wrack, which were in turn another order of magnitude higher than the levels in surf zone wrack. *Escherichia coli* levels were about one order of magnitude higher in the dry wrack than the wet wrack and over three logs higher in the wet wrack than the surf wrack. One-way ANOVA showed a statistically significant difference between the zones for both FIB ($P < 0.001$, $n = 18$).

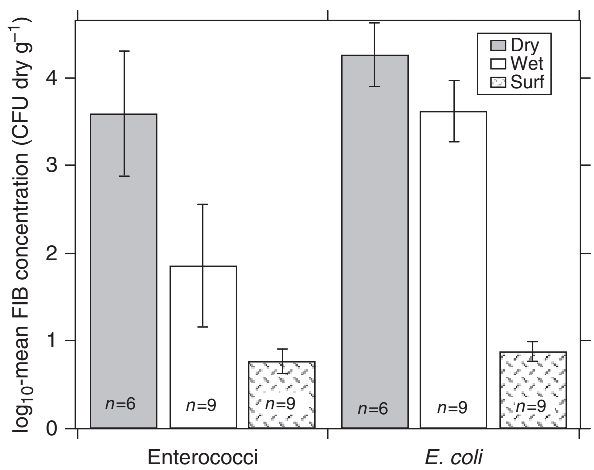

Fig. 1. Log-mean and 95% confidence intervals of FIB concentrations on wrack, normalized by dry weight from three different zones at Cowell Beach in Santa Cruz, CA.

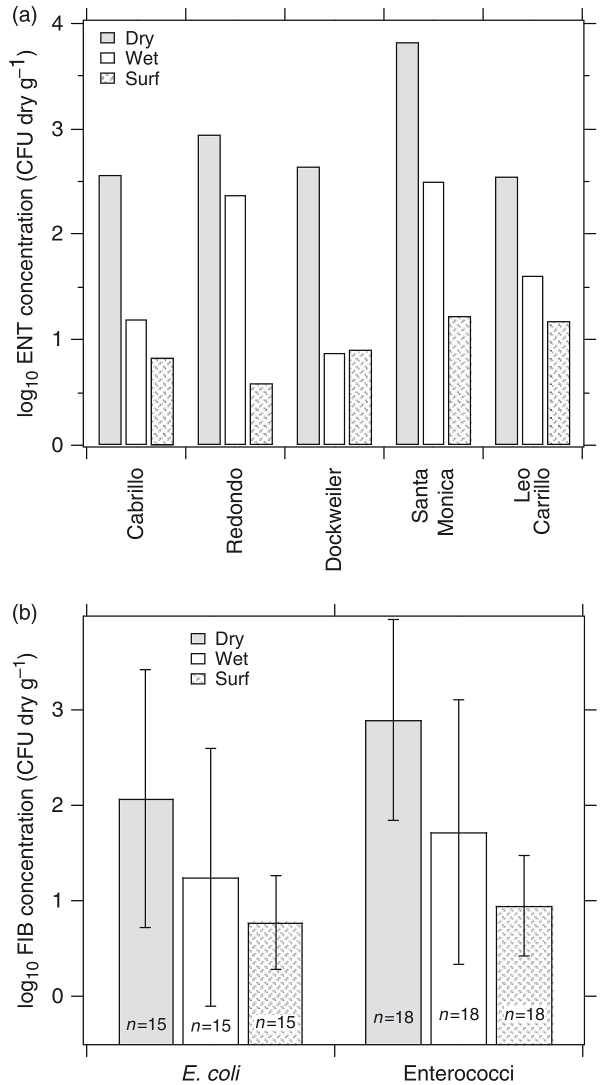

Fig. 2. (a) Enterococci concentrations in wrack from three zones at LAC beaches, $n = 3$ for each mean, normalized by dry weight. (b) Log-mean and 95% confidence intervals of FIB concentrations on wrack from three different zones from LAC beaches, normalized by dry weight.

FIB were measured in wrack collected from dry, wet, and surf zones at nine LAC beaches. Wrack from the dry zone had an average wet to dry mass ratio of 2.3 (SD of 0.5), while wrack from the wet and surf zones had average wet to dry mass ratios of 6.4 and 8.9 (SDs of 3.5 and 2.7), respectively. In five of the six beaches that had wrack from all three zones, enterococci levels were the highest in dry wrack and the second highest in wet wrack (Fig. 2a and b). Enterococci levels in dry wrack were approximately one order of magnitude greater than that in wet wrack, which was in turn over one log higher than wrack from the surf zone. The results for *E. coli* differed from those for enterococci, with less than one log separating dry and wet zone wrack, and wet and surf zone wrack. One-way repeated measures ANOVA showed a statistically significant difference between the zones for enterococci ($P < 0.01$, $n = 18$), but not for *E. coli* ($P > 0.05$, $n = 15$).

The mean enterococci and *E. coli* levels in the surf wrack for LAC beaches were very similar to the levels found at Cowell Beach (< 10 CFU dry g^{-1}). The mean concentrations on wet wrack were similar for enterococci, but *E. coli* levels were two orders of magnitude lower at LAC than the levels found at Cowell Beach. The levels on dry wrack also differed between the two sites. The mean concentrations for enterococci and *E. coli* on dry wrack from LAC beaches were approximately one and two logs lower than the levels found at Cowell Beach, respectively. However, it should be noted that different enumeration methods for the FIB were used, which may influence this comparison.

Figure 3a shows the enterococci levels for wrack, sediment directly beneath wrack, and sediment some distance from the wrack in the dry zone from the LAC beach survey (Fig. 3b displays means). In general, FIB concentrations were the highest in wrack, followed by sediment under the wrack, and sediment some distance from the wrack. A one-way repeated measures ANOVA was statistically significant between these groups for enterococci (ANOVA, $P < 0.05$, $n = 18$).

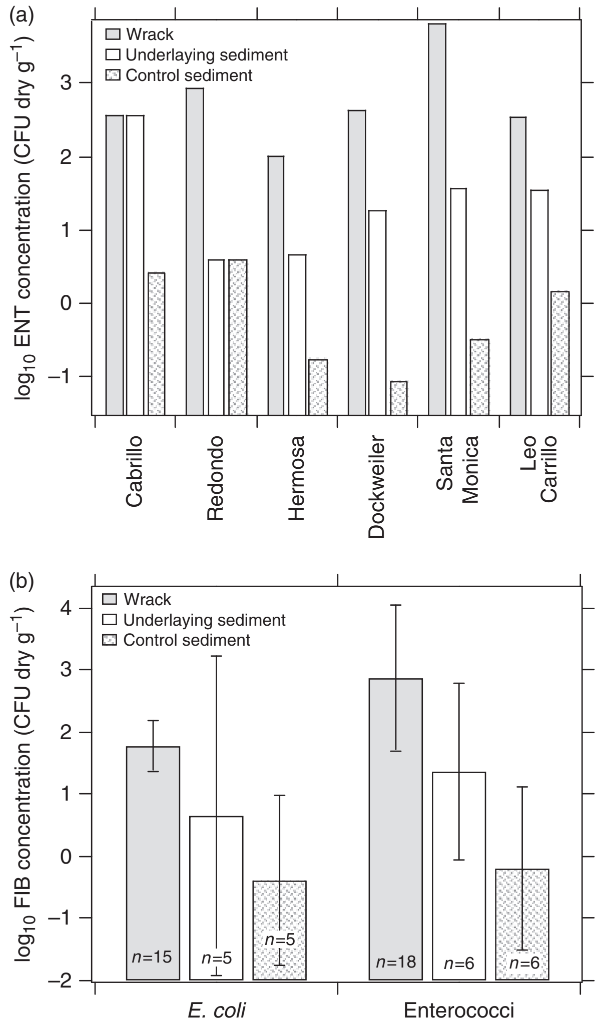

Fig. 3. (a) Enterococci concentrations on wrack, in sediment directly beneath wrack, and in sediment not covered by wrack at LAC beaches, normalized by dry weight. All samples from the dry zone. $N = 3$ for all wrack samples, $n = 1$ for all sediment samples. (b) Log-mean and 95% confidence intervals of FIB concentrations on wrack, in sediment directly beneath wrack, and in sediment not covered by wrack at LAC beaches, normalized by dry weight.

Laboratory microcosms

Microcosms consisting of Cowell Beach ocean water with native FIB populations [603 ± 1 CFU 100 mL^{-1} for *E. coli*, 191 ± 2 CFU 100 mL^{-1} for enterococci] were conducted to compare FIB survival in the presence and absence of wrack in 13 time points over 15 days. Wrack samples were taken from the surf zone, and had FIB concentrations below the detection limit. The time-series (Fig. 4a and b) showed FIB concentrations in the water to be consistently higher in the microcosms containing wrack (paired *t*-tests, $P < 0.001$, $n = 12$ for each organism). *Escherichia coli* and enterococci concentrations in the microcosms without wrack decreased by 90% by 51 and 90 h, respectively, while it took 201 and 171 h to achieve this decrease in the microcosms with wrack. Additionally, FIB in the microcosms with wrack exhibited a period of apparent regrowth before dying off, as evidenced by the increasing concentrations with time during a duration of the experiment. After an initial decrease of half an order of magnitude in the first 48 h of the wrack-containing microcosm, *E. coli* concentrations rebounded and reached nearly the starting concentration after 72 h. A similar pattern of an initial decline, followed by an increase was observed for enterococci concentrations; only the enterococci concentrations reached levels nearly an order of magnitude higher than those initially present in the microcosm. If only data during the exponential increase are included, the increase corresponds to an enterococcal growth rate of 0.11 h^{-1}. Approximately 2% of the water was removed at each time point for analysis purposes. More work will need to be carried out to confirm the regrowth of enterococci in the presence of wrack in seawater.

Three microcosm experiments with sediment containing native populations of FIB from LAC were conducted to compare FIB survival after 5 days under the following conditions: presence/absence of wrack and presence/absence of light. The wrack used was from the surf zone; FIB

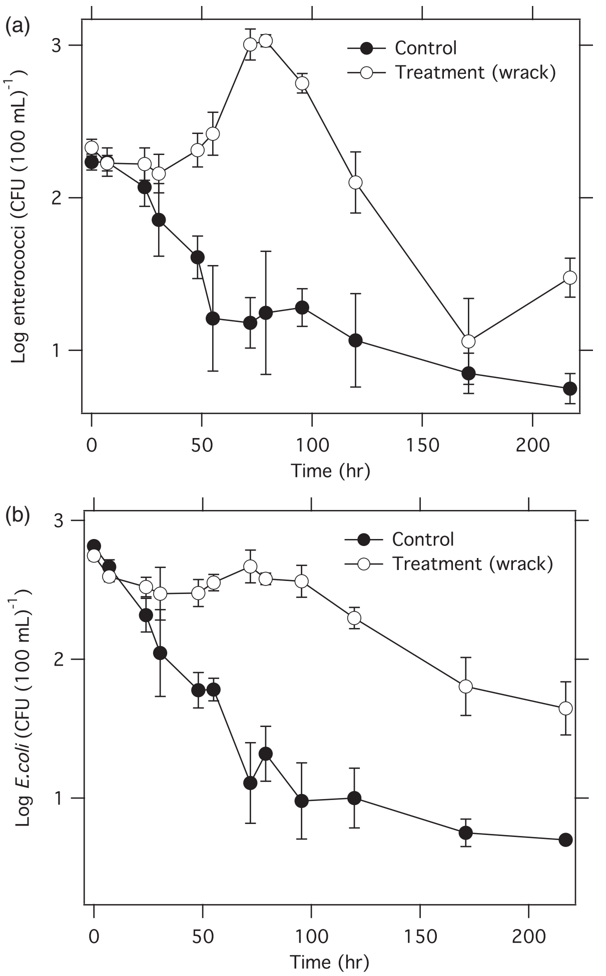

Fig. 4. Water microcosm time series for (a) *Escherichia coli* and (b) enterococci. Geometric means and 95% confidence intervals are shown of replicate time point measurements, $n = 6$ for each time point. The microcosm consisted of maintaining seawater with native FIB concentrations under two conditions: one with wrack in the water and one without wrack.

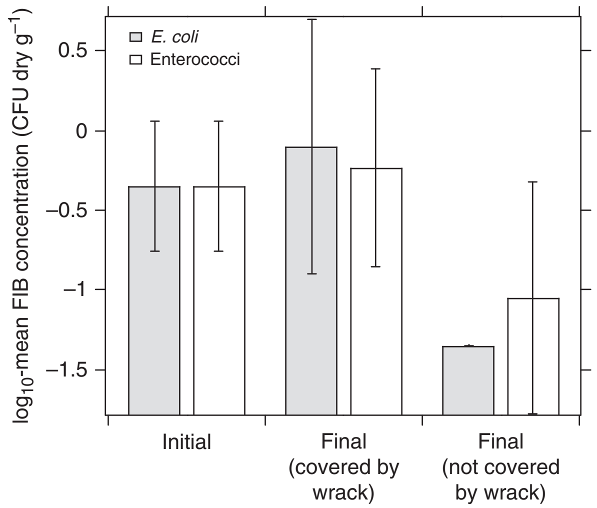

Fig. 5. First sediment microcosm, initial samples taken on 9 January 2009, remaining samples taken on 14 January 2009. Log-means and 95% confidence intervals of sediment. $N = 10$ for initial time points, $n = 5$ for others. The microcosm consisted of maintaining sediment with native FIB concentrations under two conditions: one with wrack covering the sediment and one without wrack.

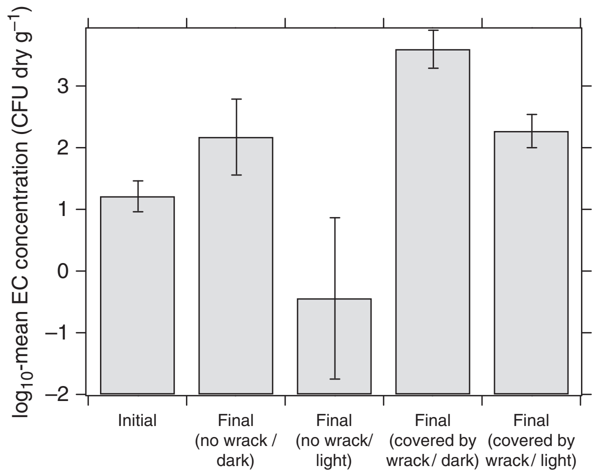

Fig. 6. Second sediment microcosm, initial samples taken on 15 May 2009, remaining samples taken on 20 May 2009, *Escherichia coli* only. Log-means and 95% confidence intervals of sediment. $N = 5$ for each mean. The microcosm consisted of maintaining sediment with native FIB concentrations under four conditions: one with wrack covering sediment and kept dark, one with wrack covering the sediment and left in the sun, one with no wrack and kept dark, and one with no wrack and left in the sun.

concentrations in the wrack were below the detection limit. The first sediment microcosm experiment (Fig. 5), which only compared effects due to presence/absence of wrack, showed that FIB concentrations in wrack-covered sand after 5 days were significantly higher than in sand not covered by wrack (ANOVA, $P < 0.05$, $n = 20$). The initial FIB concentrations were low in the sediment for this experiment – (< 1 CFU dry g^{-1}). As expected, sand with the wrack cover had a higher wet to dry ratio than the sand without cover. The wet to dry ratio for sand with wrack cover was 0.11 lower than the initial wet to dry ratio; the ratio for sand without cover was 0.28 lower than the initial ratio, indicating increased drying in the uncovered microcosm.

The second and third microcosm experiments (Figs 6 and 7) examined the effects of wrack and sunlight on FIB concentrations in sediments. Unfortunately, after 5 days, enterococci concentrations exceeded countable limits in the second microcosm, and *E. coli* concentrations were below the detection limit after 5 days in the third microcosm; hence those data are excluded from the analysis. Thus, only *E. coli* data are presented for the second experiment, and only enterococci data are presented for the third experiment.

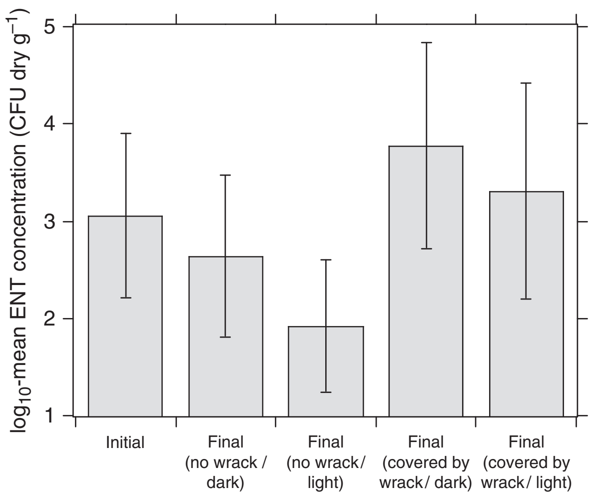

Fig. 7. Third sediment microcosm, initial samples taken on 1 July 2009, remaining samples taken 6 July 2009, enterococci only. Log-means and 95% confidence intervals of sediment. $N=5$ for each mean. The microcosm consisted of maintaining sediment with native FIB concentrations under four conditions: one with wrack covering sediment and kept dark, one with wrack covering the sediment and left in the sun, one with no wrack and kept dark, and one with no wrack and left in the sun.

The second experiment had an initial concentration of 16 CFU *E. coli* dry g^{-1}. The third experiment had an initial concentration of 1137 CFU enterococci dry g^{-1}. Significant differences between the treatments (wrack covered, dark) relative to the control (uncovered, sunlit) were found for both *E. coli* and enterococci in the second and third microcosm experiments, respectively (two-way ANOVA, $P < 0.05$, $n = 25$). The dark microcosm (covered with cardboard) had higher microorganism levels than the light microcosm (no cardboard cover); the wrack-covered microcosm had higher microorganism levels than the uncovered microcosm. Sand wet to dry ratios decreased in all microcosms over the course of the experiment. In the wrack-covered microcosm, the ratio decreased by 0.05. In the exposed, sunlit microcosm with no wrack, the ratio decreased by 0.15. Differences between wet to dry ratios were minimal between dark and light microcosms.

Discussion

Survey study results

Wrack collected from the three different zones at central and southern Californian beaches exhibited a fairly consistent trend. FIB were present at the highest concentrations in the dry zone wrack, next highest in the wet zone wrack, and at the lowest levels in the surf zone wrack. The dry zone wrack represents wrack that was stranded on the beach well above the high tide line. This finding supports the hypothesis that wrack harbors FIB. The relatively high levels in dry wrack compared with wet or surf wrack would be consistent with the premise that FIB can grow on wrack and accumulate over time as wrack is stranded on the beach. However, the present study did not test for FIB growth on dry wrack; hence, this is an avenue for future work. Previous work found that the freshwater macroalga *Cladophora* was capable of harboring FIB for over 6 months under dry conditions, and allowed for regrowth with the reintroduction of moisture (Whitman *et al.*, 2003); hence something similar may occur with marine wrack. Wrack might promote survival by providing protection from UV radiation, predation, desiccation, and serving as a substrate as it decomposes (Brettar & Hofle, 1992; Davies *et al.*, 1995; Weiskel *et al.*, 1996; Byappanahalli *et al.*, 2003; Whitman *et al.*, 2003, 2004). An alternative method of normalizing FIB concentrations (to volume or surface area of plant material) would be an interesting way of comparing FIB in wrack and should be examined in future work (Badgley *et al.*, 2011). Additional testing of wrack along marine coastlines, and microscopic investigation into the distribution of FIB on or within aquatic macrophytes such as kelp would aid in our understanding of these findings. Regardless, the results of the environmental sampling survey indicate a potential for marine wrack to serve as a nonpoint source of FIB in water as high tides reach dry wrack locations. Future work should test the potential for FIB on wrack to be transported to the nearshore by the rising tide.

Effect on water

FIB concentrations in the seawater microcosms showed an appreciably higher survival rate in the microcosms that contained wrack. While the FIB concentrations in the seawater decreased in the microcosms without wrack, concentrations in the microcosms with wrack increased for several days before the concentrations began to decrease. *Escherichia coli* concentrations took 6 days longer to achieve 90% die-off in the wrack microcosms compared with the microcosms without wrack, and enterococci took three extra days. The enterococci concentration remained above the California regulatory standard of 104 CFU 100 mL^{-1} for 2 days longer in the bottle with wrack than the control bottle. These results suggest that FIB in the nearshore of beaches with high levels of wrack suspended in the water column may persist for longer than FIB in the nearshore of beaches, where there is no wrack, and thus affect the violation of water quality standards. Field studies of FIB and wrack in the nearshore have not been undertaken at marine beaches, but several have been conducted at freshwater beaches and the results are equivocal. Two studies found that FIB levels were elevated in the water column at freshwater beaches where there were high levels of freshwater macrophytes (Kleinheinz *et al.*, 2009; Vanden Heuvel *et al.*, 2009), while another study

(Englebert *et al.*, 2008) did not find a significant correlation between microbial water quality and the presence of *Cladophora* at freshwater beaches. Further field studies are needed to test the effects of wrack on waterborne FIB in the nearshore.

Effect on sediment

FIB concentrations in the sediment covered by wrack were generally elevated in both the dry and the wet zones when compared with surrounding sediment at the LAC beaches surveyed. It is possible that FIB associated with the wrack are transferred to the underlying sediment, as often, the wrack is partially buried within the sediment. The wrack then may provide FIB in the sediment additional protection from desiccation and UV exposure, which are two important natural disinfection mechanisms (Sinton *et al.*, 1999; Mika *et al.*, 2009). Wet to dry ratios were higher in sediment under the wrack than away from it at the LAC beaches surveyed ($P < 0.05$, $n = 12$), which supports the idea that wrack aids in retaining sediment moisture. Additionally, wrack may provide organic carbon and other nutrients for cells to persist and/or grow.

The results from the three sediment microcosm experiments support the hypothesis that wrack provides protection from solar effects. Comparisons of sediment from the microcosms show that sediment kept in the dark (both covered and not covered by wrack) had higher concentrations of both *E. coli* and enterococci than sediment exposed to the sun after 5 days. We did not keep track of temperature in the sediments. Further work should be carried out to test the effects of sunlight, temperature, and wrack on the persistence of indicators, as well as pathogens, in sands. Byappanahalli *et al.* (2009) found *Cladophora* to be a likely reservoir for *Salmonella* at Lake Michigan, and thus marine wrack may serve the same function. Increasing the number of samples in future experiments would help to increase the power of the analyses.

Method differences

Enterococci were enumerated using mENT media in a subset of the analysis. mENT was designed to quantify fecal streptococci of which enterococci are a subset; hence, it may overestimate enterococci concentrations. However, a number of studies have compared the performance of enterococcal medias including mENT and determined mENT performs well, with good specificity for enterococci (Pagel & Hardy, 1980; Dionisio & Borrego, 1995). Regardless, comparisons of results between measurements made with mENT and mEI should be carried out with caution. In addition, because no colonies from mENT or mEI were verified with additional biochemical or molecular assays, the concentrations reported herein are technically those of presumptive enterococci.

Role of kelp in the beach ecosystem

Although this work illustrates a potential role for kelp in adversely affecting beach water quality as determined by concentrations of enterococci and *E. coli*, it should be noted that wrack plays an important role in the beach ecosystem by providing nutrients to the beach food web. Sea birds, invertebrates, and insects all rely on kelp as a food source. Beach grooming to remove stranded kelp has been shown to adversely impact the beach ecosystem (Dugan & Hubbard, 2010). Thus, a decision to remove wrack from a beach should only be undertaken after careful consideration of both water quality and ecosystem needs.

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