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AMPLIFICATION OF INDICATOR BACTERIA IN ORGANIC DEBRIS ON SOUTHERN CALIFORNIA BEACHES

Prepared by:

Andrew Martin
Stephen Gruber
Weston Solutions, Inc. (WESTON®)

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AMPLIFICATION OF INDICATOR BACTERIA IN ORGANIC DEBRIS ON SOUTHERN CALIFORNIA BEACHES

Andrew Martin and Stephen Gruber, Weston Solutions, Inc., Carlsbad, CA

INTRODUCTION

Certain recreational beaches in southern California frequently exceed state water quality standards for indicator bacteria (total coliform, fecal coliform, and enterococcus). In San Diego County, two sites have been particularly problematic: Mission Bay, a large coastal embayment; and Dog Beach at the mouth of the San Diego River. Recent studies designed to investigate sources of indicator bacteria at these sites suggested that densities of indicator bacteria can be amplified through extended survival and reproduction in organic debris deposited on area beaches. This process was most prevalent in two common features of recreational beaches: organic debris deposited on the beach in the form of a wrack line and tidally influenced storm drains where organic debris frequently accumulates. Field investigations showed that the wrack line acts as a bacterial reservoir that can impact receiving waters. Indicator bacteria were concentrated in the organic debris deposited on the beach during spring tides, maintained in the wrack above the water line during neap tides, then released back to the receiving waters during subsequent spring tides. At some locations, this process was considered to be a significant cause of bacterial water quality standard exceedances. In laboratory experiments that simulated tidally influenced storm drains, bacterial amplification was even more dramatic. Fecal coliform and enterococcus bacteria were shown to reproduce rapidly under conditions typical of coastal storm drains, with densities increasing three to four logs in 48 hours. The results have potential implications for managing recreational beach water quality in southern California.

HIGH-TIDE WASHING SURVEYS

The appearance that bacterial levels increased following high tides resulted in a study that focused sampling efforts around the high tide. High tides tend to wash the wrack line collecting on the beach (the wrack line is composed of marine vegetation, typically kelp and eelgrass, and other debris found deposited at the extent of the last high tide). Observations on the beach showed that dog feces tend to be within the wrack line as well. Therefore, it seemed that the source of elevated bacteria levels may be the washing of dog feces in the wrack line. On Dog Beach, four surveys were conducted that collected samples approximately 3 hours prior to high tide, during high tide, and approximately 3 hours following high tide. Samples were also collected in three specific regions of the beach, west (surf influenced), north (strong tidal current flow, steep beach, narrow river channel), and east (variable tidal current flow, flat beach, mudflats). The west and north regions were further segmented into areas with observed higher and lower concentrations of dog feces in the wrack line.

The results showed a significant difference between pre- and during high-tide samples. Samples collected at high tide were significantly higher than those collected 3 hours prior, with concentrations of all three bacterial indicators typically greater by an order of magnitude or more (Figure 1). Interestingly, samples collected in areas with high concentrations of dog feces versus areas with low concentrations of dog feces were not significantly different from each other. Regrowth of bacteria on the wrack line with an unknown mechanism of transport (i.e., flies or tidal washing) was assumed to be the likely cause of elevated bacterial concentrations in areas without apparent dog feces. Consequently, tidal washing of the wrack line was a contributing factor to elevated bacterial levels.

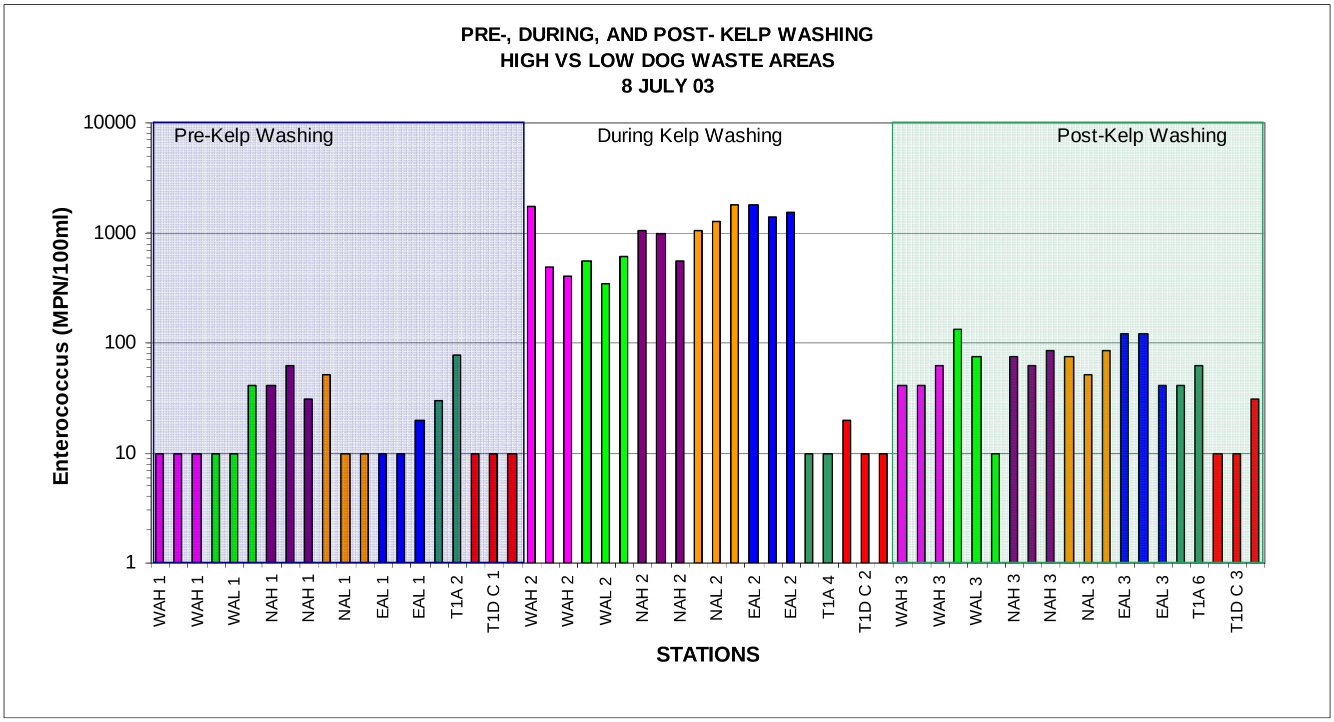

Figure 1. Results of High-Tide Washing Survey, enterococcus, July 8, 2003.

WRACK LINE SAMPLING

The results of the high-tide washing studies suggested that the washing of the wrack material at high tide was resulting in elevated bacterial densities in the nearshore water quality. Therefore, wrack line material at Dog Beach and beaches in Mission Bay was sampled and analyzed to determine the range of bacterial densities present on this medium. At Dog Beach, samples were collected of freshly deposited and decomposing kelp and eelgrass from the wrack line. Samples were further segregated into those with and without the apparent presence of dog fecal matter. Samples of freshly deposited and decomposing wrack material were also collected at a reference beach where dogs were not permitted to be on the beach.

The analytical results showed that decomposing marine vegetation, with or without the apparent presence of dog feces, had levels of bacterial contamination that were among the highest levels observed during the entire year-long study at Dog Beach (maximum of 144,527 MPN/gram for total and fecal coliforms and 40,268 MPN/gram for enterococcus) (Figure 2). Wrack material collected from the reference beach also had elevated levels of bacterial indicators (maximum of

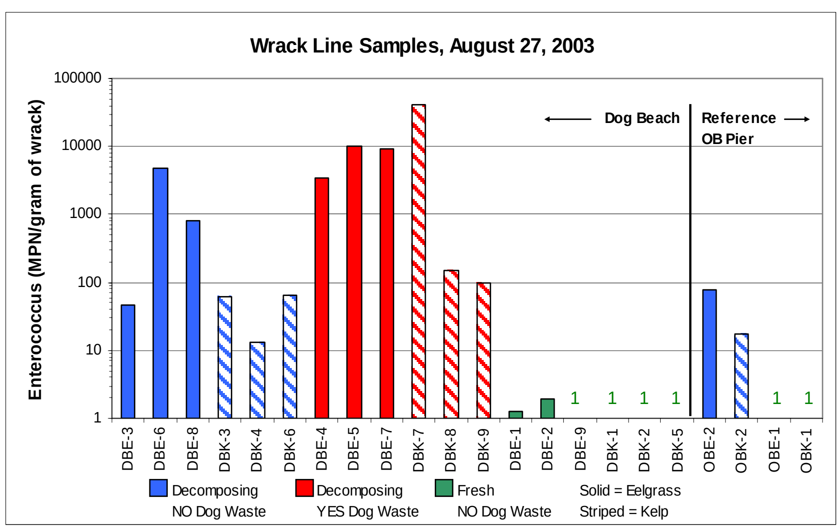

Figure 2. Enterococcus densities in wrack at Dog Beach, August 27, 2003.

9,255 MPN/gram for total coliform, 1,329 MPN/gram for fecal coliform, and >2,327 MPN/gram for enterococcus). At both Dog Beach and the reference beach, freshly deposited marine vegetation did not have similarly elevated levels of bacterial contamination (results were typically non-detect).

In a similar study at Mission Bay, sampling of the wrack line was conducted in order to determine the extent to which bacterial densities increased or were maintained within the wrack line environment and to assess the potential for the wrack line to contribute bacteria to the receiving waters. Five stations were sampled at each of two beaches: Riviera Shores and Visitors' Center for 11 days during a neap tide. At Riviera Shores and Visitors' Center, bacterial densities in the wrack material were maintained during the entire study period. Most notably, enterococcus levels were consistently greater than 10,000 MPN/gram. Bacterial densities at Riviera Shores were also maintained; however, they were typically an order of magnitude less than at the Visitors' Center. These studies confirmed the wrack line provides an environment conducive to the maintenance and possibly the growth of both enterococcus and fecal coliform bacteria. Figure 3 illustrates the results for sampling conducted at the Visitors' Center.

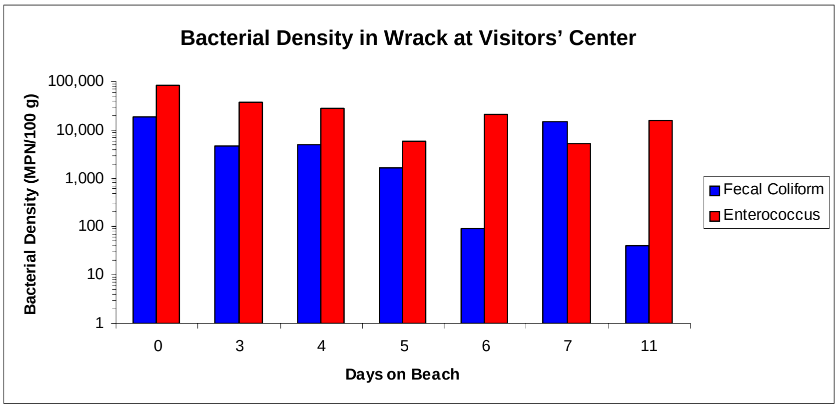

Figure 3. Mean Fecal Coliform and enterococcus densities in wrack at Visitors' Center from February 6 through 17, 2004.

In both studies at Dog Beach and in Mission Bay, the source of the bacterial contamination to the marine vegetation was assumed to be dog or bird feces. Tidal washing of wrack material could be the primary mechanism for transporting bacteria in high concentrations into the water column. Although these field studies did not specifically quantify the ability of bacteria to regrow in this environment, a laboratory-controlled study assessed the potential for bacterial amplification under conditions typically found inside a coastal storm drain.

LABORATORY EXPERIMENT

The objective of the laboratory experiment was to assess the potential for bacterial amplification under conditions typically found inside a coastal storm drain. Microcosms were created, representing three environments: 15%, 70%, and 100% seawater. Organic material was added to half of the microcosms representing each environment. Eelgrass obtained from a clean beach was rinsed and soaked in sterile deionized water and UV irradiated for 10 minutes. A total of 25 g was then aseptically transferred to two flasks of each water type, leaving two flasks from each water type with sterile water only (Table 1). One negative control of 15% seawater without eelgrass was also created. The microcosm design is summarized in Table 1.

Table 1. Summary of storm drain simulation microcosm. Percentages refer to the salinity in each flask.

15% Seawater (salinity = 5ppm)	70% Seawater (salinity = 23ppm)	100% Seawater (salinity = 32ppm)	Negative Controls
3 replicates with eelgrass	2 replicates with eelgrass	2 replicates with eelgrass	--
2 replicates without eelgrass	2 replicates without eelgrass	2 replicates without	1 sterile water only

Indicator bacteria were added to all flasks except the negative controls in the following approximate concentrations: fecal coliform at 10,000 MPN/100 mL and enterococci at 1,000 MPN/100 mL. All flasks were wrapped in aluminum foil to block out ultraviolet light and maintained at 15º C. Samples were collected for bacterial enumeration immediately after inoculation (time/day 0), 12 hours later, then each 24 hours thereafter for the first 5 days, and every 2 to 4 days for the remainder of the 27-day study.

Figure 4 shows the results of the laboratory study. In summary, prolonged survival of enterococcus in the presence of eelgrass in all three salinities tested was maintained at a density slightly above the state's AB411 water quality criteria of 104 MPN/100 mL. In the 70% and 100% seawater microcosms without the presence of organic material, bacterial densities declined rapidly to non-detect levels within 4 days. In the 15% seawater environment without the presence of eelgrass, bacterial densities were maintained or slightly decreased over the 27-day study. Similarly, bacterial densities were maintained or slightly decreased in the 70% and 100% seawater environments with the presence of eelgrass. In the 15% seawater microcosm with eelgrass present, bacterial densities increased by several orders of magnitude during the first 3 days. These elevated densities were maintained for 1 week before declining; however, elevated densities were observed throughout the duration of the study.

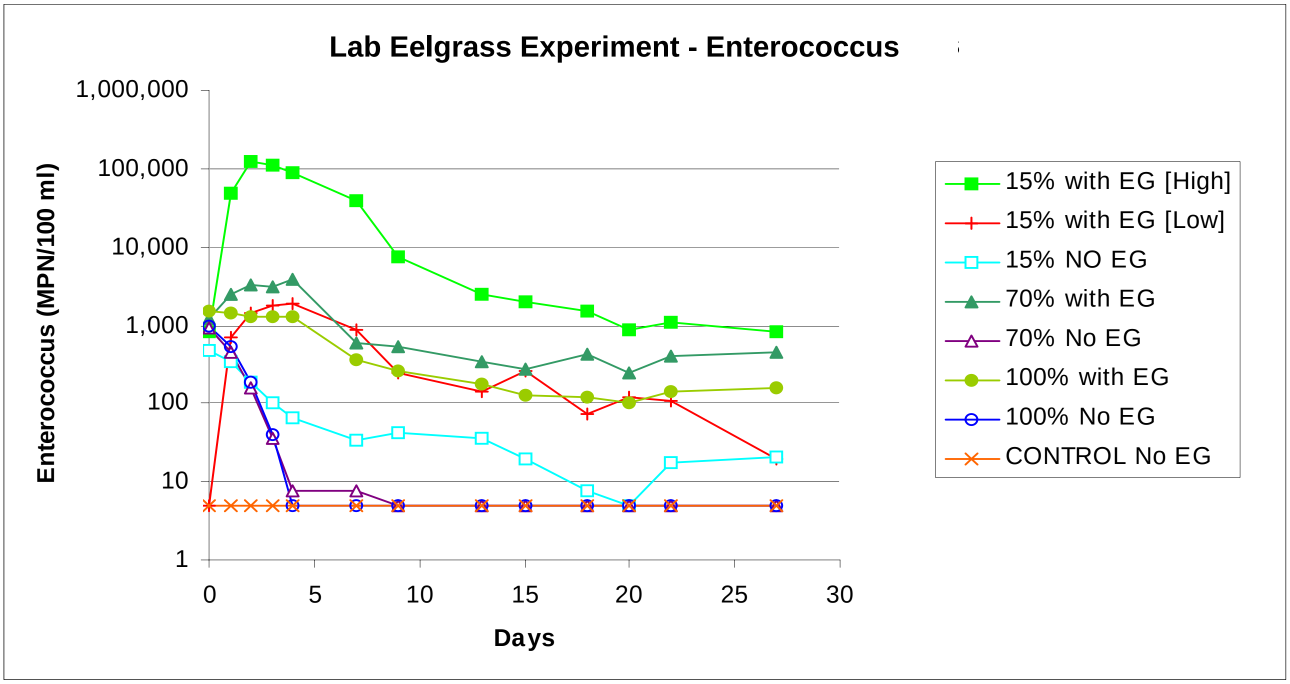

Figure 4. Enterococcus densities over time under simulated storm drain conditions. The dashed red line represents the AB411 criterion for enterococcus of 104 MPN/100 mL.

RIBOTYPING ANALYSIS

Microbial source tracking (MST) was performed to determine the animal hosts that contributed bacteria to receiving waters and wrack line. MST was conducted using *E. coli* Ribotyping by a contracted laboratory at the University of Washington's Institute for Environmental Health. To accomplish this, wrack-derived *E. coli* isolates were analyzed by the Ribotyping assay, and identifier codes based on the genetic fingerprint pattern were assigned to each Ribotype derived from the sample. The following results were found:

- A total of 80% of isolates obtained in receiving waters at Riviera Shores originated from avian sources. The remaining 20% originated from mammals (11%) or other unknown sources (9%).
- A total of 67% of isolates obtained in receiving waters at Visitors' Center originated from avian sources (Figure 5). The remaining 33% originated from canine (13%), mammal (8%), or other unknown sources (12%).
- A total of 45% of isolates obtained from the wrack line at Visitors' Center originated from avian sources. Canine sources accounted for 26% of the isolates. The remaining isolates originated from mammal (6%) or other unknown sources (23%).
- A pair-wise comparison found 64% of the receiving water isolates at Visitors' Center matched wrack line isolates. Seventy-three percent of these matches were consistent with avian, canine, or other animal-derived isolates, suggesting these hosts as the original input of bacteria to the wrack line.

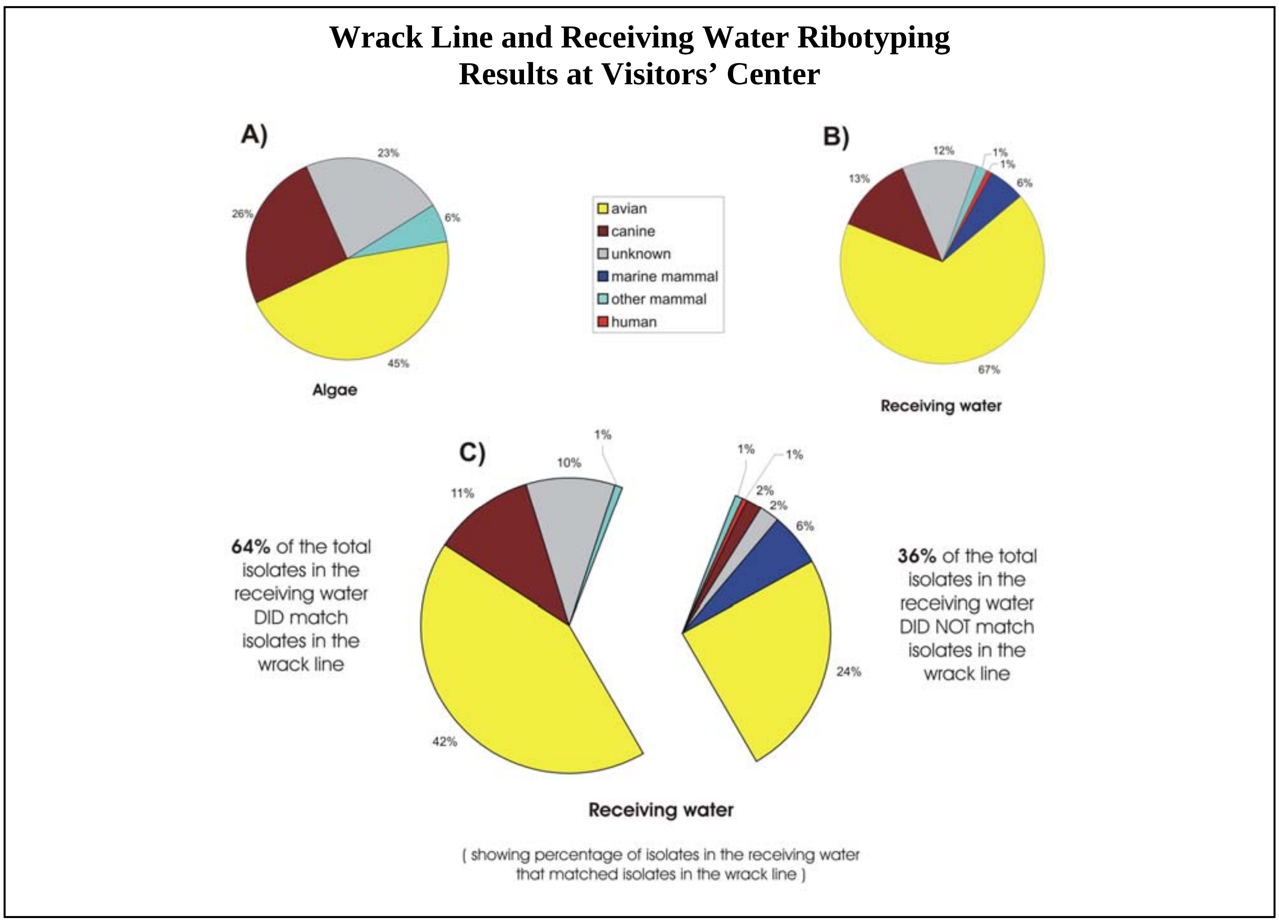

Figure 5. Results of Ribotyping analysis at Visitors' Center showing the origin of bacterial isolates in the wrack (A), receiving water (B), and the proportion of isolates in receiving water that matched those in wrack (C).

CONCLUSIONS

Visual observations and laboratory analysis of water samples collected at Dog Beach and throughout Mission Bay Park led to the hypothesis that decaying eelgrass and kelp deposited during peak flooding spring tides may be acting as amplifiers and could potentially be contributing to indicator bacteria exceedances to the accompanying receiving water. Samples of organic debris collected at several beaches confirmed elevated bacterial densities were present on the wrack material.

Simulation of storm drain conditions in the laboratory showed that growth and maintenance of fecal coliforms and enterococcus was significant in fresh water and brackish environments. In addition, survival of fecal coliform and enterococcus was significantly greater in brackish and saltwater when organic debris (i.e., wrack material) was present. Tidal flushing of the wrack line at high tide and storm drains could transport these elevated bacterial densities into the marine environment and potentially degrade water quality.

The strong isolate match between the bacteria identified in the wrack material and the bacteria in the receiving waters during washing of the wrack line identifies tidal washing of the wrack line as the transport mechanism for elevated bacterial densities into the water column.