APPENDIX F

Technical Note:
Bacterial and Coliphage Degradation Experiments in Fresh and Seawater

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Introduction

An estimated 50 million swimmers visit Santa Monica Bay's Beaches for sun and surf on an annual basis. However, Santa Monica Bay has also been listed as an impaired water body, and is on the federal 303 (d) list, making it the subject of intense scrutiny and the focus of Total Maximum Daily Load (TMDL) for indicator bacteria. The TMDL process will require that indicator bacteria be tightly controlled in order to protect public health of those using the beaches for body contact recreation. In order to control the amount of fecal contamination that reaches the beaches of Santa Monica Bay, we must first understand the fate, transport, dilution and dispersion of fecal material in the watershed and along the beach. This is because varying environmental conditions, such as temperature, sunlight (UV irradiation), nutrient levels and/or suspended solids concentrations, can potentially alter the survivability and persistence of indicator bacteria.

In order to understand the fate and transport of indicator bacteria in the Santa Monica Bay watershed, a multidisciplinary study was started that investigated hydrodynamics, physical and biological oceanography, and microbiology in order to build a dynamic water quality model. Microbiological modeling is important because degradation of indicator bacteria can reduce concentrations during transport and dispersion of fecal contaminated discharges. Although previous investigators have addressed degradation of bacterial indicators, these studies have focused upon single indicators, single environmental factors and/or did not study degradation in natural seawater; none of them were conducted to mimic conditions in or near Santa Monica Bay.

The goal of this project is to define the degradation rates of indicator bacteria under varying environmental conditions. Our approach measured the rates of bacterial degradation in the laboratory in response to varying levels of natural stressors.

Methods

There were three week-long experiments; December 1999, April 2000, and August 2000, which were designed to examine the relationship between rates of bacterial degradation and various combinations of environmental parameters. The first experiment, in December 2000, examined the effects of temperature, nutrients, bacterial concentration, and total suspended solids (TSS) in seawater. The second experiment, in April of 2000, examined the effects of temperature, inoculant type, and bacterial concentration in

seawater. The third experiment, in August of 2000, examined the effects of UV light, TSS, inoculant type, and bacterial concentration.

All experimental treatments were conducted to most effectively mimic conditions that naturally occur in Santa Monica Bay watersheds. The bacterial concentrations used in all of the experiments, high and low, were estimated to approximate the concentration of bacteria from a sewage spill, and at the AB 411 threshold for that particular indicator, respectively. There were three TSS concentrations used: ambient- imitated the natural TSS load found in coastal seawater, medium- imitated the level of TSS found in storm drain effluent during a low-flow period, and high-imitated the level of TSS found in storm drain effluent during a high-flow period. Nutrient levels were manipulated, specifically those of nitrate, nitrite, ammonia, silicate, and phosphate concentrations, to represent the ambient concentrations in seawater (low), and nutrient concentrations that would be found in storm drain effluent (high). Two temperatures were used for incubations, which approximated winter ocean temperatures in SMB (14 C), and summer ocean temperatures (20 degrees C). The experiments were also performed specifically with media from Santa Monica Bay; the seawater was collected near Malibu and the freshwater was collected from Malibu Creek.

The bacterial concentration inoculant was either a raw influent, or advanced secondary treated sewage sample collected from either Hyperion effluent, or a sample collected from the Los Angeles River, a flowing storm drain. The TSS inoculant consisted of a silt/mud mixture taken from a local creek bed (Malibu Creek). We used material from a relatively "rural" site, so as to reduce the chance of adding toxic material to our samples. The silt/mud was autoclaved, rinsed and resuspended in 0.02 μm filtered freshwater and large particles were allowed to settle out before addition to the sample bottles. Ultra-pure nutrient stocks will be added to the seawater samples so as to effectively reduce the chance of sample contamination.

For each of the three experiments, samples from each of the treatment bottles were collected at 0 h, 6 h, 12 h, 24 h, 48 h, 72 h, 96 h. Sampling times depend on logistical constraints and were sometimes adjusted based upon degradation rates observed. At each time, densities of three bacterial indicators, total coliforms, *E. coli* and enterococci, were determined using chromogenic substrate tests (Colilert and Enterolert). In some experiments, additional membrane filtration measurements were performed to assess if degradation rates were similar between *E. coli* and fecal coliforms, and to determine the comparability between Idexx kits and Membrane Filtration. In a subset of samples analyzed by both the chromogenic substrate kits and membrane filtration, we analyzed the decay of infectivity and presence of MS2 coliphage by loss of plaque forming units (PFU).

Results

Experiment 1

There were significant effects from temperature and initial bacterial concentrations on bacterial degradation rates (ANOVA $p<0.05$). At the higher temperature, bacteria

degraded significantly more rapidly than at the lower temperature. This occurred across all three different indicator types. No significant interactions were observed between either nutrient levels or TSS, and rates of bacterial indicator degradation (ANOVA $p>0.05$). All three indicator bacteria types demonstrated a lag in degradation for 24 hours, meaning that more rapid rates of degradation were observed after 24 hours had passed. Indicator bacteria did not degrade at significantly different rates, with overall rates of degradation of about 0.50-1.0 d^{-1}. The results of the first experiment indicated that temperature plays an important role in rates of degradation, but that rates of degradation were not fast enough to effect rates of plume dispersion along the coast, i.e. degradation occurs on the order of hours/days rather than minutes/hours.

Experiment 2

There were no significant differences among rates of degradation of indicators from the different types of sewage or storm drain inoculants (ANOVA $p <0.05$). Similar to experiment 1, temperature and initial bacterial concentration played the largest roles in the rates of degradation. Indicator bacteria still demonstrated a lag in degradation at the beginning of the experiments, where very slow rates of degradation occurred for the first 24 hours, and then rates appeared to increase. Rates of coliphage degradation were initially only found in high numbers in the samples inoculated with storm drain effluent. Rates of degradation of coliphage ranged from a 2 to 5 % per hr., which is similar to the rates of degradation observed for the indicator bacteria. Slower rates of coliphage degradation were associated with the lower temperature, but the relationship among temperatures was not significant.

Experiment 3

Both UV light and the type of inoculant were important to rates of degradation. Even though UV light appeared to effect rates of degradation dramatically, the rates of indicator bacteria degradation were still on the order of hours/days rather than minutes/hours. An interesting result of this experiment was to note that overall rates of indicator bacteria degradation in either freshwater or seawater were similar. However, it appears that indicator bacteria degradation in freshwater does not demonstrate an initial lag and that the pattern of degradation is linear, rather than exponential. Further examination of the data will yield a better knowledge of the patterns and differences between the two. In experiment 3, it was difficult to analyze the data for total coliforms because the expected values were misbracketed and ended up with qualified values (greater than and less than values). Rates of degradation between fecal coliforms and enterococci, however, appeared to be significantly different from one another, especially in high UV treatments.

Discussion

Our experiments demonstrated that indicator bacteria degradation is most strongly affected by temperature, UV light, initial bacterial indicator concentration, and in certain cases, the type of inoculant. TSS and nutrient levels were not found to differentially affect rates of degradation. It will be important in future studies to identify which, temperature or UV light, is more important to rates of degradation in Santa Monica Bay

seawater. Logistical constraints prohibited us from being able to successfully manipulate temperature and UV light in the same set of experiments. Rates of degradation of indicator bacteria were found to be an important factor to consider for whole watershed studies, and whole watershed models, but are not important to consider in the modeling of on-shore plume dispersion, since dilution rates are much more rapid at that interface.