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Survival of Bacterial Indicator Species on Environmental Impervious Surfaces

Bradford M. Wilson and Robert Pitt

Because of historic difficulties in the measurement of sewage borne pathogens, the microbiological quality of stormwater runoff is often characterized on the basis of bacterial indicator species. These species are assumed to derive from a common (sewage) source with pathogens of interest, and to arrive in, survive in, and move through watershed environments in numbers that correlate with the health risk from those pathogens. Commonly used indicator species (especially *Escherichia coli* and *Enterococcus* spp., or enterococci), however, may derive from sources other than sewage, and survive in the (non-enteric) environment at rates divergent from those of the pathogens they are presumed to indicate (National Research Council, 2004).

Field and Samadpour (2007) provide a critical review of both the the *indicator paradigm* (our current reliance on fecal indicator bacteria, FIBs) and an alternative monitoring regimen utilizing fecal source tracking (FST) methods. While noting the inadequate state of the art for direct measurements of pathogens, the authors find deficiencies in the correlations of FIBs to specific pathogens, and of FIBs to epidemiological measures of human health. They ascribe the deficiencies in the indicator paradigm to its inability to identify the source hosts of environmental FIBs. Landscape survival of FIBs and the ratio of FIBs to human pathogens deposited on the landscape are dependent on the source of the feces. More specifically, though zoonotic infections from non-human sources occur, correlation between human health threat and FIB presence suffers when major fecal sources other than sewage are present. (The

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authors proceed to find current state FST methods alone also deficient: they propose a multi-level combination of expanded source and epidemiological surveys, and pathogen, FIB and FST testing, while noting the expense and the laboratory retooling that would be required for such an approach.)

Continued reliance on the use of FIBs to manage the microbiological risk of environmental waters would be better informed by knowledge of the nonhuman contributions of FIBs to stormwater. In an ongoing effort to model background (i.e. of non-sewage origin) discharges of indicator species from stormwater source areas in which the presence of sewage contamination can be precluded, in the Tuscaloosa, Alabama area, a model for the environmentally relevant survival of indicator species (*E. coli* and enterococci) on impervious surfaces within the environment is presented.

14.1 Methods

14.1.1 Bacterial Cultivation and Enumeration

A full factorial study (2^3, temperature/moisture/UVB exposure, the latter being ultraviolet B radiation) of the indicator species' environmental survival factors was performed for each taxon (enterococci and *E. coli*). Pet feces slurries (1 mL) were applied to salt passivated paving blocks and incubated in controlled environmental chambers (freezerless refrigerators fitted with commercial biological oxygen demand, BOD, controllers and heaters for temperature control, desiccant or humidifiers for moisture control, and UVB enhanced fluorescents with Lexan panels to split the chambers into UV exposed and UV shielded regions) at conditions encompassing those likely to be found in Tuscaloosa. The raw concrete paving blocks had been prepared by an overnight soak in mild brine (0.25 cup, 62.5 mL, table salt into 40 gal, 151.4 L, trash can of tap water), followed by thorough tap water rinse and air drying, to provide an unreactive, passive surface. Slurries were produced by blending dog feces with distilled water (to assure microbiological purity and the absence of bactericidal components) and immediately applied (with a 3 mL sterile disposal syringe) to the passive blocks (to quickly relieve any potential osmotic stress of the distilled water). No additional nutrients (other than fecal materials) were added. Active control of temperature (40 °F and 90 °F, 4.4 °C and 32 °C, cool or warm) held the parameter steady (±2 °F, 1.1 °C) over the study period. Relative humidity (25% and 80%, dry or wet) varied over about ±4%. UV exposure was treated as present or absent (UV or dark).

Over an extended period (about two weeks), duplicate inoculated paving blocks were subjected to mechanical biofilm disruption by consistently applied and timed toothbrush abrasion (three scrubs of 1 min each, with

intervening wetting between scrubs), washing the slurry debris into sample bottles and dilution to 100 mL (with distilled water). Method development comparisons of wash-off MPN to inoculant MPN showed incomplete but consistent (within 95% confidence bands of MPN measurement) recovery of the inoculant by this abrasion–rinse technique. Washed-off samples were immediately mixed with defined substrate formulations (Colilert or Enterlert) for relief of osmotic stresses. The most probable numbers (MPN) of surviving *E. coli* and enterococci colony forming units (CFU) per 100 mL were measured using IDEXX (IDEXX Laboratories, Inc.) methods and normalized to the inoculation date (Day 0) MPN (also acquired from brush-off samples from blocks inoculated and brushed in the same way). IDEXX reagents (Colilert and Enterolert) provide for selective incubation of the taxons of interest, and colorimetric and fluorometric indicators of viable colonies within 24 h. These are recognized water assays under *Standard Methods for the Examination of Water and Wastewater* (Eaton et al., 2005:sections 9223 and 9230b, respectively). MPN measurement values with three orders of magnitude ranges (from 1 MPN/100 mL to 2 420 MPN/100 mL) are directly available with the reagents when used in conjunction with Quantitray 2000 units. Additional dilutions of each sample were incubated to ensure that all samples were quantified over even wider ranges.

14.1.2 Breakpoint Analysis

There is considerable reason to expect that the growth or decline (change in MPN over time) of bacterial populations is a first order (log-linear) relationship, arising as the sum of binary fission and death of individual cells (both dependent on the number of viable cells at any given time). This pattern, well established in textbooks currently in use (e.g. Madigan et al, 2002:142) is of the form:

$$\log (MPN / \text{initial } MPN) = k \times t \qquad (14.1)$$

where:

k = net growth constant (slope of the function), and
t = time (hours).

Changes in the slope of log(*MPN*) versus time are likely caused by a change in environmental conditions or a change in the makeup of the subject population. Introduction of a viable bacterial inoculant to a new (habitable) medium (batch style) typically results in up to four distinct phases of population behavior: lag, exponential growth, stationary, and exponential death (Madigan et al., 2002:144–5):

Lag Phase

The lag phase is characterized as a period of adaptation to the new environment, in which little or no population growth occurs, and its length is dependent on differences between the environmental history of the inoculant and the environmental conditions of the new medium. Inoculants transferred to environments similar to their historical conditions may exhibit little or no lag time; for transfers to a very different environment, lags may be considerable. Of course, if new conditions are so foreign to members of the inoculant population as to render it uninhabitable, individual cell death may occur until remnants of the inoculant population are viable (Madigan et al., 2002: 144–5).

Growth Phase

In the growth phase, the adapted (or naturally selected) population grows exponentially; population at any given time is dependent on the number of actively dividing members of the population present at previous times. Rate of growth is dependent on environmental conditions and the genetic (metabolic mechanisms available) composition of the population (Madigan et al., 2002: 144–5).

Stationary Phase

The stationary phase (in which the population is static) represents conditions in which available nutrients (either from the original inoculant or from release by the lysis of dying cells) is balanced by a buildup of refractory (and often inhibitory) waste products (Madigan et al., 2002: 144–5).

Death Phase

The death phase (dominated by waste buildup) is exponential.

Any or all of these phases may occur (or, of course, may be missed by insufficient time density of sampling) and both environmental conditions and the genetic makeup of the population are relevant (Madigan et al., 2002: 144–5). The four main environmental factors influencing bacterial growth are temperature, pH, and the availability of water and oxygen (Madigan et al., 2002: 151). For terrestrial environmental surfaces, oxygen is unlikely to be a factor. For dry weathered pavements (without liquid moisture, between rains), pH is likewise probably unimportant. An important factor in cell death, however, is that of UVB exposure (Madigan et al., 2002: 272–3), which is bactericidal, especially during cell division.

Because we cultivated our samples at constant conditions, a change in slope of log(*MPN*) versus time must be viewed as a population change. Population change may arise either through induction of new enzymes in individual cells, or through natural selection in the overall population.

Each combination ($2^3 = 8$ combinations of temperature, humidity, and UV exposure) of environmental conditions (treatments, combinations of environmental factors) was treated as a log-linear (first order) segmented (with unknown break points) model of normalized MPN with respect to time, and with continuity between the segments imposed (as shown below in Figure 14.3, for example).

The statistical analysis of such models is not straightforward. Hudson (1966) provides a graphic algorithm (for minimization of overall sum of squares of error, SSE, in the segmented model) and shows that the algorithm generally provides the maximum likelihood estimate (MLE) of the abscissa of an unknown breakpoint (*tBP*); he provides no information as to how likely that estimate may be (rendering inferences impossible). Feder (1975a; 1975b) proves that, provided the model is identified (i.e. includes no more hypothesized breakpoints than are present in the real population), and if no hypothesized tBP coincides with an abscissa of observation in the sample, then minimization of *SSE* (the MLE function) converges asymptotically to the true population breakpoint (BP).

In the unidentified case (i.e. too many BPs assumed), the MLE function becomes indeterminate (estimates are not asymptotically normal). Feder's second condition arises because a discontinuity exists in the SSE function at each observation point, rendering it non-differentiable there, allowing for a possible true BP existing between the MLE tBP and an adjacent sample observation point (i.e. the MLE function becomes unstationary). For the unstationary case, he proves that, as the number of sample observations increases, minimization of SSE of a pseudocase (in which the observation point coinciding with the tBP is removed from the dataset) still converges (at a known rate) to the true BP. Lerman (1980) adapts Feder's work into a grid-search algorithm (again, only for the identified case, and incorporating the pseudocase approach when necessary) in which proposed tBPs are mapped across the range of the observations and the SSE at each is determined. Progressive refinement (finer grain) of the grid provides the tBP (minimization of the SSE versus proposed-tBP function). The exercise also provides an estimate of the variance of that tBP estimate, corresponding to the range (which need not be continuous or symmetrical) of proposed-tBPs. The range includes all time in which SSE is less than the minimum SSE plus its associated mean square of error (minSSE + MSE). Finally, Bai and Perron (1998) derive a log-likelihood ratio by which it can be determined whether the addition of a new breakpoint to an identified model results in a new model which is also identified, and publish critical values for that ratio.

We found the grid search method amenable to spreadsheet implementation. We first modeled each treatment by simple linear regression, resulting in

a one-segment ($R = 1$, no breakpoints) model. We then hypothesized a breakpoint, and searched for it by Lerman's grid method. If the resulting MLE did not coincide with an observation point, we accepted the tBP and associated uncertainty indicated by the search (see Figure 14.1 below). We found grid search of the (asymptotically converging) pseudocase, however, problematic for the limited number of data points we had for each treatment (typically ~35). In one case, analysis of the pseudocase resulted in the tBP jumping about 100 h (and across multiple observation points, an impossible situation) because of the slower convergence of the smaller, highly variable dataset. In these cases we retained the grid derived tBP and accepted the greater uncertainty inherent; we conducted a one sided grid search solution around the tBP to establish one side of the variance range and took the adjacent observation point as the other (see Figure 14.2). Note that since we generated our grid search left-to-right (increasing t), the segment containing the discontinuity occurred between our tBP and the immediately preceding (adjacently left) observation. In both situations, the new model was tested against Bai and Perron's criteria for identification and, if it was identified, repeated the sequence. For the final model of each treatment, we numbered each tBP and intervening segments left-to-right (see Figure 14.3).

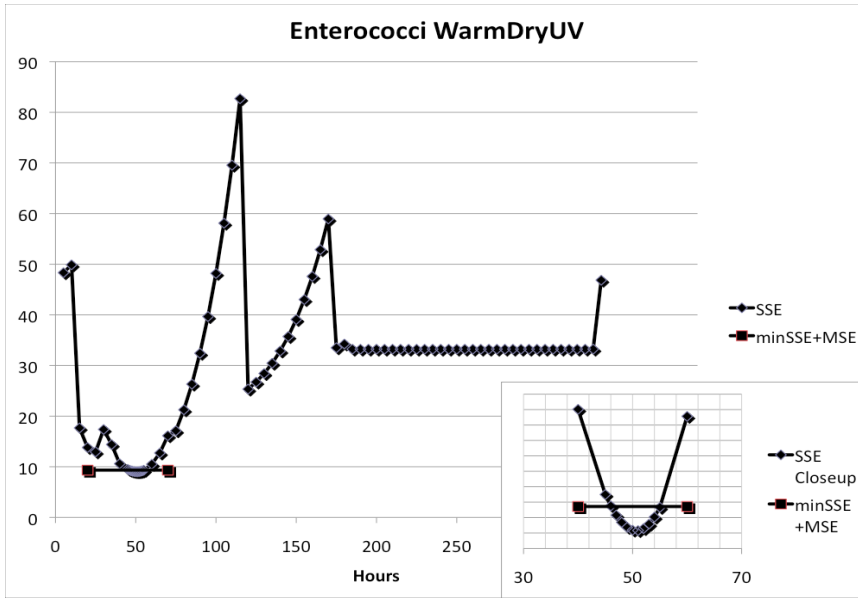

Figure 14.1 Example of graphic derivation of estimated tBP variance, normal case (with closeup inset).

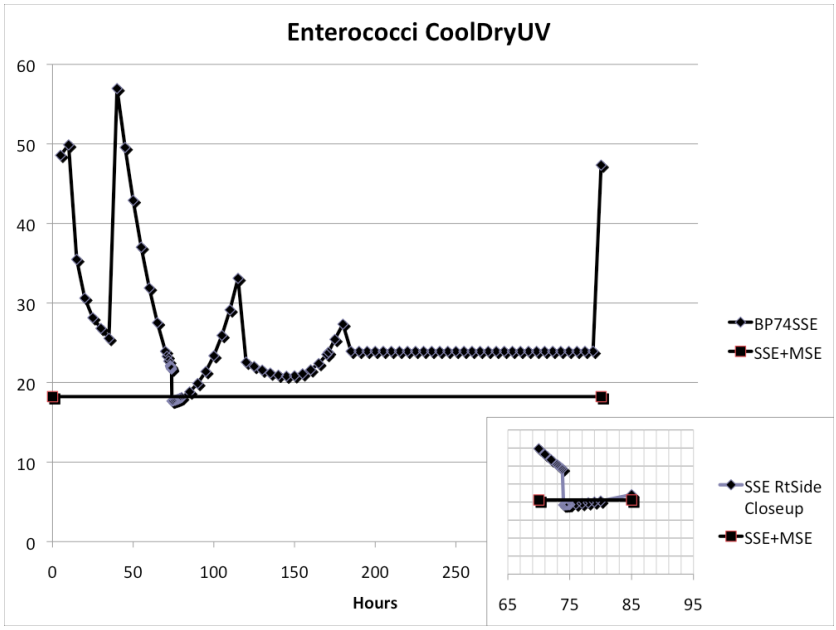

Figure 14.2 Example of right-sided graphic derivation of tBP variance, discontinuity on the left (with closeup inset).

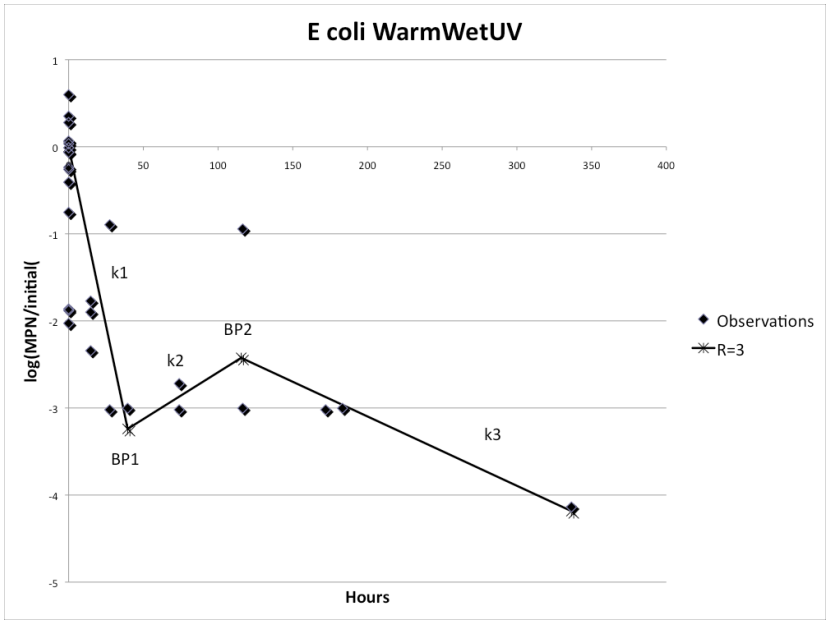

Figure 14.3 Example of a three segment (*R* = 3) treatment model with segment and breakpoint designations.

14.1.3 Environmental Factors

Each taxon (*E. coli* and the enterococci) was subjected to traditional (pooled variance) factorial analysis to rank the importance of the environmental factors (temperature, humidity, and UV exposure, coded as 1 = shaded and 0 = exposed, plus their interactions) to the abscissa of each breakpoint and to the slope (k) of each intervening segment. The tBPs, their associated uncertainties, and the k of each segment were derived directly from the breakpoint analysis above. The variance of each k was determined from log (*MPN* / initial *MPN*) / $t = k$ for each nonzero t in the segment.

This exercise was rendered problematic by the fact that different treatments (even within a taxon) differed in the number of tBPs revealed, from $R = 1$ (one segment with no BP) to $R = 3$ (three segments with two BPs). Orthogonality of the contrasts was achieved by the generation of artificial BPs within segments without breakpoints but for which tBPs were revealed in corresponding segments of other treatments. Transparency of the artificial points to the factorial analysis was achieved by assigning to them abscissae equal to the weighted average of revealed tBPs, and by assigning them zero variance. The k values of the new segments (on each side of the artificial BP) generated by this action were held to be equal, but the number of observation points (n) and the variance associated with those points were distributed (n-weighted) between the new segments.

Environmental factor effects on *tBP* or k values were deemed important if their standard errors (SE) led to conclusions of at least 90% confidence (reasonable, considering the small sample sets) that the effects were not zero, although confidence in the importance of effects was much higher (and noted) in some cases. Conclusions that effects were not zero were reached when the calculated confidence interval (CI) was smaller than the calculated effect:

$$CI = SE \times t(a) \tag{14.2}$$

where:

$t(a)$ = Student's t-table return for the appropriate degrees of freedom and

(a) = the p-value resulting in the reported confidence level (i.e. alpha).

14.1.4 Model Construction

The important environmental effects (main effects and interactions) on k and tBP values, derived above, were used to model those parameters as a function of environmental factors:

$$\textit{Model Parameter} = \textit{Mean (Parameter)} + \textit{Sum of (Effects of Environmental Factors)} \quad (14.3)$$

where:

Parameter = treatment *k* or *BP* (artificial or not) entered into the tables of contrast for the factorial analyses,

Mean(Parameter) = treatment weighted mean for that *parameter*, and

Effects of Environmental Factors (EEF) = adjustments to *Mean(Parameter)* attributable to each important environmental factor.

For 2-level factorial, effects are of the form:

$$\textit{EEF} = [\textit{Product(EF-MEF)} \times \textit{(1/2 environmental effect)}] / \textit{Product(REF)} \quad (14.4)$$

where:

EF = value of that environmental factor for an observation point,

MEF = mean of that environmental factor amongst observation points, and

REF = range (high value to low value) of an environmental factor amongst observation points.

14.2 Results and Discussion

14.2.1 *E. Coli*

Results from breakpoint analysis of the *E. coli* dataset (Figure 14.4 below) are complex. One treatment (warm/wet/dark) showed no significant tBP (not even a lag), and also exhibited an absence of any initial accelerated decline. Two treatments (warm/dry/UV and warm/wet/UV) showed two tBPs each, with an initial decline, a rebound of growth, and a subsequent second decline. Cool treatments were nearly indistinguishable from each other, and resulted in more rapid declines than warm/shade treatments. All treatments exhibiting BPs showed slower declines later in the study period than in the initial die-off

Warm conditions in general, and warm/wet/dark in particular, most closely match the primary habitat (the gut of warm blooded animals) of our enteric bacteria, and would likely impose the least stringent adaptation requirements. The fact that only warm/UV treatments elicit regrowth and three phase behavior suggests an interaction. While UVB (the primary bactericidal

band in sunlight) is not strictly ionizing radiation, it is of sufficiently high frequency to rearrange bonds in complex biomolecules.

Most importantly, UVB causes dimerization of adjacent thymine units (and other photoproducts) within bacterial genomes that inhibit the progression of (both RNA and DNA) polymerases. An unrepaired lesion within a gene prevents transcription of that gene. Each unrepaired legion also stops replication of the entire genome during fission. (Wulff and Rupert, 1962; Sinha and Hader, 2002). Hospitable (e.g., warm) conditions prompting greater cell growth and division might increase UV sensitivity until repair mechanisms are induced or tolerant strains are selected for.

The factorial analysis results (Table 14.1) are likewise complex, especially in terms of the timing of the breakpoints. Such complexity should not be unexpected considering that even the number of breakpoints is treatment specific. The fact that only *k1* shows any significant evidence of influence by environmental factors may imply adaptation (either at cellular or population level) for later segments.

Table 14.1 Important alpha, (a) ≤0.1.

E. coli k1					
Main Effects	Effects	SE(Effect)	t(a)	CI(effect	df=17
Humidity	0.061	0.00055	2.6	0.060	(a)=0.01
Interactions					
Temp/Humid	0.11	0.024	2.9	0.070	(a)=0.01
E. coli BP1					
Main Effects	Effects	SE(Effect)	t(a)	CI(effect	df=224
Temperature	2.1	0.33	2.6	0.87	(a)=.005
Humidity	3.2	0.33	2.6	0.87	(a)=.005
ShadeCode	-3.9	0.33	2.6	0.87	(a)=.005
Interactions					
Temp/Humid	12.6	0.33	2.6	0.87	(a)=.005
Temp/Shade	-5.0	0.33	2.6	0.87	(a)=.005
Humid/Shade	-6.2	0.33	2.6	0.87	(a)=.005
Temp/Humid/Shade	-2.8	0.33	2.6	0.87	(a)=.005
E. coli BP2					
Main Effects	Effects	SE(Effect)	t(a)	CI(effect	df=37
Temperature	-9.2	1.05	2.8	2.9	(a)=.005
Humidity	17.9	0.80	2.8	2.2	(a)=.005
Interactions					
Temp/Humid	17.9	0.80	2.8	2.25	(a)=.005
Humid/Shade	17.9	0.80	2.8	2.2	(a)=.005
Temp/Humid/Shade	17.9	0.80	2.8	2.2	(a)=.005

Figure 14.4 *E. coli* BP models; CFU determined by MPN (IDEXX) methods.

Our model for *E. coli* survival is therefore, for times $t \le tBP1$:

$$\log(MPN \,/\, \text{initial } MPN) = k1 \times t \tag{14.5}$$

where:

$$k1 = -0.108 + (H - 52.5) \times 0.000551 + (T - 65) \times (H - 52.5) \times 0.0000203 \tag{14.6}$$

where:

$T =$ temperature (°F)

$H =$ %relative humidity, and

$$tBP1(\text{hours}) = 21.6 + (T - 65) \times 0.0209 (H - 52.5) \times 0.0293 - (S - 0.5) \times 1.95 + (T - 65) \times (\mathrm{H} - 52.5) \times 0.00229 - (T - 65) \times (S - 0.5) \times 0.0503 - (H - 52.5) \times (S - 0.5) \times 0.0560 - (T - 65) \times (H - 52.5) \times (S - 0.5) \times 0.000506 \tag{14.7}$$

where:

$S =$ shade code (1 = shade, 0 = exposed)

Our model for *E. coli* survival is, for times $t > tBP1$ and $t \le tBP2$:

$$\log(MPN \,/\, \text{initial } MPN) = k1 \times tBP1 + 0.002214 \times (t - tBP1) \tag{14.8}$$

where:

$$tBP2 = 80.71 - (T - 65) \times 0.0924 + (H - 52.5) \times 0.163 + (T - 65) \times (\mathrm{H} - 52.5) \times 0.00326 - (H - 52.5) \times (S - 0.5) \times 0.163 - (T - 65) \times (H - 52.5) \times (S - 0.5) \times 0.00326 \quad (14.9)$$

Our model for *E. coli* survival is, for times $t > tBP2$:

$$\log(MPN / \text{initial } MPN) = k1 \times tBP1 + 0.00221 \times (tBP2) - (0.00501) \times (t - tBP2) \quad (14.10)$$

The model presented does not fully account for the variability in the observations (R^2 is only 0.42, and see Figure 14.5) of the full *E. coli* dataset. It does, however, offer improved correlations with, and better balance between, under- and over-predictions than would be provided by a simple linear regression of the same dataset (compare Figures 14.6 and 14.7, and note closely concentric trending in Figure 14.5). Residuals of the model show little evidence of any trend over time, providing some comfort in the pooled variance methods used here (Figure 14.8).

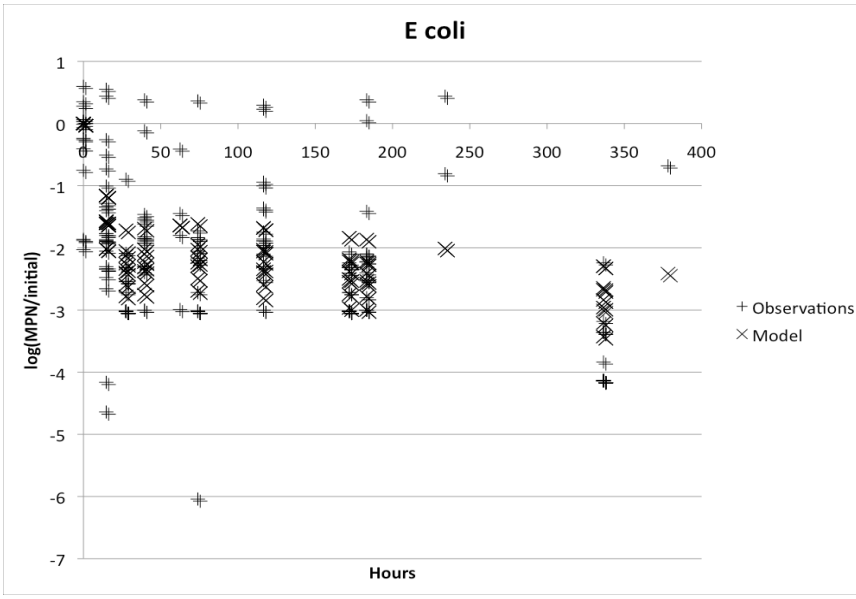

Figure 14.5 Overlay of model predictions on observations, all treatments combined.

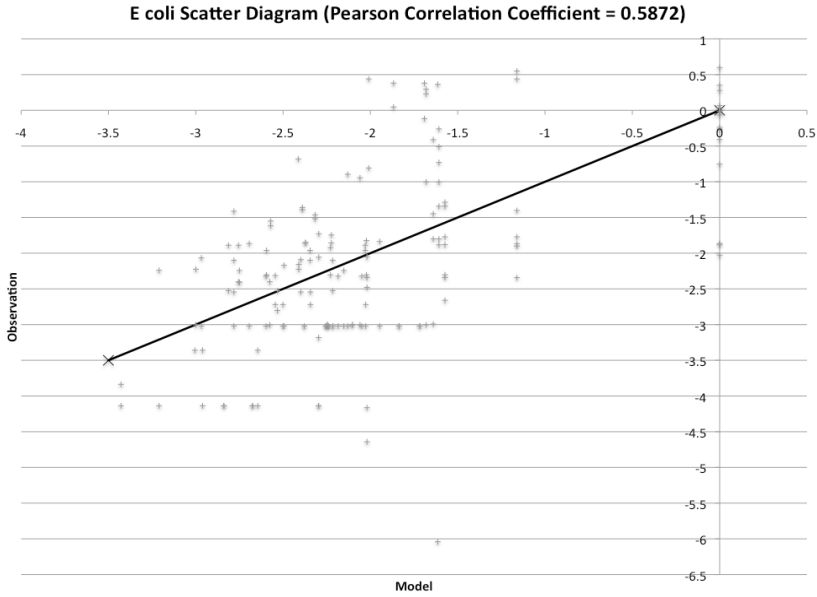

Figure 14.6 Observations vs model; line is observation = model prediction.

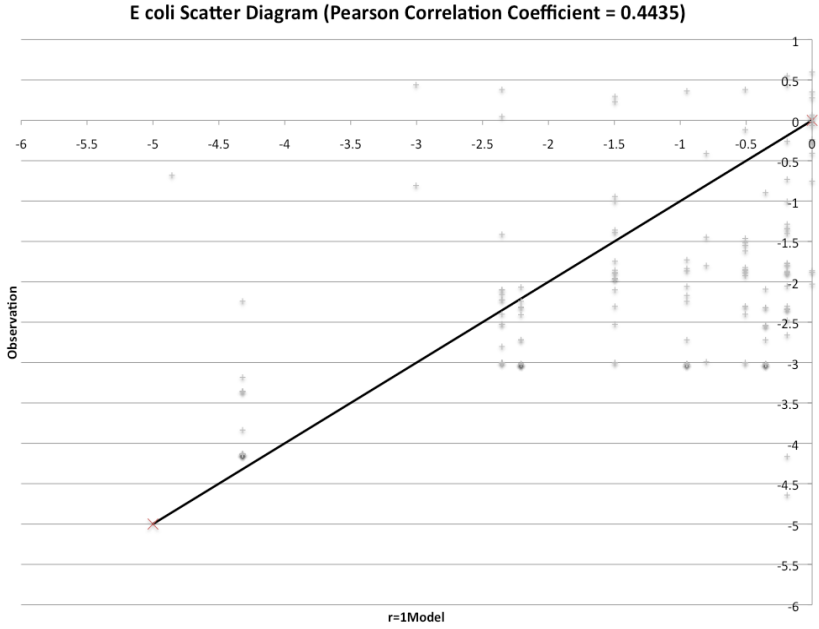

Figure 14.7 Observations vs predictions of linear regression without environmental factorial.

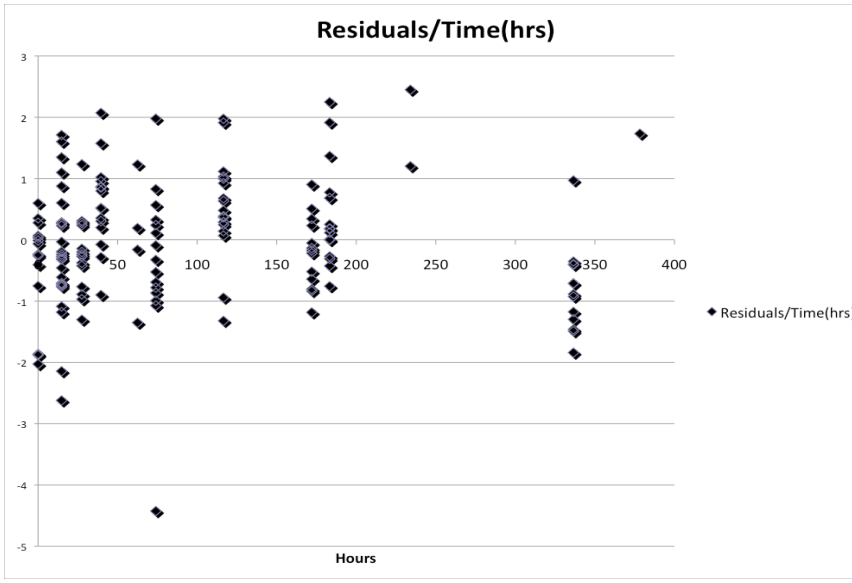

Figure 14.8 Residuals vs time for the presented *E coli* model.

The model derived parameters applied to our experimental conditions are presented in Table 14.2 below. All treatments exhibit an initial lag or die-off, the rate of which depends on the temperature and humidity. Notably, the warm/wet conditions (those most like the enteric habitat, and exerting the least pressure for adaptation) show the lowest initial rate (*k1*) of decline, but all inoculants had declined from two to three orders of magnitude within a day or so. The duration of the decline appears to be quite variable (19 h to 27 h), but should be interpreted with caution.

Recall that the BP analysis resulted in several tBPs that coincided with the first (earliest) observation point. Though the values listed in the table represent the best estimates for predictive purposes, they must be viewed mechanistically as the latest likely time for the change. The true BP1 may have occurred before the first observation. The insensitivities of *k2* and *k3* to environmental factors imply that all adaptive mechanisms available to the inoculant population had been implemented prior to (and caused) the first breakpoint. The two phase behavior subsequent to BP1 could be attributed to waste buildup in these batch systems or to accumulation of UV generated thymine dimers (and a review of the warm treatment behaviors in the original BP analysis, Figure 14.4 above, suggests that both factors are involved).

Table 14.2 *E. coli* modeled parameters, applied to experimental conditions.

	k1 (1/hours)	BP1 (hours)	k2 (1/hours)	BP2 (hours)	k3 (1/hours)
Cool/Dry/UV	-0.109	21.6	0.00221	76.8	-0.00501
Cool/Dry/Dark	-0.109	22.1	0.00221	79.0	-0.00501
Cool/Wet/UV	-0.107	21.3	0.00221	83.5	-0.00501
Cool/Wet/Dark	-0.107	19.4	0.00221	81.2	-0.00501
Warm/Dry/UV	-0.137	20.4	0.00221	71.0	-0.00501
Warm/Dry/Dark	-0.137	19.1	0.00221	77.8	-0.00501
Warm/Wet/UV	-0.0787	27.1	0.00221	91.2	-0.00501
Warm/Wet/Dark	-0.0787	22.0	0.00221	84.5	-0.00501

14.2.2 Enterococci

Treatment analyses of the breakpoints is less complex for enterococci than for *E. coli* (see Figure 14.9), although some disparity as to number and tBP values per treatment appears here as well.

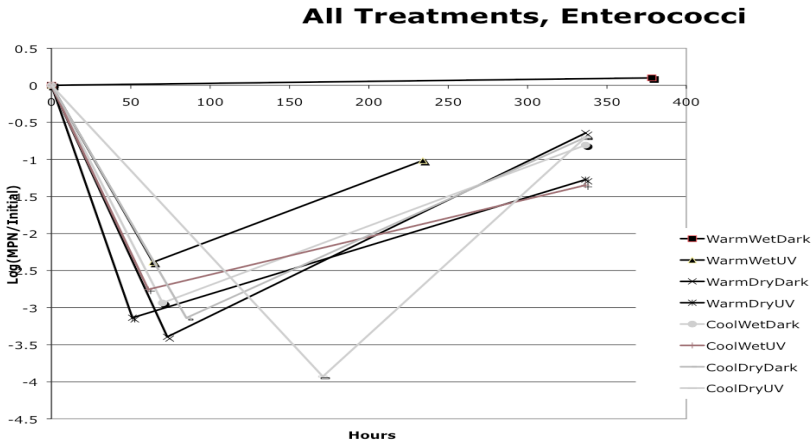

Figure 14.9 Enterococci BP models.

The warm/wet/dark treatment shows no evidence of a breakpoint (even a lag). It also displays a slope statistically indistinguishable from zero. The clear trend of greater net survival in warm treatments seen in the *E. coli* analysis is not evident here, and the timing of breakpoints in treatments (where they occur) is less varied than occurred for *E. coli*. Our assay in this case is sensitive to metabolic signals for an entire genus rather than a single species. One is tempted to argue that the greater genetic diversity of the higher taxon provides an overall greater potentiality of adaptive capacity (natural selection

acting differently on distinct species or strains in different conditions) and a greater likelihood of genes for UVB damage repair mechanisms within the initial inoculant. Remarkably, when regrowth phases are recognized, none of the treatments show a net decline of more than about one order of magnitude over a 2 week period. It also should be noted that no population is in decline at the end of the study period. As a final note, our study was unable to distinguish bacterial lysis from other fecal components of the inoculant slurry as nutrients for growth. Factorial analyses (Table 14.3) for enterococci were also simpler than for *E. coli*, but again showing greater complexity for tBP values than for intervening segments. As for the *E. coli* analyses, *k* values become insensitive to environmental factors subsequent to the tBP, implying capacity for adaptation to the secondary (non-enteric) habitat.

Table 14.3 Important factors per enterococci factorial analysis.

Enterococci k1					
Main Effects	Effects	SE (Effect)	t(a)	CI(effect)	df=56
Humidity	0.015	0.011	1.3	0.014	(a)=0.1
Shade Code	0.015	0.11	0.010		(a)=0.1
Interactions					
Temp/Humid	0.020	0.011	1.7	0.019	(a)=0.05
Temp/Shade	-0.077	0.011	2.7	0.030	(a)=0.005
Enterococci BP					
Main Effects	Effects	SE (Effect)	t(a)	CI(effect)	df=233
Temperature	-8.8	0.31	2.7	0.84	(a)=0.005
Humidity	-5.3	0.32	2.7	0.84	(a)=0.005
Shade Code	11.2	0.31	2.7	-0.84	(a)=0.005
Interactions					
Temp/Humid	8.7	0.31	2.7	0.84	(a)=0.005
Temp/Shade	1.2	0.32	2.7	0.85	(a)=0.005
Humid/Shade	-5.3	0.32	2.7	0.84	(a)=0.005
Temp/Humid/Shade	-4.3	0.32	2.7	0.85	(a)=0.005

Our model for enterococci survival is therefore:

$$\log\left(MPN / \text{initial } MPN\right) = k1 \times t \text{ for } t \leq tBP \tag{14.11}$$

where

$$\begin{aligned} k1 = -0.0356 &+ (H - 52.5) \times 0.000137 + \\ &(S - 0.5) \times 0.00727 + \\ &(T - 65) \times (H - 52.5) \times 0.00000372 - \\ &(T - 65) \times (S - 0.5) \times 0.00771 \end{aligned} \tag{14.12}$$

and

$$tBP = 68.74 - (T - 65) \times 0.881 - (H - 52.5) \times 0.0483 + (S - 0.5) \times 5.59 + (T - 65) \times (H - 52.5) \times 0.00158 + (T - 65) \times (S - 0.5) \times 0.0119 - (H - 52.5) \times (S - 0.5) \times 0.0483 - (T - 65) \times (H - 52.5) \times (S - 0.5) \times 0.000784 \quad (14.13)$$

and for $t > tBP$:

$$\log(MPN / \text{initial } MPN) = k1 \times tBP + 0.00652 \times (t - tBP) \quad (14.14)$$

Comparison of the model with observations (Figure 14.10) makes it apparent that there are other sources of variability than the environmental factors analyzed here (and R^2 is only 0.59). However, the model again provides closer (and more balanced) agreement with the data than does a simple regression (Figures 14.11 and 14.12, and closely concentric trending in Figure 14.10). Residual plot, again, provides no evidence of increase or decrease over time (Figure 14.13).

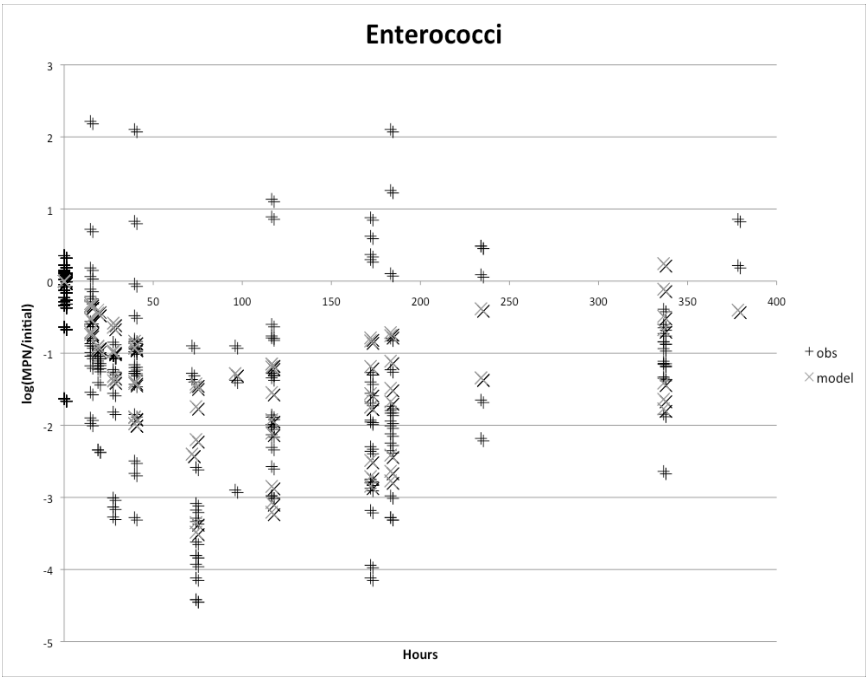

Figure 14.10 Enterococci, observations vs model comparison.

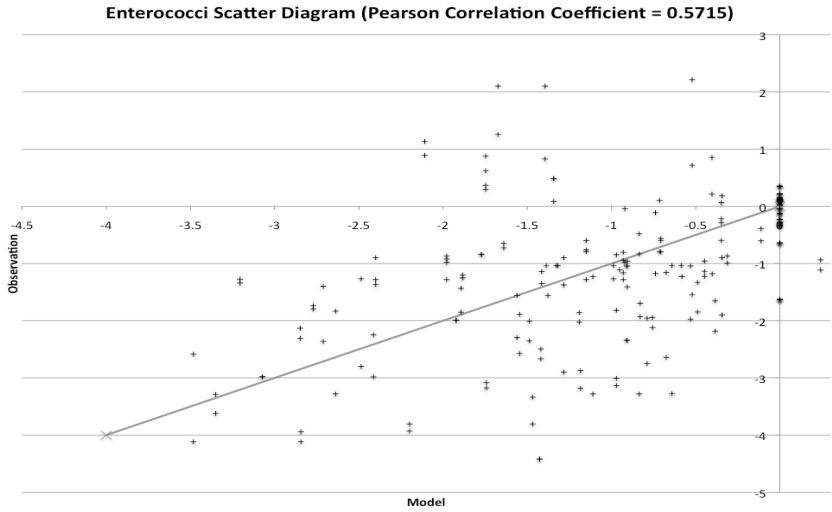

Figure 14.11 Model Predictions vs observations; line shows observation = prediction.

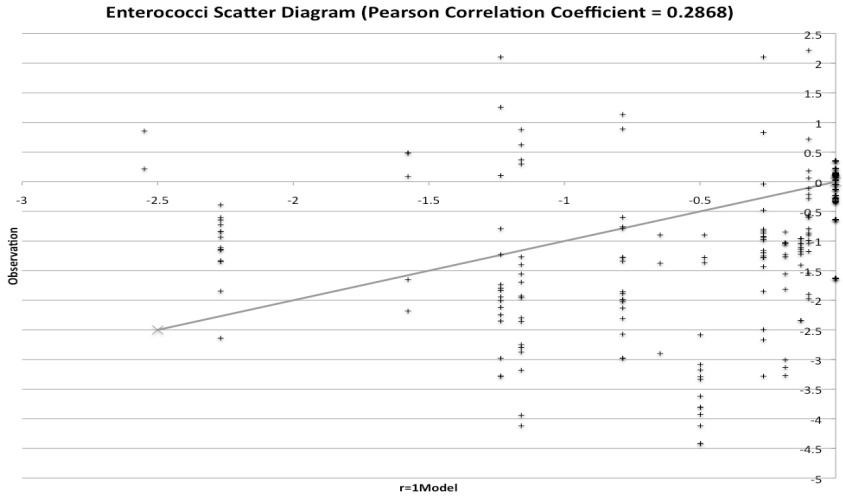

Figure 14.12 Paired observations vs predictions from a simple linear regression; line displays observation = prediction.

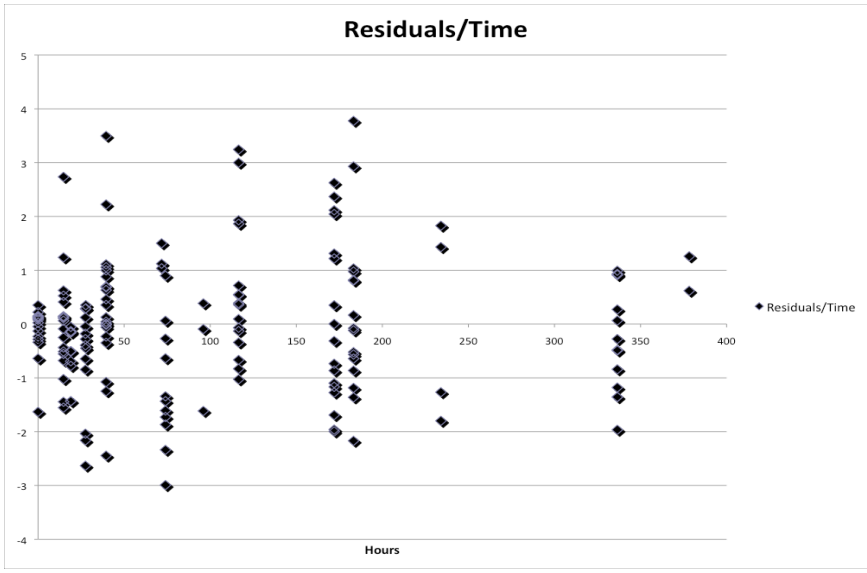

Figure 14.13 Residuals, over time, of the proposed model.

The model derived parameters applied to our experimental conditions are presented at Table 14.4. All treatments, again, exhibit an initial decline, with all three environmental factors (temperature, humidity, and UV exposure) contributing (either as main effects or within interactions). The rates of decline, however, are only about half of those suffered by *E. coli.* None of the BPs for these populations coincided with initial observations, and the adaptation phase of these inoculants lasted about three days. Even with the slower rates of decline, most inoculants had been reduced by two or three orders of magnitude in the initial period.

Table 14.4 Enterococci modeled parameters, applied to experimental conditions.

	k1 (1/hours)	BP (hours)	k2 (1/hours)
Cool/Dry/UV	-0.0501	70.0	0.00652
Cool/Dry/Dark	-0.0235	76.7	0.00652
Cool/Wet/UV	-0.0477	66.5	0.00652
Cool/Wet/Dark	-0.0211	70.5	0.00652
Warm/Dry/UV	-0.0359	63.2	0.00652
Warm/Dry/Dark	-0.0479	70.4	0.00652
Warm/Wet/UV	-0.0233	64.0	0.00652
Warm/Wet/Dark	-0.0353	68.6	0.00652

The insensitivity of *k2* to environmental effects, and the fact that it is positive (indicating net growth) implies that these organisms adapt to impervious environmental surfaces quite well. By the end of the study period (about two weeks) all inoculants had rebounded to about 10% of their original populations.

14.3 Conclusions

We developed the models presented here in support of an ongoing effort to model source area processes contributing to the background (i.e. of non-sewage origin) presence of fecal indicators in stormwater. Together with a planned similar study of survival on pervious surfaces (soils), they should contribute to a mass balance link between fecal deposition on the landscape and biological stormwater quality.

Others, however, may find the work of interest. The studied indicator organisms (especially *Enterococci* spp.) were found to be quite persistent (especially under environmental conditions that most closely approximate enteric conditions) on impervious surfaces subject to the extreme Tuscaloosa, Alabama environmental conditions. Moreover, under most conditions studied, the rate of disappearance of these organisms from the landscape slowed (or even reversed), rendering short term studies of their survival (or even the simple regression of long term studies) unreliable in predicting their environmental fate.

We hope that risk analysis of stormwater exposures, and efficient search for sources of indicators species in runoff, will be better informed by this work.

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