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Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration

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ABSTRACT

Most probable number (MPN) and colony-forming-unit (CFU) estimates of fecal coliform bacteria concentration are common measures of water quality in coastal shellfish harvesting and recreational waters. Estimating procedures for MPN and CFU have intrinsic variability and are subject to additional uncertainty arising from minor variations in experimental protocol. It has been observed empirically that the standard multiple-tube fermentation (MTF) decimal dilution analysis MPN procedure is more variable than the membrane filtration CFU procedure, and that MTF-derived MPN estimates are somewhat higher on average than CFU estimates, on split samples from the same water bodies. We construct a probabilistic model that provides a clear theoretical explanation for the variability in, and discrepancy between, MPN and CFU measurements. We then compare our model to water quality samples analyzed using both MPN and CFU procedures, and find that the (often large) observed differences between MPN and CFU values for the same water body are well within the ranges predicted by our probabilistic model. Our results indicate that MPN and CFU intra-sample variability does not stem from human error or laboratory procedure variability, but is instead a simple consequence of the probabilistic basis for calculating the MPN. These results demonstrate how probabilistic models can be used to compare samples from different analytical procedures, and to determine whether transitions from one procedure to another are likely to cause a change in quality-based management decisions.

1. Introduction

Coastal water resource management agencies frequently revise standard water quality analysis procedures based on the latest available technologies. For example, the North Carolina Department of Environmental and Natural Resources Shellfish Sanitation and Recreational Water Quality Section (NCDENR-SSS), and similar water resource management agencies, are considering replacing multiple-tube fermentation (MTF) fecal coliform analysis procedures with membrane filtration (MF) procedures because MF results, while variable, are much less so than MTF results (as commonly implemented) from the same water quality sample. NCDENR-SSS and other agencies are concerned, however, that water quality-based management decisions for a particular water body (such as approval or prohibition of shellfishing) may change after MF procedures are implemented.

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Nomenclature

Bi	binomial distribution
L	likelihood function
LN	log normal distribution
No	normal distribution
Po	Poisson distribution
V	volume of a water quality sample (ml)
argmax_c	value of c for which the given function attains its maximum value
c	fecal coliform concentration (organisms per 100 ml)
f	probability density function
i	index of dilution series level
$\log_{10}$, ln	log base 10 and natural log
m	number of different dilution series
n	number of tubes or wells in a dilution series
p	probability of a dilution series sample exhibiting fermentation or growth
v	volume of dilution series sample (ml)
x	number of samples in a dilution series exhibiting fermentation or growth

Here, we derive a theoretical model for the probability distribution of MTF and MF test results from the same water quality sample. This innovative approach allows a side-by-side comparison of alternative testing methods, accommodating their intrinsic differences (rather than assuming that these differences have no effect). Further, we find the probability distributions for the true fecal coliform concentrations associated with different possible measurement results from each procedure.

Differences, if observed, between the MTF–MF relationship predicted by our model and the MTF–MF relationship observed empirically in samples from a particular laboratory, would suggest significant extrinsic sources of uncertainty and variability (i.e. unrelated to natural spatial distribution of organisms in a sample aliquot volume) and, more importantly, an increased chance that changing standard fecal coliform analysis from MTF to MF might lead to a change in water quality-based management decisions.

Variability in MTF and MF analysis results can be divided into two categories: intrinsic stochastic variability due to the natural dispersion of bacteria within sample containers, and extrinsic variability. Intrinsic sources of variability are mostly a consequence of procedure design, and are explained later in this section. Extrinsic sources of variability include departures from expected sampling protocol, microbial cell damage (during filtration, for example) which may reduce the number of viable organisms (Kloot et al., 2006), and clumping of bacteria cells (Noble et al., 2003b). Other potential extrinsic sources of variability relate to environmental conditions at the time of sampling, including antecedent rainfall, turbidity, and season (Cabelli et al., 1983; Noble et al., 2003a). These extrinsic sources of variability are not included in our model and, if they actually contribute to MTF–MF intra-sample variability, will limit our model's ability to explain the difference between MTF and MF results.

Fecal and total coliform bacteria are indicators of potential fecal pollution and water-borne pathogenic threats to human health (Cabelli, 1983; LeClerc et al., 2001). Other bacterial measures of water quality include *Escherichia coli* (a subset of fecal coliforms), and enterococci (Noble et al., 2003a). Extensive definitions of fecal and total coliform bacteria are presented elsewhere (Rompré et al., 2002; Kloot et al., 2006). Our model is applied to monitoring data from shellfish harvesting areas in which fecal coliform is a more common measure of water quality. As a result, we discuss only fecal coliform bacteria concentrations for the rest of this paper, however the application of probabilistic models to intra-sample variability can be applied to a wide range of microbial, physical, and chemical pollutants (see, e.g. Kinzelman et al., 2003; U.S. Geological Survey, 1996; Horowitz, 1986).

MTF and MF are two common procedures for estimating fecal coliform concentrations in coastal resource waters (Eckner, 1998; Buckalew et al., 2006). MTF and MF fecal coliform analysis results are reported as most probable number (MPN) and colony-forming unit (CFU) estimates of the true fecal coliform concentration c (typically in organisms per 100 ml). Detailed descriptions of the MF microbial analysis procedure are presented in Rose et al. (1975), Rippey et al. (1987), Dufour et al. (1981), Eckner (1998), and Esham and Sizemore (1998). Similar descriptions of the MTF procedure are presented in Cochran (1950), Hurley and Roscoe (1983), Beliaeff and Mary (1993), and McBride et al. (2003).

MPN estimates derived from a standard (e.g. 5-tube $\times 3$ dilution series) MTF analysis are, by definition, the possible values of the concentration at which the likelihood function (see Appendix, Eq. (2)) attains its maximum. The likelihood function offers an indication of how strongly an observed pattern of positive tube counts from an MTF analysis support each possible value c of the concentration (McBride, 2005, pp. 12–13). The MPN estimates are highly variable because this function has a very broad peak, and so is close to its maximum value over a wide range of possible concentrations.

Additional discussion of the statistical assumptions inherent in MTF-based MPN calculations can be found in Eisenhart and Wilson (1943), Beliaeff and Mary (1993), and Klee (1993). CFU estimates are based on the number of distinguishable bacterial colonies which form on a culture plate after filtration and incubation. CFU variability is inversely proportional to the volume of sample water filtered, and therefore while CFU estimates are variable, the variability is often small compared to that of MTF-derived MPN estimates when large aliquot volumes are used. The broad likelihood function of MTF positive tube count observations and variability in the number of distinguishable bacterial growth colonies are both examples of intrinsic variability in MPN and CFU estimates, and are therefore addressed explicitly in our model.

Several recent studies document empirical relationships between fecal bacteria analysis results from different testing procedures (e.g. Eckner, 1998; Noble et al., 2003b; Kloot et al., 2006). The study by Noble et al. (2003b), for example, which

compares beach water quality analysis results using MF, MTF, and the IDEXX Quanti-Tray®2000 chromogenic substrate test (CST) kit, indicates that measurement error inherent to analytical procedures is likely to exceed differences between analytical procedures assuming standard laboratory procedures are followed; Buckalew et al. (2006) also find the intrinsic variability of these methods to exceed their differences.

Furthermore, Noble et al. (2003b) acknowledge that different test procedures are likely to yield different fecal coliform concentration estimates because they measure different metabolic process endpoints. Similar historic studies include a comparison between MF-derived estimates of enterococci and *E. coli* by Levin et al. (1975) and Dufour et al. (1981), a comparison between total coliform, fecal coliform, and fecal streptococci concentration estimates using MTF procedures by Sayler et al. (1975), and comparison between both MTF and MF estimates of *E. coli*, *Klebsiella*, and *Enterobacter* species by Dufour and Cabelli (1975). We know of no study, however, which attempts to explain the difference between standard MF and MTF procedures by modeling only intrinsic variability in MPN and CFU estimates.

The remaining sections of this paper include a description of fecal coliform water quality sampling and analysis procedures, followed by our approach to deriving a probabilistic model of the relationship between observed MPN and CFU estimates. We then present results of our analysis, including a comparison of our proposed theoretical probability distributions to observations from a recent NCDENR-SSS water quality study which included analysis for fecal coliform concentration using both MTF and MF procedures. We fit an ordinary least-squared (OLS) regression model to the NCDENR-SSS data and compare regression model fitted values and prediction intervals to our theoretical probability model. We conclude with a discussion of how our findings might be used to guide water resource area management agencies through transitions from one standard water quality analysis procedure to another.

2. Methods

2.1. *Water quality monitoring*

One-hundred and forty-four surface water quality samples were collected by NCDENR-SSS personnel at monitoring stations throughout the Newport River Estuary in Eastern North Carolina between May 2006 and January 2007 (NCDENR, 2007, unpublished data). As a designated shellfish harvesting area, the Newport River Estuary is governed by the National Shellfish Sanitation Program (NSSP) whose guidelines (National Shellfish Sanitation Program, 2003) require that its water quality standards be based on either MPN or CFU estimates of fecal coliform bacteria concentration. Water quality samples were therefore analyzed by NCDENR-SSS for fecal coliform concentration using both 5-tube decimal dilution MTF and MF analysis tests in accordance with both NSSP guidelines and industry standards (APHA, 2005).

2.2. *Theoretical probability model*

We derive a probabilistic model, addressing only intrinsic sources of variability, of the relationship between fecal coliform MTF and MF measurements from the same water quality sample. This model is theoretical because it assumes extrinsic sources of variability are insignificant. We begin by calculating the probability distribution of the MPN and CFU for any true fecal coliform concentration c (measured in organisms per 100 ml). We then implement a Bayesian analysis to derive the conditional distribution of the true fecal coliform concentration c for any recorded MPN or CFU estimate. Finally, we apply conditional probability distribution theory to yield the probability function of the MPN for any observed CFU estimate from the same sample. Details of the calculation procedures are included in the Appendix.

2.3. *OLS regression empirical model*

In addition to deriving a theoretical probability model, we fit a simple empirical log-scale OLS regression model to the NCDENR-SSS data (see Weisberg, 2005, pp. 21–30 for details on OLS regression). When all tubes in an MTF test are negative, the maximum likelihood estimate (and hence the MPN) of the true concentration c is zero (see Appendix, Eq. (1)). Because the logarithm of zero is not finite, our regression model excludes seven NCDENR-SSS data points with an MPN of 0 organisms per 100 ml, and (for similar reasons) two data points with a CFU of 0 organisms per 100 ml. The regression model also excludes the 19 NCDENR-SSS observations whose MF test results were recorded as "too numerous to count (TNTC)".

3. Results and discussion

In Fig. 1 we present expected values of the MPN (in panel A) and CFU (in panel B) for every 5th integer-valued true fecal coliform concentration c in the range $0 \leqslant c \leqslant 250$, including 95% prediction sets. The 95% prediction *set* is the finite collection of highest-probability values from a (perhaps multi-modal, as in the case of the MPN) discrete probability distribution whose cumulative probability is at least 0.95. While these sets are well represented as intervals for the CFU in panel B, it is clear (see panel A) that the likely MPN values vary widely and the 95% prediction sets is not well represented as an interval. The results in Fig. 1 illustrate that the wide variability of MPN results, a feature which might be misattributed to extrinsic variability, is really a simple consequence of the probability distribution for the MPN.

In Fig. 2 we present expected values of the true fecal coliform concentration, along with 95% credible intervals, for observable MPN estimates (in Panel A) and for every 5th observable CFU estimate (in Panel B). A Bayesian 95% *credible interval* contains the true fecal coliform concentration with a probability at least 0.95; see Casella and Berger (2002, pp. 436–437) or McBride (2005, pp. 208–209), where credible intervals are described in detail and contrasted with confidence intervals. Details of our Bayesian analysis are presented in the Appendix. The "observable MPN estimates"

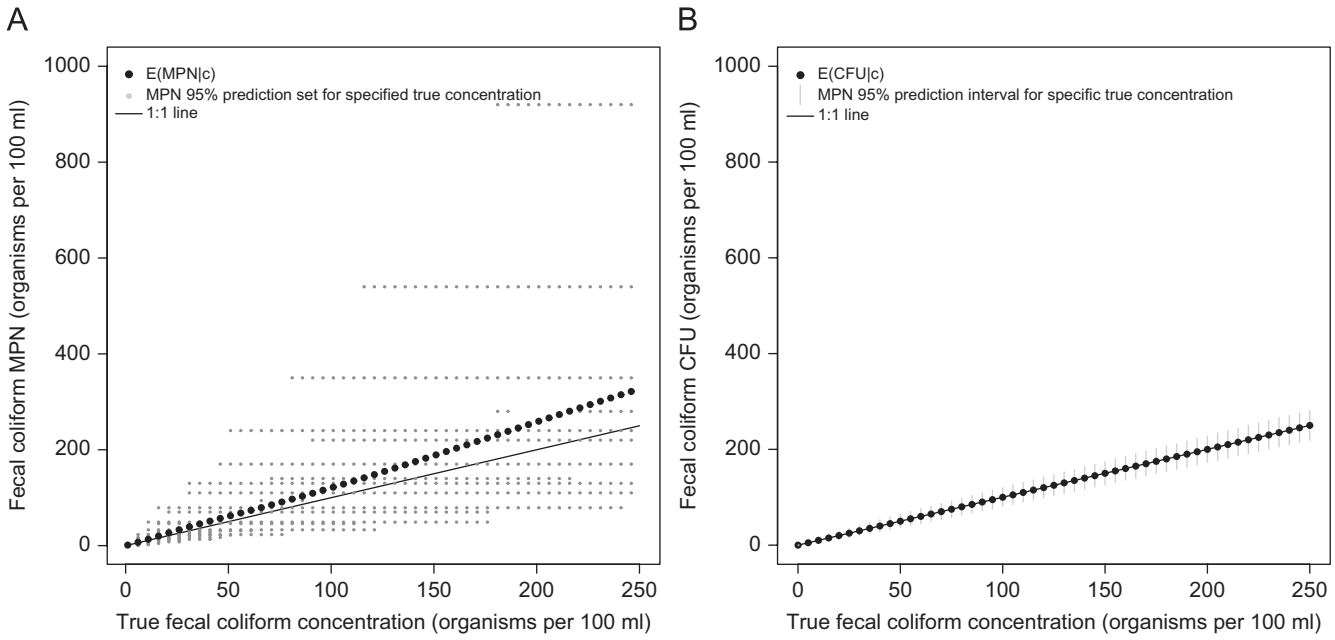

Fig. 1 – Expected values and 95% prediction sets or prediction intervals for observable fecal coliform MPN (panel A) and CFU (panel B) measurements given the true fecal coliform concentration in organisms per 100 ml. For clarity, expected values and 95% prediction sets or intervals are plotted only for every 5th integer-valued concentration c. Maximum true concentrations in each plot are based on maximum MPN and CFU observations in the NCDENR-SSS data set. CFU prediction intervals are based on an MF sample aliquot volume of 100 ml.

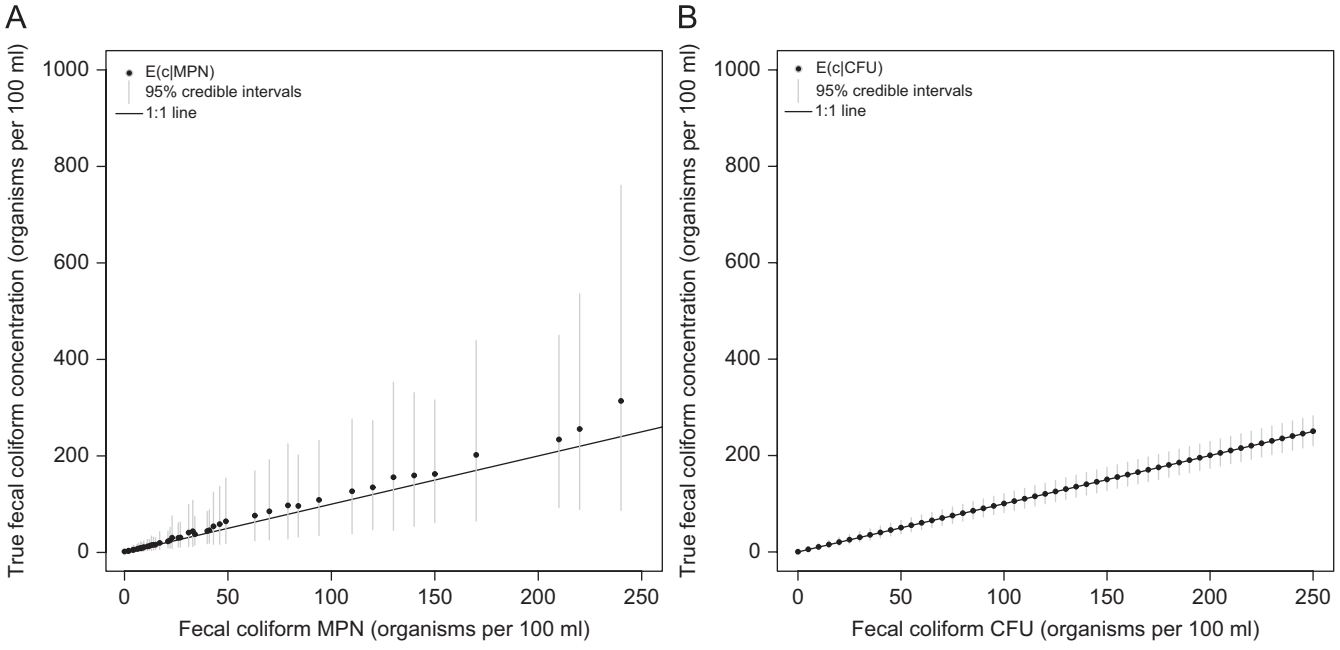

Fig. 2 – Expected value and 95% credible intervals for the fecal coliform true concentration given MPN (panel A) and CFU (panel B) estimates in organisms per 100 ml. For clarity, panel A includes only the 51 observable MPN estimates presented in standard laboratory analysis MTF conversion tables for the 5-tube serial dilution analysis procedure (see, e.g. Woodward, 1957) and panel B includes only every 5th observable CFU value based on an MF test with a sample aliquot volume of 100 ml.

are those which can possibly arise from the (NSSP standard) 5-tube fermentation serial dilution analysis (the most likely ones are presented, for example, in tables in Woodward, 1957); for a sample aliquot volume of 100 ml (per NCDENR-SSS operating protocol), the observable CFU estimates are all nonnegative integers. Lengths of credible intervals depend on the numbers of tubes used, for MPN, and on aliquot volume, for CFU (see Appendix, Eq. (5)); thus, although the confidence

intervals for the CFU method are narrower than those for the MPN method for any fixed sample volume (as suggested by the relative interval lengths in panels A and B of Fig. 2), intervals could be made narrower for either method by using more tubes (for MPN) or a greater volume (for CFU).

In Fig. 3 we present OLS regression model fitted values and theoretical probability model expected values of the MPN for CFU estimates observed in the NCDENR-SSS study. In addition, we present MPN 95% prediction intervals and prediction sets for the regression model and probabilistic model, respectively. Observations from the NCDENR-SSS study are also plotted in Fig. 3.

Prediction intervals in panel A of Fig. 3 are based on standard assumptions regarding the distribution of OLS linear regression model fitted value residuals (see Weisberg, 2005), and are presented to contrast with the true discrete multimodal distribution of the MPN presented in both panel B of Fig. 3, and in detail in Fig. 4. Fig. 4 includes the full theoretical

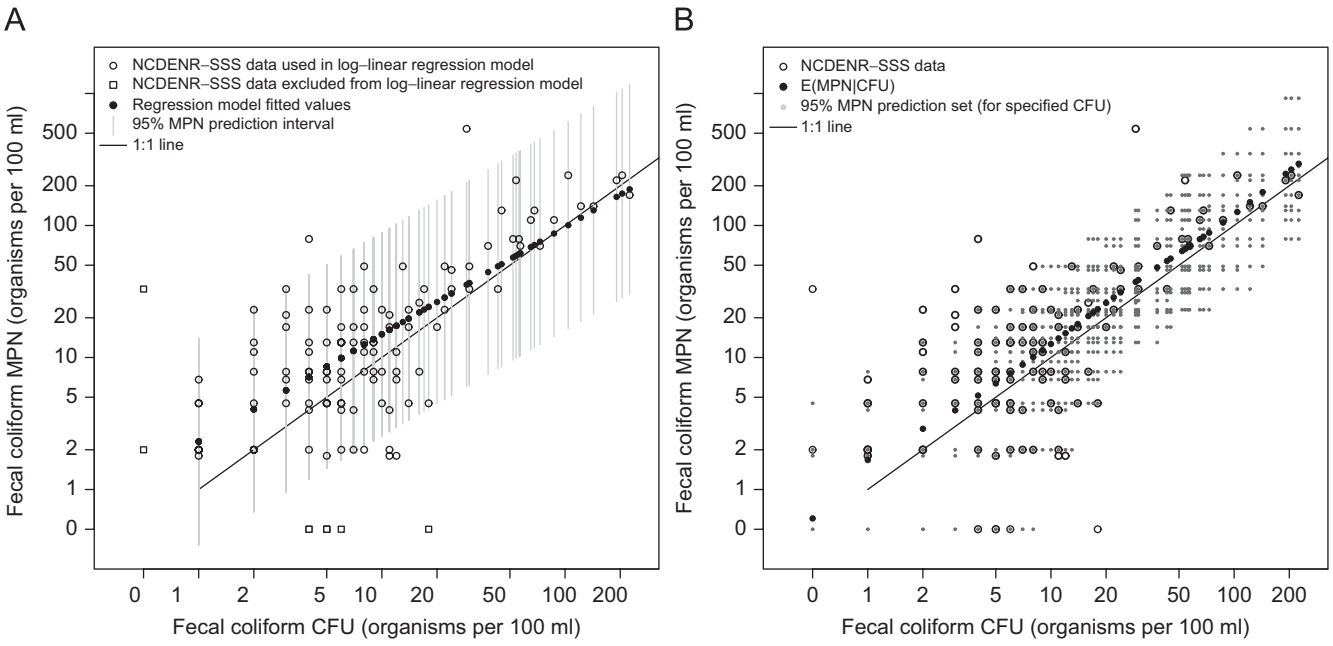

Fig. 3 – Empirical linear regression model (panel A) and theoretical probability model (panel B) of the relationship between fecal coliform MPN and CFU estimates from the same water quality sample.

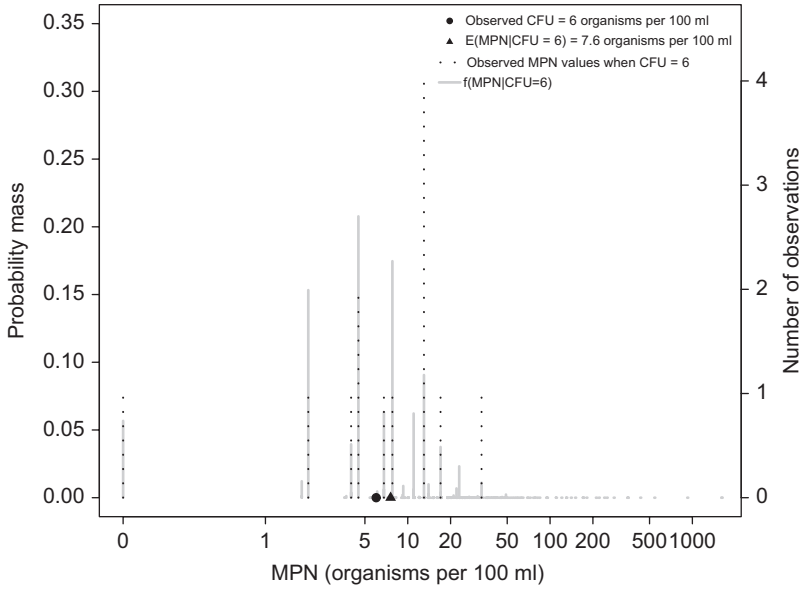

Fig. 4 – Observed values, expected values, and the theoretical probability mass function of the MPN for a CFU measurement from the same water quality sample. Observed values are from recent NCDENR-SSS study.

probability distribution of the MPN for an observed CFU value of six organisms per 100 ml along with a histogram of MPN estimates from 13 of the NCDENR-SSS water quality samples with a CFU estimate of 6 organisms per 100 ml. Figs. 3 and 4 demonstrate not only that the most likely MPN estimates for a given water quality sample are a discrete subset of non-consecutive observable MPN estimates, but also that the NCDENR-SSS observations are entirely consistent with our theoretical probability model. Furthermore, our theoretical probability model explains why the MPN is a positively-biased estimate of fecal coliform concentration (Garthright, 1993, 1997).

Despite differences between regression model fitted values (panel A of Fig. 3) and expected values from our theoretical probability model (panel B of Fig. 3), we expect empirical regression model fitted values to approach expected values of the MPN for a specific CFU as sample size increases. Differences, if any, between large-sample empirical regression model fitted values and our theoretical model expected values might suggest significant non-probabilistic (i.e. extrinsic) sources of variability. Exploring comparisons between our proposed probabilistic model and regression models fit to very large data sets is an area for future research.

4. Conclusions

We derived a theoretical model of the MPN probability distribution for any observed CFU estimate from the same water quality sample. Recent water quality samples collected and analyzed by NCDENR-SSS for fecal coliform concentration using both MTF and MF analysis tests yielded MPN and CFU estimates entirely consistent with our theoretical probabilistic model. Our results indicate that MPN and CFU intra-sample variability does not stem from human error or laboratory procedure variability, but is instead a simple consequence of the probabilistic basis for calculating the MPN.

We anticipate this study will serve as a stepping stone towards future research on whether different fecal coliform analysis procedures might lead to different water quality standard violation frequencies for the same water body. Method-dependent differences, if any, might propagate into coastal resource water management decisions through two undesirable pathways. First, analysis of water quality samples from a coastal resource water might, depending on the analysis procedure used, result in different management actions (such as closing or opening a shellfish harvesting area). Second, if fecal coliform concentration estimates vary depending on whether MTF or MF procedures are used, potential benefits of merging historic MPN and new CFU data sets would be limited (Noble et al., 2003b). Future research on the probabilistic basis for current water quality standard violations, coupled with the modeling tools presented in this paper, could provide answers to these research questions.

Other suggested studies stemming from our research include, but are not limited to, quantifying membrane filtration-related fecal coliform thinning and contamination rates, exploring environmental effects on fecal coliform concentration estimate bias, and determining how measuring different coliform bacteria metabolic output effects fecal coliform concentration estimates.

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Appendix

Assuming fecal coliform organisms at concentration c (in organisms per 100 ml) are well mixed in a water sample, it is commonly assumed that aliquots of volume v_i ml from the water sample contain a Poisson $\mathrm{Po}(cv_i/100)$ distributed number of fecal coliform organisms (McCrady, 1915; Greenwood and Yule, 1917; Deman, 1977; Russek and Colwell, 1983; Best and Rayner, 1985; Woomer et al., 1990; Briones and Reichardt, 1999). Out of n_i serial dilution analysis tubes, the numbers of positive tubes x_i are independent binomial $\mathrm{Bi}(n_i, p_i)$ random variables with $p_i = 1 - \exp(-cv_i/100)$ (for more on using Poisson and binomial distributions in environmental data analysis, see Ott, 1995, pp. 93–113 and 127–137). The MPN for m dilution series can therefore be expressed as

$$\mathrm{MPN} = \underset{c}{\operatorname{argmax}}\left[\prod_{i=1}^{m}(1-\mathrm{e}^{-cv_i/100})^{x_i}(\mathrm{e}^{-cv_i/100})^{n_i-x_i}\right] \tag{1}$$

and the conditional probability distribution of positive tube counts $X = \{x_i\}$, given true fecal coliform concentration c, is:

$$f(x|c) = \prod_{i=1}^{m}\binom{n_i}{x_i}[1-\mathrm{e}^{-cv_i/100}]^{x_i}[\mathrm{e}^{-cv_i/100}]^{n_i-x_i} \tag{2}$$

The Poisson-distributed CFU observation $Y \sim \mathrm{Po}(\lambda)$ with mean $\lambda = cV/100$ for sample aliquot volume V ml has conditional probability distribution, given true fecal coliform concentration c, given by

$$f(y|c) = \frac{1}{y!}(cV/100)^y \mathrm{e}^{-cV/100} \quad \text{for } y \in 0, 1, 2, \ldots \tag{3}$$

The posterior probability distribution of the true fecal coliform concentration c for an observed tube count combination x, using Jeffreys' scale-invariant "reference" prior distribution $\pi(c) \propto 1/\sqrt{c}$ (Jeffreys, 1946; Bernardo and Ramon, 1998), is given by

$$f(c|x) \propto c^{-1/2}\mathrm{e}^{-(c/100)\sum_{i=1}^{m} v_i(n_i-x_i)}\prod_{i=1}^{m}(1-\mathrm{e}^{-cv_i/100})^{x_i}, \quad c>0 \tag{4}$$

Using the same Jeffreys' prior distribution, the posterior distribution of c for a given CFU observation y is:

$$f(c|y) \propto c^{y-1/2}e^{-cV/100}, \quad c>0 \tag{5}$$

which we recognize as a Gamma $Ga(\alpha,\lambda)$ distribution with shape parameter $\alpha = y + 1/2$ and rate parameter $\lambda = V/100$.

Finally we calculate the probability distribution of the positive tube count vector $x = (x_1,\ldots,x_m)$, $1 \leqslant x_i \leqslant n_i$ for any CFU observation y, $\mathbb{P}[X = x|Y = y]$, by combining Eqs. (2) and (5):

$$\begin{aligned} f(x|y) &= \int_0^\infty f(x|c)f(c|y)\,dc \\ &= \frac{(V/100)^{y+1/2}}{\Gamma(y+1/2)}\int_0^\infty c^{y-1/2}e^{-(c/100)[V+\sum_{i=1}^m v_i(n_i-x_i)]}\prod_{i=1}^m\binom{n_i}{x_i}(1-e^{-cv_i/100})^{x_i}\,dc \end{aligned} \tag{6}$$

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