

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/23147268>

An Assessment of Fecal Indicator Bacteria-Based Water Quality Standards

ARTICLE in ENVIRONMENTAL SCIENCE AND TECHNOLOGY · AUGUST 2008

Impact Factor: 5.33 · DOI: 10.1021/es703144k · Source: PubMed

CITATIONS

22

READS

60

4 AUTHORS:



[Andrew D. Gronewold](#)

National Oceanic and Atmospheric Admini...

42 PUBLICATIONS 230 CITATIONS

SEE PROFILE



[Mark E. Borsuk](#)

Dartmouth College

92 PUBLICATIONS 2,403 CITATIONS

SEE PROFILE



[Robert L. Wolpert](#)

Duke University

122 PUBLICATIONS 1,828 CITATIONS

SEE PROFILE



[Kenneth H Reckhow](#)

Duke University

140 PUBLICATIONS 3,219 CITATIONS

SEE PROFILE

An Assessment of Fecal Indicator Bacteria-Based Water Quality Standards

ANDREW D. GRONEWOLD,^{*,†}
MARK E. BORSUK,[‡]
ROBERT L. WOLPERT,^{†,§} AND
KENNETH H. RECKHOW[†]

Nicholas School of the Environment, Duke University,
Durham, North Carolina 27708, Thayer School of
Engineering, Dartmouth College, Hanover, New Hampshire
03755, and Department of Statistical Science, Duke
University, Durham, North Carolina, 27708

Received December 16, 2007. Revised manuscript received
April 1, 2008. Accepted April 4, 2008.

Fecal indicator bacteria (FIB) are commonly used to assess the threat of pathogen contamination in coastal and inland waters. Unlike most measures of pollutant levels however, FIB concentration metrics, such as most probable number (MPN) and colony-forming units (CFU), are not direct measures of the true *in situ* concentration distribution. Therefore, there is the potential for inconsistencies among model and sample-based water quality assessments, such as those used in the Total Maximum Daily Load (TMDL) program. To address this problem, we present an innovative approach to assessing pathogen contamination based on water quality standards that impose limits on parameters of the actual underlying FIB concentration distribution, rather than on MPN or CFU values. Such concentration-based standards link more explicitly to human health considerations, are independent of the analytical procedures employed, and are consistent with the outcomes of most predictive water quality models. We demonstrate how compliance with concentration-based standards can be inferred from traditional MPN values using a Bayesian inference procedure. This methodology, applicable to a wide range of FIB-based water quality assessments, is illustrated here using fecal coliform data from shellfish harvesting waters in the Newport River Estuary, North Carolina. Results indicate that areas determined to be compliant according to the current methods-based standards may actually have an unacceptably high probability of being in violation of concentration-based standards.

1. Introduction

Section 303(d) of the United States Clean Water Act requires that states assess the condition of surface waters and report those which fail to meet ambient water quality standards (1, 2). These are added to the United States Environmental Protection Agency (USEPA) list of impaired waters (3) and can only be removed after the performance of a Total Maximum Daily Load (TMDL) assessment (4, 5) followed by sample-based verification that the standards are being met. The primary objective of a TMDL assessment is to determine

the maximum allowable pollutant load from point, nonpoint, and natural sources, including a margin of safety (MOS), which can be discharged into a receiving water without violating water quality standards (2, 4). Such predictive assessments are usually based on an empirical or mechanistic water quality model relating pollutant loading levels to water body concentrations (6, 7).

The latest official assessment of U.S. water quality data (8) indicates that pathogens are the leading cause of coastal shoreline standard violations (275 total miles impaired) and the second leading cause of violations in rivers and streams (82,100 total miles impaired). Fecal indicator bacteria (FIB), such as fecal coliform, are commonly used to assess potential pathogen contamination in coastal waters, and concentration estimates are based on indirect measures of microbial density. These are typically reported as either the most probable number (MPN) or the number of colony-forming units (CFU) per unit volume. As a result, MPN- and CFU-based estimates of FIB form the basis of numeric water quality standards for several designated uses including drinking water supply (9), recreational use (10), and shellfish harvesting (11).

Unlike most measures of pollutant levels, the probability distributions of MPN and CFU do not directly correspond to the true *in situ* concentration distribution. The true FIB concentration at a fixed point over time is typically believed to have a continuous unimodal (often log-normal) probability distribution (12, 13). The probability distributions of corresponding MPN and CFU values for the same monitoring location, however, are discrete and, in the case of the MPN, often multimodal. In addition, MPN and CFU variability depends on the number and volume of sample aliquots.

Accurately predicting MPN- or CFU-based standard violations from FIB loading levels should therefore involve explicit acknowledgment of how a model-predicted receiving water concentration translates into an MPN or CFU value, and then how frequently those MPN and CFU values violate standards (5, 14). A review of current modeling practice, however, indicates that such an approach is seldom, if ever, implemented.

We present a three-part approach to assessing water body impairment from model-based predictions of FIB concentration, using MPN- and CFU-based fecal coliform standards in shellfish harvesting waters as an example. First, we simulate the frequency with which a model-generated distribution of the true FIB concentration c yields MPN and CFU values that violate shellfish harvesting area water quality standards. Second, we identify the distributional parameters of c expected to lead to water quality standard violations no more than a specified percentage of the time (e.g., 10%). We propose that these parameters be used as the basis for FIB-based standards because they do not depend on the analytical method employed, are more closely related to the true water body concentration, and are consistent with the outcomes of most water quality models. In the third part, we demonstrate how compliance with such concentration-based standards can be inferred from traditional MPN and CFU values using a Bayesian approach. We demonstrate this procedure using data from the most recent assessment of shellfish harvesting waters in the Newport River Estuary of Eastern North Carolina.

1.1. Background on Current FIB-Based Water Quality Standards. Model Ordinance in the Guide for the Control of Molluscan Shellfish, prepared by the National Shellfish Sanitation Program (NSSP), includes recommended water quality criteria for shellfish-growing waters based on numeric limits on MPN- and CFU-based measures of fecal coliform

* Corresponding author e-mail: adg12@duke.edu.

[†] Nicholas School of the Environment, Duke University.

[‡] Thayer School of Engineering, Dartmouth College.

[§] Department of Statistical Science, Duke University.

TABLE 1. NSSP Shellfish Harvesting Area Fecal Coliform Water Quality Standards Based on a Minimum of 30 Randomly Collected Samples

basis for standard	standard		
	q_{50}	μ_{geo}	q_{90}
n MPN observations from 5-tube MTF procedure	14	14	43
n CFU observations from MF procedure	14	14	31

concentration (11). States which participate in the NSSP, and which are also members of the Interstate Shellfish Sanitation Conference, enforce the Model Ordinance as a minimum requirement for sanitary control of shellfish (11). While similar FIB-based water quality standards are enforced in surface waters with other designated uses, such as recreational use (10) and drinking water supply (9), we illustrate our proposed methodology using NSSP water quality standards as an example.

While several MPN- and CFU-based FIB analysis procedures are in common use (for a wide range of water body designated uses), the three cited in NSSP-recommended standards for shellfish growing waters (see ref (11), Chapter 2, Subsection IV.02) are the 5-tube multiple tube fermentation (MTF), the 3-tube MTF, and the membrane filtration (MF) procedure for fecal coliform bacteria. Here, we explore only the 5-tube MTF and the MF procedures because they are far more common than the 3-tube MTF procedure. The standard 5-tube MTF procedure involves diluting a water quality sample into 3 sets of 5 tubes, with each set of 5 containing either 10 mL, 1 mL, or 0.1 mL of the original sample (alternative dilutions may be used depending on the expected concentration of the sample (15)). The MPN is then the maximum likelihood estimate (MLE) of the true fecal coliform concentration based on the number of positive tubes observed in each dilution series after a period of incubation (15–18). A positive tube is one containing visible gas, which serves as an indication of bacterial lactose fermentation. The MF procedure involves filtering a water quality sample and counting the number of bacteria colonies emerging from the filter on a growth plate after a period of incubation. The number of colonies is divided by the sample aliquot volume and reported as the CFU (19–23).

MPN and CFU values are intrinsically variable and subject to additional uncertainty arising from minor variations in experimental protocol. NSSP criteria address MPN and CFU variability by recommending that a water body assessment be based on a minimum number n of MPN or CFU values, and by recommending separate numeric standards for the median, geometric mean, and 90th percentile of those values (Table 1). For example, the NSSP criteria for MPN values from a standard 5-tube MTF decimal dilution analysis state that a growing area is classified as “Approved” if a “minimum of 30 of the most recent randomly collected samples” have an MPN median and geometric mean no greater than 14 organisms per 100 mL, and an estimated MPN 90th percentile no greater than 43 organisms per 100 mL (11). Ninetieth percentiles are calculated from a log-normal distribution parametrized by the mean and variance of the natural logarithm of the n MPN or CFU values (11).

A common goal of model-based TMDL assessments is to evaluate pollution loading mitigation strategies which will result in compliance with water quality standards. Despite a growing body of research on the role of models and model uncertainty in water resources management (7, 14, 24), we know of no water quality models which explicitly acknowledge how natural variability in FIB concentrations, combined with intrinsic analytic uncertainty, propagates into MPN- or

CFU-based water quality standard violations and associated management decisions. The following section describes our attempt to fill this gap.

2. Methods

Most water quality standards, including those based on FIB concentrations, imply that long-term pollutant concentrations at a monitoring station are adequately characterized by an assumed stationary log-normal probability distribution (6, 13). The 30 fecal coliform samples required by NSSP guidelines, however, are usually collected at bimonthly intervals, implying that shellfish harvesting area water quality assessments may take 4–5 years to complete. Changes in fecal coliform sources and delivery routes during that time are likely to translate into changes in the fecal coliform concentration distribution. Here, we assume a constant fecal coliform concentration probability distribution at a monitoring station throughout the assessment period. While we recognize this is a potentially restrictive assumption, it is consistent with current management practice and allows our results to be easily compared with recommended water quality criteria. Developing strategies for assessing a water body based on a changing pollutant concentration probability distribution is an area for additional research (25).

We also assume that water quality samples are either simulated (as in the case of a model forecast) or collected based on a systematic random sampling program. In other words, we assume that the 30 samples (as required by NSSP guidelines) are collected under environmental conditions (such as rainfall and tide, for example) indicative of long-term distributions. Furthermore, we presume that the NSSP criteria for an “Approved” classification implies compliance with shellfish harvesting area water quality standards in NSSP guidelines, Chapter 2, subsection 02F (“Standards for approved classification of growing areas affected by non-point sources”) (11).

2.1. Translation of Model-Predicted Concentrations to MPN- and CFU-Based Standard Compliance. As a starting point, we assume that uncertainty in a water quality model or variability in the environment leads to a probability distribution on the fecal coliform concentration c (in organisms per 100 mL) that is log-normal, $LN(\mu_c, \sigma_c)$, where μ_c and σ_c are the mean and standard deviation, respectively, of the natural logarithm of c . Therefore, we began our analysis by simulating 300,000 concentrations drawn at random from this distribution. We then randomly grouped the 300,000 simulations into 10,000 “samples” of size 30. We repeated the simulation for values of μ_c evenly spaced between 0 and 3.5 in $\log(\text{organisms per 100 mL})$ (at intervals of 0.05), and values of σ_c evenly spaced between 0 and 3.0 in $\log(\text{organisms per 100 mL})$ (at intervals of 0.1), and all combinations thereof. The ranges of values for μ_c and σ_c were selected based on a preliminary analysis of MPN values from the Newport River Estuary. For each of the 300,000 fecal coliform concentration values, we randomly drew an MPN and a CFU value from the conditional sampling distribution. For the MPN values, we used a Poisson/binomial probability model (12, 15, 16, 26–31) written as

$$p_i = 1 - e^{-cv/100} \quad (1)$$

$$MPN = \underset{c}{\operatorname{argmax}} \left[\prod_{i=1}^d p_i^{x_i} (1 - p_i)^{m_i - x_i} \right] \quad (2)$$

where p_i = probability of a positive tube in dilution series i , c = fecal coliform concentration (in organisms per 100 mL), v_i = volume of original sample in dilution series i (mL), d = number of dilution series (often 3), x_i = number of positive tubes in dilution series i ($i \in [1, \dots, d]$), and m_i = number of tubes in dilution series i (often 5).

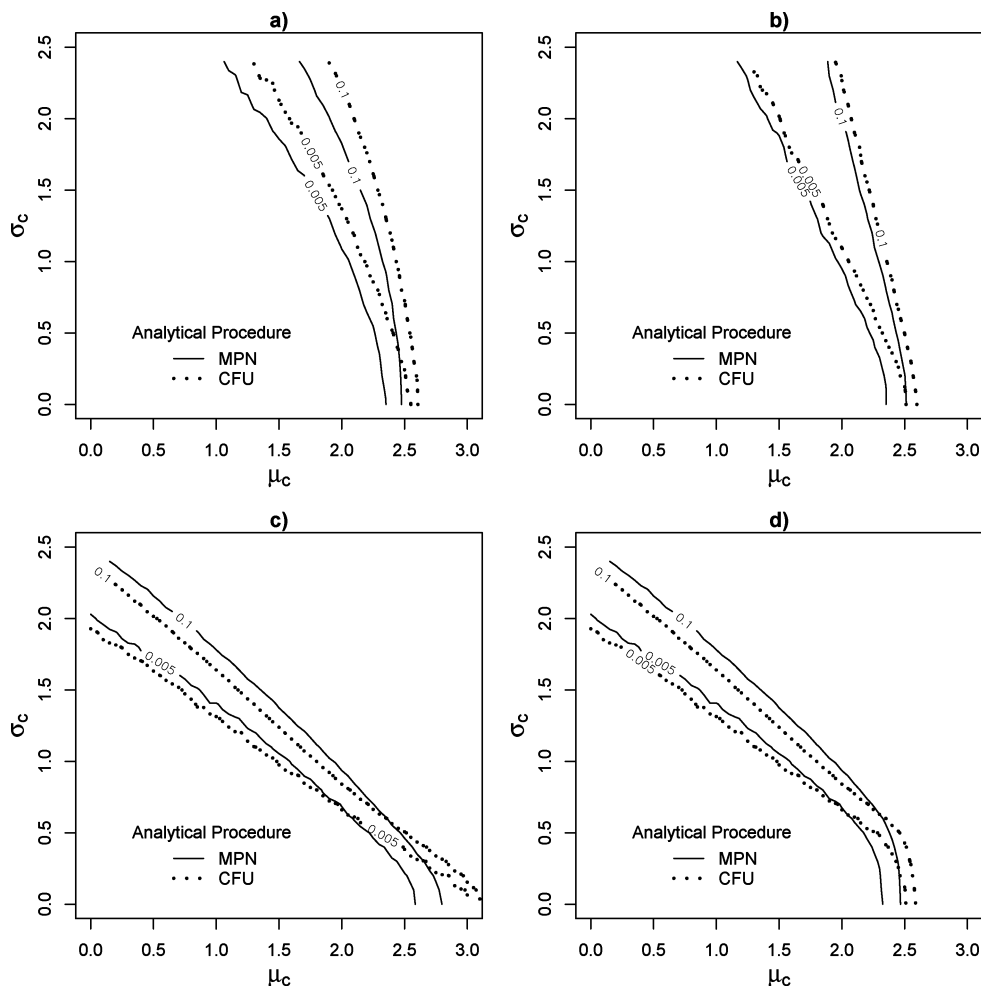


FIGURE 1. Combinations of the mean μ_c and standard deviation σ_c of the log-transformed fecal coliform concentration distribution which yielded MPN (solid lines) or CFU (dotted lines) samples in violation of the NSSP median standard (panel a), geometric mean standard (panel b), 90th percentile standard (panel c), or any standard (panel d) with a frequency of either 0.005 or 0.1. The zone of violations is in the upper right of each panel.

CFU values were generated using a Poisson $Po(\lambda)$ probability model (32):

$$\lambda = cV/100$$

$$y\lambda \sim Po(y\lambda) = \lambda^y e^{-\lambda} / y! \quad (3)$$

$$CFU = 100y/V \quad (4)$$

where y = number of identifiable colony forming units, and V = sample aliquot volume.

Previous authors have suggested that the Poisson distribution underestimates the variability of microorganisms in both natural waters and sample aliquots and recommend using the negative binomial distribution instead (32–37). However, the Poisson probabilities in our eqs 1 and 3 represent only the conditional probabilities of positive tubes and CFU values given the true fecal coliform concentration c . Because we also assume a log-normal distribution on c , the marginal distributions of positive tubes and CFU values are very similar to the negative binomial. It is possible, however, that the conditional distributions represented by eqs 1 and 3 may also be more widely dispersed than Poisson due to departures in laboratory procedure from standard protocol, clumping of bacteria cells, and similar issues (34, 38). We explored probability models more dispersed than the Poisson and found that our analysis would only change significantly if the methodological variability (e.g., measurement error, cell damage, filter bypass) were 33% greater than that described by the Poisson distribution (see Supporting

Information for details). We believe that variability of this magnitude is highly unlikely when standard laboratory procedures are carefully followed.

For each (μ_c, σ_c) pair, we recorded the proportion of the 10,000 simulated size-30 samples which violated the geometric mean standard, the median standard, the 90th percentile standard, or any of the three standards for both MPN and CFU values (see Table 1). Simulations and calculations were performed using the statistics and graphics software program R (39).

2.2. Determination of Concentration-Based Standards.

Guidelines prepared by USEPA for assessing water quality impairment suggest that a waterbody does not support its designated use if 10% of samples violate associated designated use criteria (40, 41). NSSP guidelines for shellfish harvesting waters address this provision by setting numeric limits on statistics of a set of at least 30 consecutive water quality samples. According to NSSP guidelines, any size-30 sample which violates any of the standards (i.e., geometric mean, median, or 90th percentile) indicates impairment. Given our probabilistic framework, however, it is effectively impossible to obtain a predicted fecal coliform concentration distribution that will violate standards with zero probability. We therefore identified (μ_c, σ_c) pairs that would violate standards no more than 0.5% of the time. We also identified those pairs violating standards no more than 10% of the time for purposes of comparison.

To formulate our results in the form of a more readily implementable regulatory standard, we statistically estimated the equation of the line in (μ_c, σ_c) space that divides violating from nonviolating concentration distributions. This was done by fitting a regression model to points on the violation boundary (see Supporting Information for details).

2.3. Translation of MPN and CFU Values to Concentration-Based Standard Compliance. Assessing compliance with concentration-based standards from traditional MPN and CFU observations requires “reversing” the logic of the probabilistic expressions given in eqs 2, 3, and 4 through a process of Bayesian inference (42, 43). This involves using these equations, together with the log-normal distribution on c , as the likelihood functions for μ_c and σ_c and then multiplying these likelihoods by prior distributions on μ_c and σ_c . Following approaches of refs 44–47, we use diffuse prior distributions for μ_c and σ_c in the model of J independent observations of the concentration c at a given monitoring station:

$$\begin{aligned} c_j | \mu_c, \sigma_c &\stackrel{iid}{\sim} \text{LN}(c_j | \mu_c, \sigma_c), j = 1, \dots, J \\ \mu_c &\sim \text{Normal}(\mu_0 = 0, \sigma_c = 1\,000\,000) \\ \sigma_c &\sim \text{Uniform}(\text{lower} = 0, \text{upper} = 100) \end{aligned} \quad (5)$$

We then implemented a Markov-chain Monte Carlo (MCMC) procedure to generate samples from the joint (μ_c, σ_c) posterior distribution using the Bayesian software package WinBUGS (48). Posterior distributions used in our analysis are based on 1000 MCMC samples collected after convergence, as indicated by a potential scale reduction factor $\hat{R} = 1.0$ (44).

Although we used very diffuse priors in this analysis, the Bayesian approach is still preferable to a frequentist method because it yields a full posterior distribution for the distributional parameters, rather than mere point estimates. This allows us to express our results in terms of both “confidence of compliance” (6, 31) and in terms of the posterior probability of a sample of size n revealing a violation. We define confidence of compliance (CC) in this case as the posterior probability that the actual values of μ_c and σ_c are below our proposed violation boundaries. We estimated CC by integrating the joint (μ_c, σ_c) posterior probability density function over all possible μ_c and σ_c values that fall below these boundaries. The posterior probability of a sample revealing a violation is estimated by using MCMC samples from the full joint (μ_c, σ_c) posterior distribution to generate samples of fecal coliform concentration from the log-normal distribution. These concentrations are then propagated through eqs 1 and 2 to generate size-30 sample sets of MPN values, from which violation probabilities can be calculated.

2.4. Application to the Newport River Estuary. We applied our Bayesian inference procedure and concentration-based (μ_c, σ_c) standards to water quality monitoring data collected from the Newport River Estuary, NC, during the most recent assessment period (2000–2005). The NC Department of Environment and Natural Resources, Shellfish Sanitation and Recreational Water Quality Section (NCDENRSS) collects ambient fecal coliform samples from this estuary (and other shellfish harvesting waters throughout North Carolina) at roughly bimonthly intervals and analyzes each sample using the 5-tube MTF procedure. The Newport River Estuary is historically a productive shellfish harvesting area. However, all of its segments and tributaries are either permanently or conditionally closed to shellfishing based on poor water quality or proximity to known or potential sources of fecal contamination. As a result, the estuary and its tributaries comprise 28 of the designated shellfish harvesting areas in North Carolina which are currently included in the USEPA 303(d) list and therefore require a TMDL assessment. Our goal in analyzing these data is to determine whether our concentration-based approach would

have yielded a different impairment decision than that obtained using the MPN-based standard, and therefore whether water quality data provide quantitative support for including the Newport River (and its tributaries) on the 303(d) list.

3. Results

3.1. Data Simulations. Simulation results indicate that (for a given μ_c and σ_c) samples will violate NSSP water quality standards at different frequencies depending on whether they are assessed using the MPN-based standard or the CFU-based standard (Figure 1). Additionally, the relative stringency of the two standards will depend on the value of σ_c . Specifically, application of the 5-tube MTF procedure (and associated MPN standard) to a particular sample is less likely to lead to a violation than application of the MF procedure (and CFU standard) when σ_c is greater than 0.65, and more likely to lead to a violation when σ_c is less than 0.65 (Figure 1d). Results also indicate that samples with values in the range of those collected at the Newport River shellfish harvesting area monitoring stations are more likely to be in violation of the NSSP 90th percentile standards (Figure 1c) than either the median or geometric mean standards (Figure 1a and b).

3.2. Concentration-Based Standards. Our regression-based estimation of the lines that divide violating from nonviolating values in the joint (μ_c, σ_c) space reveals that “compliant” values of μ_c and σ_c (i.e., those expected to violate any of the three MPN or CFU-based standards no more than 0.5% of the time) are defined by the following inequalities for the MPN procedure:

$$\mu_c \leq \begin{cases} \frac{2.65 - \sigma_c^{1.39}}{1.04} & \text{for } \sigma_c > 0.65 \\ \frac{2.44 - \sigma_c^{2.61}}{1.05} & \text{for } \sigma_c \leq 0.65 \end{cases}$$

and by the following inequalities for the CFU procedure:

$$\mu_c \leq \begin{cases} \frac{1.98 - \sigma_c^{1.03}}{0.66} & \text{for } \sigma_c > 0.65 \\ \frac{1.65 - \sigma_c^{2.35}}{0.66} & \text{for } \sigma_c \leq 0.65 \end{cases}$$

Thus, with point estimates of μ_c and σ_c determined for a particular site from either a simulation model or sample-based inference procedure, the frequency-based compliance status of that site can be established in a straightforward way. Of course, when there is uncertainty about μ_c and σ_c , then, rather than a binary compliance determination, a CC is the result, as demonstrated in the next section.

3.3. Newport River Estuary Assessment. Application of our concentration-based assessment to the MPN data of the Newport River Estuary resulted in eight stations having an assessed CC less than 1%, whether employing the NSSP MPN or CFU standard (Table 2). Samples collected between 2000 and 2005 actually revealed MPN standard violations at all eight of these stations. While violations were not found at any of the other stations during this period, our Bayesian inference procedure indicates a low CC with the MPN standard (less than 25%) at four other stations as well (Stns. 8, 8A, 25, and 84). This reflects the stringency of the NSSP guideline that no violations, ever, will be tolerated (which we operationalized as a violation frequency of less than 0.5%). Thus, a station such as station 25, which did not actually reveal a violation during the 2000–2005 assessment period, still has a pattern of MPN values that indicate it would be highly unlikely (3% probability) to comply with the standard if enough samples were collected. On the other hand, we can

TABLE 2. Estimated Confidence of Compliance (CC), Posterior Probability of Violating any MPN Standard, and Observed Violations for Monitoring Stations in the Newport River Estuary during the 2000–2005 Assessment Period

station	CC (%)		posterior probability of size-30 sample violating any MPN standard	violated any MPN standard during the 2000–2005 assessment period?
	MPN	CFU		
3	52	39	5	no
4	44	33	6	no
4A	<1	<1	53	yes
4B	<1	<1	85	yes
5A	<1	<1	80	yes
7	<1	<1	55	yes
8	14	9	18	no
8A	15	12	15	no
9	93	89	<1	no
10	100	100	<1	no
11	53	41	5	no
14A	51	40	6	no
16A	32	20	12	no
18	62	50	3	no
24	80	71	1	no
25	3	2	32	no
27A	<1	<1	49	yes
28	96	93	<1	no
29	<1	<1	58	yes
35	80	73	1	no
41	<1	<1	89	yes
41A	<1	<1	72	yes
55	60	49	4	no
56	78	67	1	no
83	47	35	5	no
84	13	6	17	no
85	94	91	<1	no
86	87	81	1	no

be fairly confident that stations with a high CC will not exhibit a violation, even with the collection of many more samples.

The marginal probability that a water quality sample of size 30 (the minimum required by NSSP) will reveal an MPN violation at a particular location can be assessed by applying (i) the posterior distribution of μ_c and σ_c describing knowledge of uncertainty about the concentration at that location, (ii) the log-normal distribution of c capturing environmental variability, and (iii) the Poisson/binomial distribution representing MPN sampling variability. Results (Table 2) indicate that, indeed, those stations with a roughly 50% or greater assessed (posterior) probability of exhibiting a violation actually did violate at least one of the three NSSP standards (Table 1) during the 2000–2005 assessment period. All of the remaining stations have a probability of violation less than 33%, and samples from these stations did not show a violation.

4. Discussion

Given the inherent differences between actual *in situ* FIB concentrations and results of laboratory analyses, forecasting water quality standard compliance in response to TMDL levels should involve a two-step process. First, water body FIB concentrations are predicted from changes in loading, presumably using a water quality model. Then, these concentrations are translated into laboratory results corresponding to a particular method and compared to relevant standards. Despite the relative transparency of this approach, nearly all models published as decision support tools for FIB and pathogen TMDL assessment treat the predicted *in situ* concentration as if it were equivalent to the corresponding laboratory result (49–51). Thus, an important source of uncertainty and potential bias in model predictions is ignored.

In an effort to address the above problem, we have outlined the probabilistic expressions required to translate FIB concentrations into either MPN- or CFU-equivalent measurements. Using simulations, we found that the existing NSSP fecal coliform standards corresponding to these two methods are inconsistent, meaning that a given distribution of actual water body concentrations may be found to be in violation of the CFU-based standard, but not the MPN-based standard. This is clearly an undesirable situation.

Rather than recommend a revision to the numeric limits of the existing MPN- and CFU-based standards, we suggest that FIB standards be based on the actual *in situ* concentration. The various laboratory analysis techniques would then be explicitly understood to be imperfect measures of this concentration, each with their own uncertainties. This would have a number of important benefits. First, and foremost, such standards would have an explicit connection to the protection of human health. This is because impairment decisions would be based on our best understanding of actual conditions in the water body, rather than on the uncertain outcome of a laboratory analysis.

Second, a focus on *in situ* concentrations would lead to a single set of universal standards which are independent of the analytical procedure used. We demonstrated how the distribution of the actual fecal coliform concentration in shellfish harvesting waters can be inferred from MPN or CFU measurements using a Bayesian approach. Similar inference could be performed on any laboratory method, for any designated use category, by incorporating method-specific measures of uncertainty and designated use-specific standards of quality. In fact, by estimating the “second-order” uncertainty in the concentration distribution itself, in the form of uncertainty in μ_c and σ_c (see Figure 2), the Bayesian approach provides an incentive for states to employ or develop more precise laboratory analysis methods. Methods that have a lower measurement error will lead to a greater “confidence of compliance” at a nonviolating site than methods that are more uncertain.

Finally, a concentration-based standard would encourage a more consistent and accurate treatment of FIB concentration data in models forming the basis for TMDL determinations. In addition to precluding the need for modelers to probabilistically translate their predictions into units of MPN or CFU, a focus on concentration would facilitate proper loading specification and model calibration. Currently, FIB loading rates in models are often expressed in units of MPN or CFU per unit volume (52). However, the discrepancy between MPN- and CFU-based determinations of FIB concentration coming from a particular loading source and the true FIB contribution of that source suggest that such an approach is inappropriate. In fact, using MPN or CFU values as FIB loading rates will lead to model predictions that represent neither the true FIB concentration nor MPN- or CFU-based estimates of the true concentration (53). Similarly, using MPN or CFU determinations to calibrate a FIB water quality model does not imply that the end points of the model are correct MPN or CFU values. The concentration-based perspective adopted here provides a potential solution to these common, but flawed, FIB water quality modeling practices.

Our results show that a new concentration-based FIB standard can be conveniently expressed in terms of the mean, μ_c , and standard deviation, σ_c , of the log-transformed concentration distribution. We gave examples of such a standard that are compatible with the stringency imposed by the present MPN or CFU standards. Of course, rather than arbitrarily deciding to match one or the other of these method-based standards, it would be more logical for the NSSP to select an *in situ* concentration threshold that should not, in theory, be exceeded more than a specified

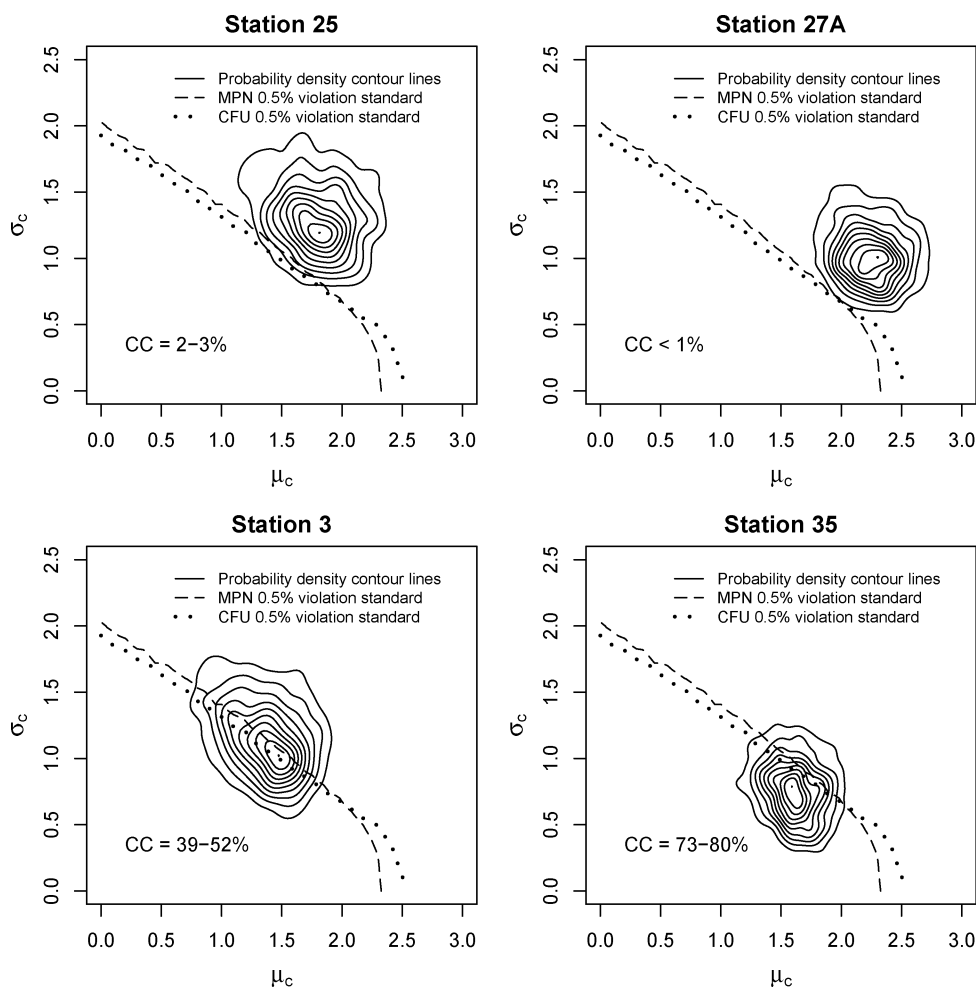


FIGURE 2. Joint posterior probability density contour lines (solid lines) for four monitoring stations in the Newport River Estuary. Dashed and dotted lines indicate combinations of the mean μ_c and standard deviation σ_c of the log-transformed fecal coliform concentration distribution which violate our concentration-based standards no more than 0.5% of the time using MPN or CFU standards as the reference. Confidences of compliance (CC) are given in the lower left of each panel for both MPN- and CFU-based standards.

amount of time. Any chosen sampling plan and laboratory analysis technique would then provide an estimate of this exceedance frequency, together with the uncertainty in this estimate expressed as the “confidence of compliance.” NSSP would therefore also need to specify the level of confidence required to avoid shellfish harvesting area closure. Our results for the Newport River Estuary indicate that requiring a CC of 50% or greater would not be unreasonable. All stations with a CC greater than or equal 50% according to the MPN standard were determined to be in compliance according to samples collected during the 2000–2005 assessment period. A CC threshold also protects against possible inconsistencies in the current standards under which a water body may have an unacceptably high (e.g., greater than 10%) probability of violating existing NSSP standards yet, because of the inherent limitations in using only 30 samples, yield a compliant water sample set (e.g., stations 8, 8A, 16A, 25, and 84).

Water quality models used for TMDL assessment should also be held to CC standards, though perhaps not as stringent as those applied to monitoring programs. An approach to making CC determinations based on model-based parameter uncertainty has been presented previously (6), and is consistent with the approach taken here. This allows for straightforward translation between concentration-based standards, water quality analysis techniques, and model

predictions, accounting for the variability and uncertainty characteristics of each.

Acknowledgments

This study was supported with funds from the North Carolina Division of Water Quality (Contract EW05049) through the USEPA 319 program, and through grants from the National Science Foundation (NSF Grants DMS-0112069 and DMS-0422400). M.E.B. was partially supported by a grant from USEPA Office of Research and Development’s Advanced Monitoring Initiative (AMI) Pilot Projects Focused on GEOSS (Global Earth Observation System of Systems). In addition, A.D.G. is grateful to the Water Environment Federation (WEF), the North Carolina Association of Environmental Professionals (NCAEP), and Quantitative Environmental Analysis (QEA), LLC for scholarship support. We thank NCDENR-SSS (particularly Patti Fowler, Andy Haines, Shannon Jenkins, and J.D. Potts) for making their water quality monitoring data available, and for answering questions throughout the preparation of this manuscript. Craig Stow, Song Qian, Rachel Noble, Sean McMahon, and Conrad Lamon provided valuable suggestions, as did two anonymous reviewers.

Supporting Information Available

Supporting Information is available for the analysis of alternative probability models (Section 2.1), and regression models (Section 3.2). This information is available free of charge via the Internet at <http://pubs.acs.org>.

Literature Cited

- (1) Smith, E. P.; Ye, K. Y.; Hughes, C.; Shabman, L. A. Statistical assessment of violations of water quality standards under section 303(d) of the Clean Water Act. *Environ. Sci. Technol.* **2001**, *35*, 606–612.
- (2) Houck, O. A.; *The Clean Water Act TMDL Program: Law, Policy, and Implementation*, 2nd ed.; Environmental Law Institute: Washington, DC, 2002.
- (3) U.S. Environmental Protection Agency. *Guidance for 2006 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d), 305(b) and 314 of the Clean Water Act*; USEPA: Washington, DC, 2005..
- (4) National Research Council. *Assessing the TMDL Approach to Water Quality Management*; NRC: Washington, DC, 2001.
- (5) Cooter, W. S. Clean Water Act assessment processes in relation to changing U.S. Environmental Protection Agency management strategies. *Environ. Sci. Technol.* **2004**, *38*, 5265–5273.
- (6) Borsuk, M. E.; Stow, C. A.; Reckhow, K. H. Predicting the frequency of water quality standard violations: A probabilistic approach for TMDL development. *Environ. Sci. Technol.* **2002**, *36*, 2109–2115.
- (7) Benham, B. L.; Baffaut, C.; Zeckoski, R. W.; Mankin, K. R.; Pachepsky, Y. A.; Sadeghi, A. M.; Brannan, K. M.; Soupir, M. L.; Habersack, M. J. Modeling bacteria fate and transport in watersheds to support TMDLs. *T. ASABE* **2006**, *49*, 987–1002.
- (8) U.S. Environmental Protection Agency. *National Water Quality Inventory: Report to Congress (2002 Reporting Cycle)*; EPA 841-R-07-001; USEPA: Washington, DC, 2007.
- (9) U.S. Environmental Protection Agency. Code of Federal Regulations: Title 40, Chapter 1, Part 141, 2005.
- (10) NC Department of Environment and Natural Resources. *Coastal Recreational Waters Monitoring, Evaluation, and Notification Rules*; 15A NCAC 18A 0.3400; 2004.
- (11) National Shellfish Sanitation Program. *Guide for the Control of Molluscan Shellfish*; 2005.
- (12) Thomas, H. A. Statistical Analysis of Coliform Data. *Sewage Ind. Wastes* **1955**, *27*, 212–222.
- (13) Ott, W. *Environmental Statistics and Data Analysis*; Lewis Publishers: Boca Raton, FL, 1995.
- (14) Shirmohammadi, A.; Chaubey, I.; Harmel, R. D.; Bosch, D. D.; Munoz-Carpena, R.; Dharmasri, C.; Sexton, A.; Arabi, M.; Wolfe, M. L.; Frankenberger, J.; Graff, C.; Sohrabi, T. M. Uncertainty in TMDL models. *T. ASABE* **2006**, *49*, 1033–1049.
- (15) Cochran, W. G. Estimation of Bacterial Densities by Means of the 'Most Probable Number'. *Biometrics* **1950**, *6*, 105–116.
- (16) McCrady, M. H. The Numerical Interpretation of Fermentation Tube Results. *J. Infect. Dis.* **1915**, *17*, 183–212.
- (17) de Man, J. C. MPN Tables For More Than One Test. *Eur. J. Appl. Microbiol.* **1977**, *4*, 307–316.
- (18) Klee, A. J. A Computer Program For the Determination of Most Probable Number and Its Confidence Limits. *J. Microbiol. Meth.* **1993**, *18*, 91–98.
- (19) Dufour, A. P.; Cabelli, V. J. Membrane-Filter Procedure for Enumerating Component Genera of Coliform Group in Seawater. *Appl. Microbiol.* **1975**, *29*, 826–833.
- (20) Dufour, A. P.; Strickland, E. R.; Cabelli, V. J. Membrane-Filter Method for Enumerating *Escherichia coli*. *Appl. Environ. Microbiol.* **1981**, *41*, 1152–1158.
- (21) Rippey, S. R.; Adams, W. N.; Watkins, W. D. Enumeration of Fecal-Coliforms and *Escherichia coli* in Marine and Estuarine Waters - an Alternative to the APHA-MPN Approach. *J. Water Pollut. Control Fed.* **1987**, *59*, 795–798.
- (22) Esham, E. C.; Sizemore, R. K. Evaluation of two techniques: mFC and mTEC for determining distributions of fecal pollution in small, North Carolina tidal creeks. *Water Air Soil Pollut.* **1998**, *106*, 179–197.
- (23) Rompré, A.; Servais, P.; Baudart, J.; de Roubin, M.-R.; Laurent, P. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *J. Microbiol. Meth.* **2002**, *49*, 31–54.
- (24) Refsgaard, J. C.; van der Sluijs, J. P.; Hojberg, A. L.; Vanrolleghem, P. A. Uncertainty in the environmental modelling process - A framework and guidance. *Environ. Modell. Software* **2007**, *22*, 1543–1556.
- (25) Milly, P. C.; Betancourt, J.; Falkenmark, M.; Hirsch, R. M.; Kundzewicz, Z. W.; Lettenmaier, D. P.; Stouffer, R. J. Stationarity Is Dead: Whither Water Management. *Science* **2008**, *319*, 573–574.
- (26) Greenwood, M.; Yule, G. U. On the Statistical Interpretation of Some Bacteriological Methods Employed in Water Analysis. *J. Hyg.* **1917**, *16*, 36–54.
- (27) Thomas, H. A. On Averaging Results of Coliform Tests. *Boston Soc. Civil Engrs. J.* **1952**, *39*, 253–270.
- (28) Hurley, M. A.; Roscoe, M. E. Automated Statistical Analysis of Microbial Enumeration by Dilution Series. *Appl. Environ. Microbiol.* **1983**, *55*, 159–164.
- (29) Haas, C. N. Estimation of microbial densities from dilution count experiments. *Appl. Environ. Microbiol.* **1989**, *55*, 1934–1942.
- (30) Beliaeff, B.; Mary, J.-Y. The Most Probable Number Estimate and Its Confidence-Limits. *Water Res.* **1993**, *27*, 799–805.
- (31) McBride, G. B.; McWhirter, J. L.; Dalgety, M. H. Uncertainty in most probable number calculations for microbiological assays. *J. AOAC Int.* **2003**, *86*, 1084–1088.
- (32) Eisenhart, C.; Wilson, P. W. Statistical Methods and Control in Bacteriology. *Bacteriol. Rev.* **1943**, *7*, 57–137.
- (33) Pipes, W. O.; Ward, P.; Ahn, S. H. Frequency Distributions for Coliform Bacteria in Water. *J. Am. Water Works Assoc.* **1977**, *69*, 664–668.
- (34) El-Shaarawi, A. H.; Esterby, S. R.; Dutka, B. J. Bacterial Density in Water Determined by Poisson or Negative Binomial Distributions. *Appl. Environ. Microbiol.* **1981**, *41*, 107–116.
- (35) Christian, R. R.; Pipes, W. O. Frequency Distributions of Coliforms in Water Distribution Systems. *Appl. Environ. Microbiol.* **1983**, *45*, 603–609.
- (36) Haas, C. N.; Heller, B. Test of the validity of the Poisson assumption for analysis of Most-Probable-Number results. *Appl. Environ. Microbiol.* **1988**, *54*, 2996–3002.
- (37) Teunis, P. F. M.; Medema, G. J.; Kruidenier, L.; Havelaar, A. H. Assessment of the risk of infection by *Cryptosporidium* or *Giardia* in drinking water from a surface water source. *Water Res.* **1997**, *31*, 1333–1346.
- (38) Russek, E.; Colwell, R. R. Computation of Most Probable Numbers. *Appl. Environ. Microbiol.* **1983**, *45*, 1646–1650.
- (39) Ihaka, R.; Gentleman, R. R. A Language for Data Analysis and Graphics. *J. Comput. Graph. Stat.* **1996**, *5*, 299–314.
- (40) Reckhow, K. H.; Arhonditsis, G. B.; Kenney, M. A.; Hauser, L.; Tribo, J.; Wu, C.; Elcock, K. J.; Steinberg, L. J.; Stow, C. A.; McBride, S. J. A predictive approach to nutrient criteria. *Environ. Sci. Technol.* **2005**, *39*, 2913–2919.
- (41) U.S. Environmental Protection Agency. *Guidelines for the Preparation of the Comprehensive State Water Quality Assessments (305(b) Reports) and Electronic Updates: Report Contents*; Technical Report; USEPA: Washington, DC, 1997.
- (42) Berry, D. A. *Statistics: a Bayesian Perspective*; Duxbury Press: Belmont, CA, 1996.
- (43) Bolstad, W. M. *Introduction to Bayesian Statistics*; Wiley-Interscience: Hoboken, NJ, 2004.
- (44) Gelman, A. J.; Carlin, J. B.; Stern, H. S.; Rubin, D. B. *Bayesian Data Analysis*; Chapman & Hall/CRC: Boca Raton, FL, 2004.
- (45) Gelman, A. J. Prior distributions for variance parameters in hierarchical models (Comment on Article by Browne and Draper). *Bayesian Anal.* **2006**, *1*, 515–534.
- (46) Van Dongen, S. Prior specification in Bayesian statistics: Three cautionary tales. *J. Theor. Biol.* **2006**, *242*, 90–100.
- (47) Qian, S. S.; Reckhow, K. H. Combining model results and monitoring data for water quality assessment. *Environ. Sci. Technol.* **2007**, *41*, 5008–5013.
- (48) Lunn, D. J.; Thomas, A.; Best, N. G.; Spiegelhalter, D. J. WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. *Stat. Comput.* **2000**, *10*, 325–337.
- (49) Shen, J.; Sun, S.-C.; Wang, T.-P. Development of the fecal coliform total maximum daily load using Loading Simulation Program C++ and tidal prism model in estuarine shellfish growing areas: A case study in the Nassawadox coastal embayment, Virginia. *J. Environ. Sci. Health, A* **2005**, *40*, 1791–1807.
- (50) U.S. Environmental Protection Agency. *Decision Rationale Letter: Total Maximum Daily Load for Shellfish Harvest and Primary Contact Use Impairments in the Poquoson River, Back Creek and Back River Watersheds, York County, Virginia*; USEPA, 2006.
- (51) U.S. Environmental Protection Agency. *Decision Rationale Letter: Total Maximum Daily Load for Shellfish Harvest and Primary Contact Use Impairments in the Onancock Creek, Locklies and Mill Creek Watersheds, Accomack and Middlesex Counties, Virginia*; USEPA, 2006.
- (52) Alderisio, K. A.; DeLuca, N. Seasonal enumeration of fecal coliform bacteria from the feces of ring-billed gulls (*Larus delawarensis*) and Canada geese (*Branta canadensis*). *Appl. Environ. Microbiol.* **1999**, *65*, 5628–5630.
- (53) Sanders, B. F.; Arega, F.; Sutula, M. Modeling the dry-weather tidal cycling of fecal indicator bacteria in surface waters of an intertidal wetland. *Water Res.* **2005**, *39*, 3394–3408.

ES703144K