

ACCOMMODATING CHANGE OF BACTERIAL INDICATORS IN LONG TERM WATER QUALITY DATASETS¹

*Curtis G. Cude*²

ABSTRACT: In 1996, the State of Oregon adopted a water quality standard based on *Escherichia coli* (*E. coli*), recognizing *E. coli* as an indicator of pathogenic potential. The Oregon Department of Environmental Quality (DEQ) began analysis for *E. coli* that same year. The Oregon DEQ continued collection and analysis of fecal coliform (a prior indicator organism) for data input to bacterial loading models and the Oregon Water Quality Index (OWQI). The OWQI is a primary indicator of general water quality for the Oregon DEQ and the Oregon Progress Board. The objective of this study was to develop a regression relationship between fecal coliform and *E. coli*. This relationship would fill data gaps and extend water quality models and indicators. Water quality policy is better informed by the ability of these extended water quality models to determine whether water quality meets present or would have met past bacterial standards. Monitoring resources spent on dual bacterial analyses could be conserved. This study also showed that changes to OWQI values (as a result of changing bacterial indicators) were minimal, and corresponded to improved characterization of water quality with respect to pathogenic potential.

(**KEY TERMS:** water quality; fecal coliform; *Escherichia coli*; modeling; statistical analysis; water quality monitoring; water quality index; water quality standards.)

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INTRODUCTION

It is not practical or feasible to test for all of the disease causing organisms present in surface water. Therefore, organisms that correlate with the presence of human pathogens and the risk of disease are selected as indicators of potential pathogenic contamination. An ideal indicator organism should be associated

with sources of human pathogens, equally or more resistant to disinfection than are pathogens, unable to grow in aquatic environments, applicable to all types of water, and remain quantifiable after infectious levels of pathogens have disappeared (Oregon DEQ, 1994). The choice of indicator organism used in Oregon's water quality standards changed over time from total coliform to fecal coliform to enterococci as new studies determined which indicator correlated best with human illness. In 1996, Oregon adopted an *Escherichia coli* (*E. coli*) standard for fresh and estuarine waters not subject to commercial shellfish harvest and a fecal coliform standard for marine and estuarine/shellfish producing waters. The Oregon Department of Environmental Quality (DEQ) collected water samples for analysis of fecal coliform starting 1967 and for *E. coli* starting 1996.

This change of freshwater bacterial standards over time and the interface of standards in estuaries presented challenges to the comparison of water quality data to standards. The change in standards affected bacterial loading models and the Oregon Water Quality Index (OWQI). Bacterial loading models must account for standards violations of either *E. coli* or fecal coliform, and sometimes for both in estuaries. If a waterbody was placed on the Water Quality Impairment (303(d)) list for fecal coliform, the Oregon DEQ must continue to compare water quality to that standard. If bacterial quantity is of concern in marine or estuarine/shellfish-producing waters, the Oregon DEQ must evaluate the incoming freshwater streams. It was feasible but costly to continue collection and analysis for both bacteria. However, even when both

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²Natural Resource Specialist, Oregon Department of Environmental Quality, MSD/BSO, 811 S.W. Sixth Avenue, Portland, Oregon 97204 (E-Mail: cude.curtis@deq.state.or.us).

were routinely collected, data for the preferred analyte were still occasionally unavailable. An extrapolation of one bacterium to the other was desired to fill in data gaps.

The OWQI was introduced in the 1970s (Dunnette, 1979) and modified in the 1990s (Cude, 2001). The OWQI is a single number that expresses water quality by integrating measurements of eight water quality variables: temperature, dissolved oxygen, biochemical oxygen demand, pH, ammonia+nitrate nitrogen, total phosphorus, total solids, and fecal coliform. The eight variables are transformed to unitless subindex numbers before aggregation into the combined OWQI score. The index provides a simple and concise method for expressing water quality status and trends in Oregon's rivers and streams for general recreational use (e.g., fishing and swimming). The OWQI is Oregon DEQ's primary indicator of trends in water quality and is used for strategic planning (Oregon DEQ, 2002a), performance measurement (Oregon DEQ, 2002b), and public communications (Oregon DEQ, 2002c). The OWQI also serves as the benchmark indicator of stream water quality for the Oregon Progress Board (Oregon Progress Board, 2003).

One recognizable inconsistency in the OWQI was the use of fecal coliform while *E. coli* was recognized as Oregon's standard indicator organism. If changes were made to this important indicator, the significance and magnitude of change must be determined. A significant change of great magnitude would produce a discontinuity in the OWQI, prohibiting monotonic trend analyses and comparison of OWQI data before and after the switch. The OWQI would be unavailable for use as an indicator until enough data were made available to calculate trends post-change. This period of unavailability would be on the order of three to five years. The Oregon DEQ policymakers would need to seek an alternative water quality indicator for the interim.

Previous studies compared fecal coliform and *E. coli* in different ways. Van Ess and Harding (1999) tested selected bacterial, physical, and chemical variables at two natural freshwater swimming areas in western Oregon. While the study compared results of fecal coliform and *E. coli* to each other and to federal and state regulatory levels, no relationship was developed between the two variables. Elmund *et al.* (1999) compared *E. coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency. They examined correlations between these variables and recommended that stream standards and discharge permits be revised in support of *E. coli* based standards. Kroening (1999) investigated fecal coliform and *E. coli* in the St. Croix National Scenic Byway, compared these variables to state and federal

standards, and examined correlations between each of these variables and stream discharge. Rasmussen and Ziegler (2003) developed extensive comparisons and continuous estimates of fecal coliform and *E. coli* in selected Kansas streams. They compared these variables to state and federal standards and developed regression relationships between fecal coliform and *E. coli* to extend this comparison for other water quality models. These regression relationships were site-specific and range limited, and *E. coli*/fecal coliform ratios were used to extend modeling. Additionally, regression relationships were developed between the bacterial variables and turbidity, so that continuous turbidity monitors could be used to estimate bacterial density to calculate flow duration curves, and annual and seasonal loads and yields. Francy *et al.* (1993), explored *E. coli* and fecal coliform as indicators of recreational water quality in Ohio. Their study compared these variables to state and federal standards and developed regression relationships between fecal coliform and *E. coli*. Furthermore, analysis of covariance testing determined how data from individual sites could be pooled, allowing for the development of regression relationships between the two variables that covered a wide geographic area.

The primary objective of the study reported herein was to determine whether a regression relationship could be developed to define the relationship between fecal coliform and *E. coli* for Oregon's rivers and streams. The shift in emphasis from fecal coliform to *E. coli* represents a potential discontinuity in the sampling record generated at long term monitoring stations. The regression relationship would fill data gaps, extend water quality models, and cover as wide a geographical range as practical. Methods used by Francy *et al.* (1993) supported development of the relationship.

SAMPLE COLLECTION AND ANALYSIS

Oregon DEQ personnel collected water samples for analysis between February 1996 and February 2000. Samples were collected in sterile containers in a manner that minimized contamination. Samples were refrigerated at approximately 4°C and processed within 30 hours after collection at the Oregon Public Health Laboratory in Portland, Oregon, as per Oregon DEQ Laboratory standard protocol (Oregon DEQ, 1997). The mTEC procedure (American Public Health Association *et al.*, 1998, Section 9213 D.3) was used for all *E. coli* samples. The standard membrane filtration procedure (American Public Health Association *et al.*, 1998, Section 9222 D) was used for all fecal

coliform samples. Data were analyzed using S-Plus 2000 Professional Version for Windows (MathSoft, 1999) and WQHydro (Aroner, 2001).

All samples were collected from sample stations representing ambient rivers and streams in Oregon. Some of these stations were included in the fixed station ambient stream monitoring network (henceforth referred to as the “monitoring network”). The monitoring network (152 sites as of 2003) provided conventional pollutant data for trending, standard compliance, and problem identification. Sites were selected to represent all major rivers in the state and provide statewide geographical representation. Most sites reflected the integrated water quality impacts from point and nonpoint source activities as well as the natural geological, hydrological, and biological impacts on water quality for the watershed they represent. Sampling frequency for monitoring network stations was generally bimonthly.

PREDICTION OF FECAL COLIFORM FROM *E. COLI* RESULTS

The primary objective of this study was to develop a regression relationship between fecal coliform and *E. coli*, with a benefit of allowing for the cessation of fecal coliform monitoring simultaneous with *E. coli* monitoring. The use of data from the monitoring network ensured that each station contributed 15 to 30 samples to the regression relationship. The data were base-10 logarithmically transformed and classified as belonging to one of 16 hydrological basins. Linear regression relationships were developed to predict $\log_{10}(\text{fecal coliform})$ from $\log_{10}(E. coli)$ with monitoring station as a factor, with hydrological basin as a factor, and with no spatial factor.

Analysis of variance (ANOVA) determined that neither monitoring station nor hydrologic basin was an important factor ($\alpha = 0.95$). Thus, a single regression relationship was applied to all stations that monitor microbiological quality in Oregon.

The product of the ANOVA was a linear regression relationship ($r^2 = 0.754$, $p < 0.001$), developed using all available paired samples. This relationship was expressed as

$$\log_{10}(\text{fecal coliform}) = 0.260 + 0.946 * (\log_{10}(E. coli)) \quad (1)$$

The precision ($\alpha = 0.95$, 874 degrees of freedom) of the regression estimates was calculated by the

method of least squares (Mac Berthouex and Brown, 1994)

$$\begin{aligned} y\text{-intercept: } & 0.260 \pm 0.0768 \\ \text{slope: } & 0.946 \pm 0.0358 \end{aligned}$$

The regression relationship was simplified by taking the anti-log of both sides of Equation (1). The result was expressed as

$$\text{Fecal coliform} = 1.82 * (E. coli)^{0.946} \quad (2)$$

The inverse of Equation (2) can be used to estimate *E. coli* concentrations from fecal coliform data for use in bacterial loading models. This inverse relationship was expressed as

$$E. coli = 0.531 * (\text{Fecal coliform})^{1.06} \quad (3)$$

APPLICATION TO BACTERIAL LOADING MODELS

The Oregon DEQ is responsible for developing total maximum daily loads (TMDLs) for waterbodies that are water quality limited with respect to specific pollutants. Bacteria loading models are developed to determine the bacterial load that can be allocated to nonpoint sources, and the relative contribution of point and nonpoint sources, while still meeting water quality standards. Bacterial loading models must account for standards violations of either *E. coli* or fecal coliform, and sometimes for both in estuaries.

Bacteria concentrations are inherently variable. Analysis of 521 paired (replicate) samples collected in Oregon freshwater streams between February 1996 and February 2000 revealed a standard error of 0.36 for log-transformed fecal coliform and 0.33 for log-transformed *E. coli* (bacteria concentrations are log-normally distributed in this dataset). Although the relationship was significant ($\alpha = 0.05$), bacterial concentration estimates in environmental samples were not very precise, as indicated by substantial variability among replicate samples. Concentrations of *E. coli* measured in tributaries were converted, using Equation (2), to fecal coliform concentrations for TMDL development in shellfish producing Nestucca Bay (Oregon DEQ, 2002d). Concentrations of fecal coliform bacteria measured at estuarine river mouths were converted to *E. coli* concentrations using Equation (3) for development of the North Coast Subbasins TMDL (Oregon DEQ, 2003).

MODIFICATION TO OREGON WATER QUALITY INDEX

Oregon Water Quality Index scores were calculated at each monitoring network station for each sample with sufficient data (i.e., values present for all OWQI variables). The OWQI can be treated as an individual variable representing general water quality at the given station and used in a variety of temporal and spatial data analyses. Supporting information for any OWQI analysis can be determined by inspecting subindices and underlying raw data (e.g., Cude, 2002).

The transformation for the fecal coliform subindex (SIFC), originally developed by Dunnette (1979), is given by

$$\begin{aligned} \text{FC} \leq 50 \text{ \#/100 mL:} \\ \text{SI}_{\text{FC}} = 98 \end{aligned} \quad (4)$$

$$\begin{aligned} 50 \text{ \#/100 mL} < \text{FC} \leq 1600 \text{ \#/100 mL:} \\ \text{SI}_{\text{FC}} = 98 * \exp((\text{FC}-50) * -9.9178\text{E-}4) \end{aligned} \quad (5)$$

$$\begin{aligned} 1600 \text{ \#/100 mL} < \text{FC:} \\ \text{SI}_{\text{FC}} = 10 \end{aligned} \quad (6)$$

where FC represents fecal coliform concentration and “#” represents either most probable number (MPN) or colony forming units (CFU). Past Oregon DEQ studies showed that these differing fecal coliform methods provide comparable results for ambient water quality (L. Caton, February 10, 2000, Oregon DEQ, personal communication).

To permit greater flexibility, the OWQI could be calculated with either fecal coliform or *E. coli*, generating a bacterial subindex (SI_{BACT}). This can be accomplished by using *E. coli* to calculate fecal coliform concentrations (Equation 2) and then calculating SI_{BACT} using Equations 4 through 6. Alternatively, SI_{BACT} can be calculated using *E. coli* directly

$$\begin{aligned} \text{Ec} \leq 33 \text{ \#/100 mL:} \\ \text{SI}_{\text{BACT}} = 98 \end{aligned} \quad (7)$$

$$\begin{aligned} 33 \text{ \#/100 mL} < \text{Ec} \leq 1300 \text{ \#/100 mL:} \\ \text{SI}_{\text{BACT}} = 98 * \exp(4.96\text{E-}2 \\ - (1.81\text{E-}3 * (\text{Ec}^{\wedge}0.946))) \end{aligned} \quad (8)$$

$$\begin{aligned} 1300 \text{ \#/100 mL} < \text{Ec:} \\ \text{SI}_{\text{BACT}} = 10 \end{aligned} \quad (9)$$

where *Ec* represents *E. coli* concentration and “#” represents either MPN or CFU. Oregon DEQ studies showed that these differing *E. coli* methods provide

comparable results for ambient water quality (L. Caton, C. Cude, and S. Schwind, 2000, unpublished draft report, *Comparison of Escherichia Coli Methods: Membrane Filtration vs. Quantitray*, Oregon DEQ, Portland, Oregon). Equations (7) through (9) (including the *E. coli* concentration boundary values) were derived by substituting Equation (2) for “FC” in Equations (4) through (6). A graphical representation of SI_{BACT} calculated using fecal coliform versus *E. coli* is presented in Figure 1.

Figure 1 displays the range of SI_{BACT} values calculated using fecal coliform at the boundaries of the standard error (0.36 for log-transformed fecal coliform). SI_{BACT} calculated using *E. coli* (including values at the boundaries of the standard error, 0.33 for log-transformed *E. coli*, not plotted here) is within the standard error for fecal coliform.

Sensitivity Analysis

SI_{BACT} and OWQI values were calculated using paired measurements of *E. coli* and fecal coliform to determine whether the use of *E. coli* versus fecal coliform data had a significant influence on subindex and OWQI results.

The OWQI is calculated with the unweighted harmonic square mean formula

$$\text{OWQI} = \sqrt{\frac{n}{\sum_{i=1}^n \frac{1}{\text{SI}_i^2}}} \quad (10)$$

where n is the number of subindices and SI_i is subindex i . This formula allows the most impaired variable to impart the greatest influence on the water quality index and acknowledges that different water quality variables will pose differing significance to overall water quality at different times and locations.

For this analysis, it was assumed that all other OWQI subindices were fixed at a value of 100. This ensured that variable SI_{BACT} results introduced maximum variability in OWQI results. The formula for calculating the OWQI for this analysis became

$$\text{OWQI} = \sqrt{\frac{8}{\left(\frac{1}{\text{SI}_{\text{BACT}}^2} + \frac{7}{100^2}\right)}} \quad (11)$$

where SI_{BACT} is the bacterial subindex.

Figures 2 and 3 allow a visual examination of the differences between the paired groups (i.e., SI_{BACT} calculated with fecal coliform versus *E. coli* and

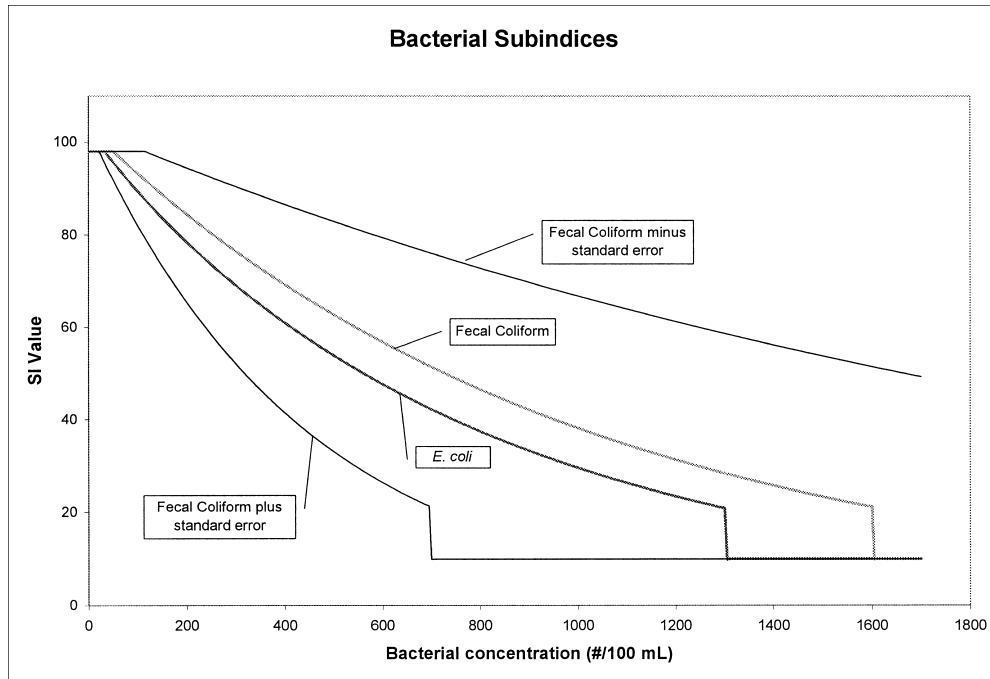


Figure 1. Comparison of Bacterial Subindices.

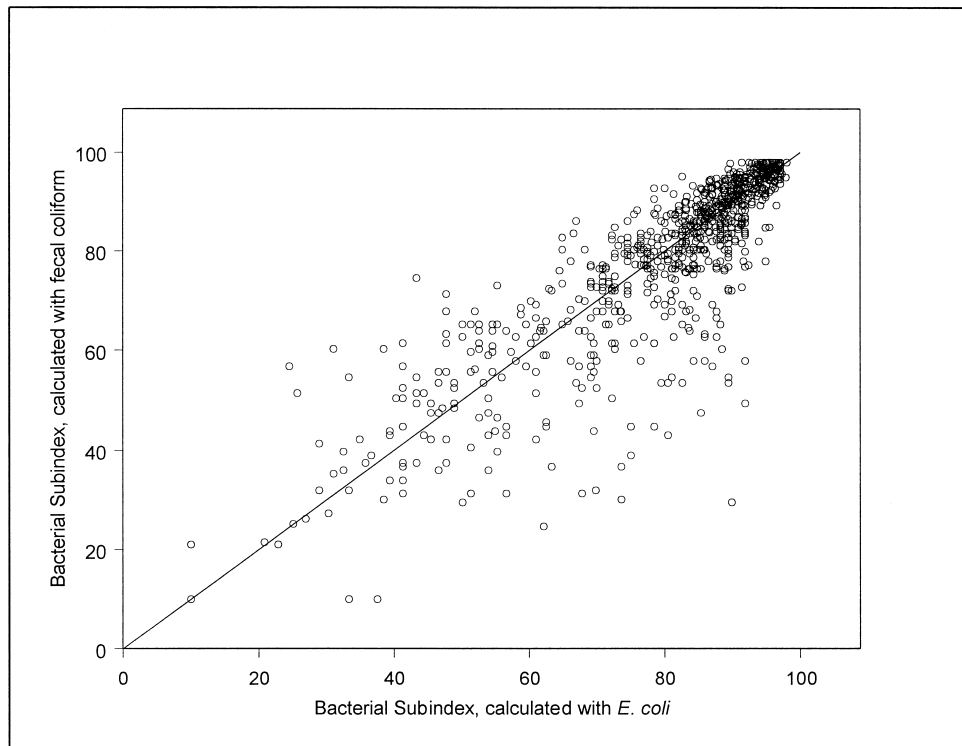


Figure 2. Comparison of SIBACT Calculated With *E. coli* Versus Fecal Coliform.

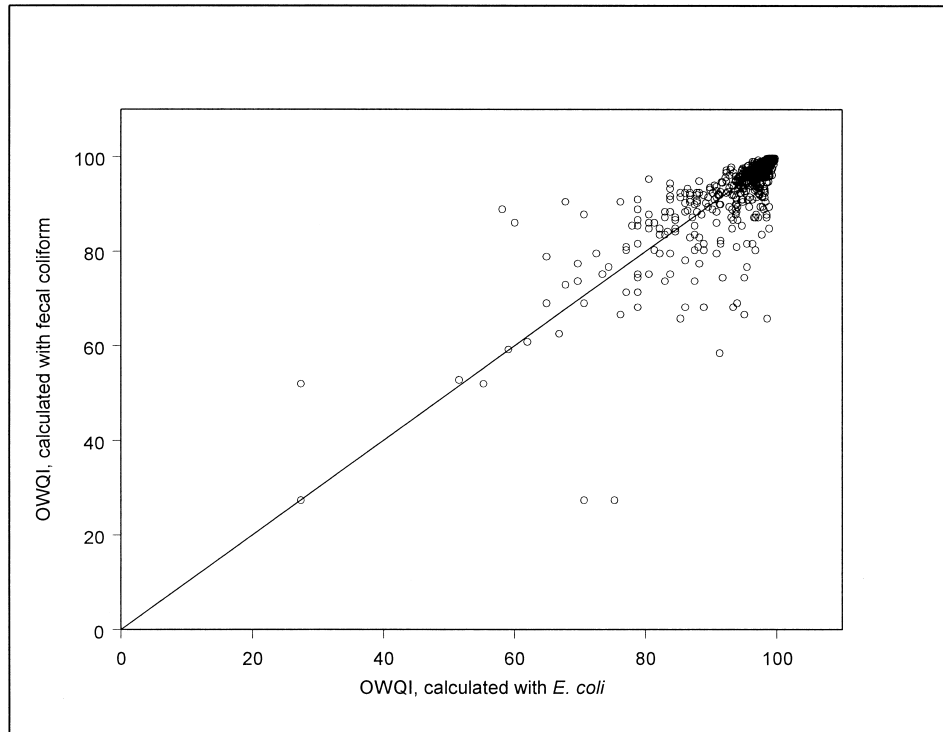


Figure 3. Comparison of OWQI Calculated With *E. coli* Versus Fecal Coliform.

OWQI calculated with the two alternate forms of SI_{BACT}). Values plotted on the diagonal line in each figure indicate perfectly corresponding values. These figures show less differences at lower concentrations of bacteria (and thus higher index values, comprising the majority of the data) and greater differences at higher concentrations of bacteria.

The Anderson-Darling k-Sample test (Scholz and Stephens, 1987) was used to compare the paired empirical distribution functions (EDFs) because the test does not assume equal variances. The test evaluates the more general null hypothesis that all samples have the same distribution against the alternative that the samples differ in central tendency and/or in variability. In both cases (paired SI_{BACT} and paired OWQI EDFs), the null hypothesis was rejected ($\alpha = 0.01$).

All sample datasets were normalized to 0.0 by subtracting from each observation its respective within-sample median and the tests were recomputed on the normalized data (Aroner, 2000). If the null hypothesis of the retest was not rejected, this would suggest that the original hypothesis rejection was due to a difference in central tendency. However, in both cases (paired SI_{BACT} and paired OWQI EDFs), the null hypothesis was rejected ($\alpha = 0.01$), suggesting that the samples differ in their variability as well as central tendency.

Average differences and average absolute differences between the groups were calculated to quantify the differences in central tendency. SI_{BACT} and OWQI values calculated using *E. coli* were slightly higher than when using fecal coliform (average difference: 1.05 SI points and 0.55 OWQI points; average absolute difference: 5.92 SI points and 2.21 OWQI points). The magnitude of difference (and absolute difference) was greater for SI_{BACT} calculations than for OWQI calculations because the OWQI calculation dampens the variability of individual parameters.

OWQI Modification Summary

SI_{BACT} and OWQI values were calculated using paired measurements of fecal coliform and *E. coli* data. There is significant difference in central tendency and dispersion between paired SI_{BACT} and OWQI sample sets. The SI_{BACT} and OWQI values calculated using *E. coli* tended to be slightly higher than those calculated from fecal coliform. The magnitude of the average difference was small, on the order of one SI_{BACT} or OWQI unit. While the magnitude of average absolute differences was greater, standard deviation of SI_{BACT} and OWQI values in this dataset was on the order of 17 and 8 index units, respectively.

Some difference is expected, as represented in the regression relationship presented in Equation (2). This difference represents an improved indication of pathogenic potential within the context of general water quality as represented in the OWQI. Since the difference lies within the expected range of variability, either fecal coliform or *E. coli* results may be used without producing a discontinuity in the OWQI, although caution should be exercised when interpreting results.

CONCLUSIONS

The primary objective of this study was to determine whether a regression relationship could be developed between fecal coliform and *E. coli* for Oregon's rivers and streams. This study used data collected from long term monitoring stations located in a variety of ecoregions and land uses throughout Oregon. ANOVA determined that relationships incorporating either site-specific parameters or hydrologic basin-specific parameters did not add significant information to the final regression relationship. One relationship (Equation 2) can be applied to all monitoring stations representing ambient rivers and streams in Oregon.

This relationship enables the Oregon DEQ to continue to meet water quality assessment and policy objectives: comparison of water quality to standards, assessment of water quality status and trends, and determination of spatial distribution and composition of pollution. This relationship can be used in extrapolation of one bacterium to the other to fill data gaps in bacterial loading models. There is some risk associated with relying on this regression relationship, but the risk must be evaluated within the context of the inherent variability of bacteria concentrations. An economic benefit to use of the relationship is long-term conservation of resources previously spent on dual bacterial analyses. Such conservation is attained by reducing or removing the obligation to continually monitor for both types of bacteria.

This relationship presents minimal disruption to Oregon DEQ's primary water quality trend indicator, the OWQI. While the difference between calculating the OWQI with fecal coliform and with *E. coli* is significant, the magnitude is small and well within the expected variability. Some difference is expected, since the change in bacterial indicators reflects an improved understanding of correlations between bacteria and human illness.

The methods used to develop this relationship could be applied in the future should a new bacterial

indicator be developed. This method could be explored for use in situations where other indicators within an index (e.g., indicators of oxygen demand) may change.

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