Appendix F
Bacteria Analytical Methods

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FECAL COLIFORM TO *E. COLI* CONVERSION

DEQ developed the Molalla-Pudding TMDL using DEQ-collected *E. coli* and fecal coliform data and *E. coli* data collected by the Marion Soil and Water Conservation District. DEQ data was collected between 1968 and 2006 and all fecal coliform data (collected from 1968 – 2001) were converted to *E. coli* equivalents via the equation developed by DEQ based on regression analysis of a large state-wide data set from DEQ's ambient monitoring program (Cude, 2005):

$$\textit{E. coli} \text{ count} = 0.53087 * \text{Fecal Coliform count}^{1.05652}$$

Most DEQ data were collected between 1989 -1993 and 2005-2006. Marion SWCD data were collected and analyzed based on DEQ sampling, analysis, and quality assurance protocols between 2002 and 2006.

To evaluate the validity of including converted fecal coliform data with *E. coli* data, DEQ reviewed all available bacteria data from the Pudding River site with the largest data set, Pudding River at Highway 99E (river mile 7.3). The data set spanned the years 1973 – 2006, with only 1 sample per year for 1973, 1975, and 1979, between two and 12 samples/year (not counting quality assurance duplicates) in the 1980s and 90s, and approximately bimonthly samples between 2000 and 2006. Up until 1996, DEQ analyzed bacteria samples for fecal coliform. Between February 1996 and February 2000, DEQ performed fecal coliform and *E. coli* analysis on all bacteria samples. And from 2001 on, DEQ analyzed samples for *E. coli* only.

DEQ divided the data into two data sets: the first included data collected up until January 1, 1996 and the second included data collected after January 1, 1996. DEQ used a seasonal step trend analysis in the program WQ Hydro (Aroner, 1997) to evaluate the difference between the two sample sets. The data was thinned to one sample per month to eliminate bias from more intensive sampling around particular events.
Table F- 1 compares the medians of the Pudding River at Highway 99E data set split into pre-January 1996 and post January1996 sample sets, and two scenarios:

1. Data thinned to one sample/month and evaluated between 1973 - 1996 (with fecal coliform data converted to E. coli equivalents) and after 1996. The period 1996 – 2002 includes both converted fecal coliform and *E. coli* analysis for each sample.

2. Same as scenario 1, but deleting the converted fecal coliform data analyzed between 1996 and 2002.

Table F- 1: Comparison of medians of "before" and "after" transition to E. coli as indicator bacteria species in 1996.

	Median *E. coli* (counts/100 ml) 1973 – 1995	**Median *E. coli* (counts/100 mL) 1995 – 2006**	**Significance**	**Sample Thinning**
Scenario 1	65	45	2xP = 0.4427 Not significant (80% confidence level)	1 sample/month
Scenario 2	65	44	2xP = 0.4329 Not significant (80% confidence level)	1 sample/month, fecal coliform data post 1996 removed

In each case, the difference between the pre-1996 data and post-1996 data is not significant (80% confidence interval). For this analysis, the null hypothesis, H_o, is that there is no trend and 2xP in Table F- 1 is the probability of incorrectly concluding there is a trend when none actually existed. The 2xP value is compared to a pre-determined error level which represents the acceptable

chance of an incorrect conclusion, known as the significance level (α, or alpha). A significance level of 0.20 (alternatively know as a confidence level of 80%) indicates that, for a two-tailed test, a 0.10 maximum probability of error in concluding that a significant increasing trend exists and a 0.10 maximum probability of error in concluding that a significant decreasing trend exists (for an overall error potential of 0.20) is acceptable. For example, for Scenario 1, since the 2xP of 0.4427 is not less than 0.20, for an 80% confidence level the null hypothesis of no trend cannot be rejected. Therefore, the conclusion is that there is no trend. The 2xP result of 0.4427 indicates that there is a 44.27% probability that a trend does not exist in the population and that any observed sample trend is the result of random sampling variability (Aroner, 1997).

The maximum 2xP level that is selected should be based on the relative consequences of (1) failing to detect a population trend (a Type II error, β), versus (2) falsely concluding that a population trend exists (a Type I error, α, or 2xP). The consequence of erroneously concluding that there is no difference (e.g. trend) between the two data sets would be to include the converted fecal coliform data in with the *E. coli* data. This decision would be conservative because the median of the converted fecal coliform data is higher than the *E. coli* data. Therefore, not rejecting the null hypoethesis (that no trend exists) with a relatively high 2xP value is appropriate.

Figure F- 1 and Figure F- 2 illustrate the relationship between the two data sets. WQHydro uses a Seasonal Hodges Lehman calculation (Hirsch,1988) to quantify the magnitude of the difference between the two data sets. Within each month, the median of the differences between all possible pairings of pre-January 1996 and post-January 1996 data is calculated. The median of all the monthly median differences is the Seasonal Hodges Lehman estimator (ΔY). In Scenario 1, the ΔY = -5.5 and in Scenario 2, the ΔY = -6. Figures A1 and A2 represent the ΔY with two horizontal lines equally spaced above and below the average of the medians of the two data sets (Scenario 1 average median = 55; Scenario 2 average median = 54.5) . DEQ concluded that combining the pre- and post-January 1996 data sets would not introduce bias into the analysis.

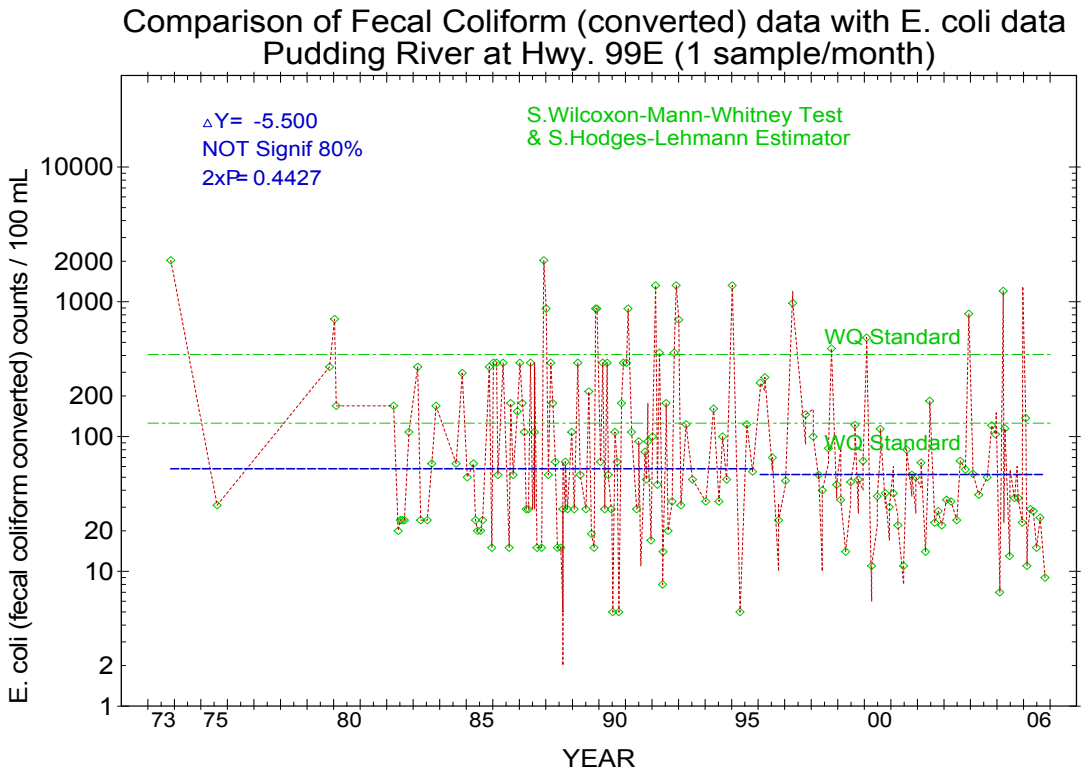

Figure F- 1: Comparison of medians of pre-1996 fecal coliform data (converted to *E. coli*) with post 1996 data (mixture of *E. coli* data and fecal coliform data converted to E. coli).
Data collected at river mile 7.3 and thinned to one sample/month.

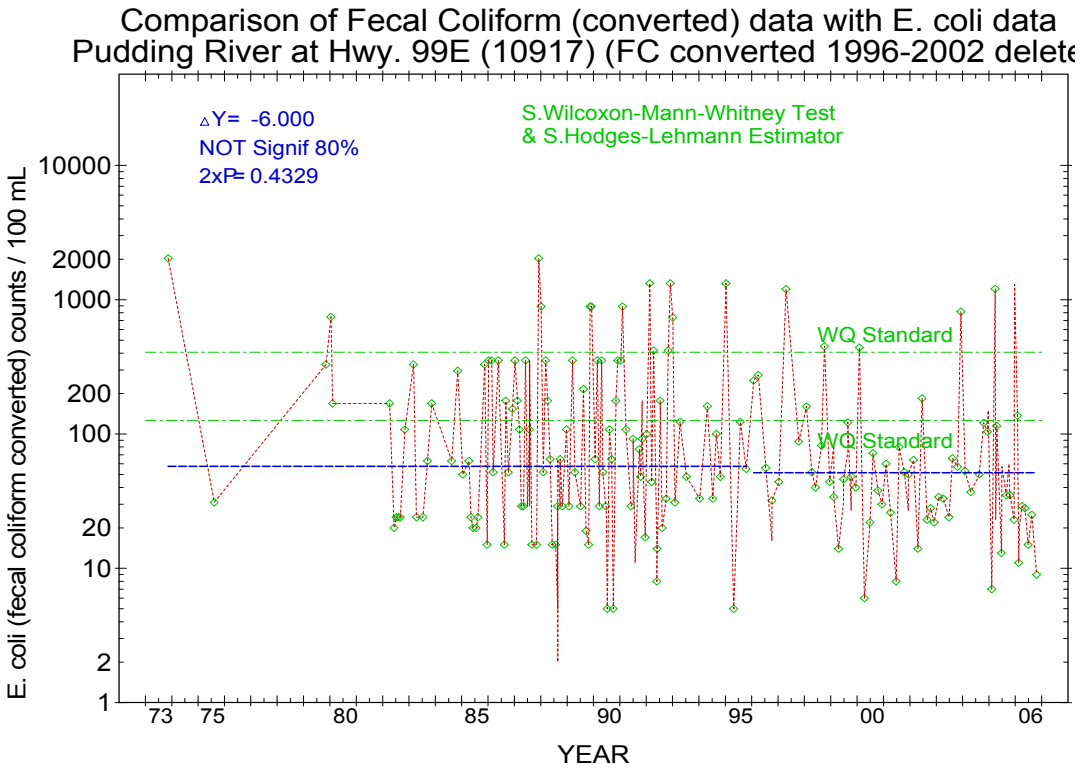

Figure F- 2: Comparison of medians of pre-1996 fecal coliform data (converted to *E. coli*), with post 1996 data (only *E. coli* data).
Data collected at river mile 7.3 and thinned to one sample/month.

BOX AND WHISKER PLOTS

DEQ used box-and whisker-plots (box plots) to assess the longitudinal and temporal variability of bacteria counts. Box plots illustrate the spread of bacteria counts measured at a site, including extreme values (outliers). Box plots use the median as a measure of central tendency and the interquartile range (the 25th percentile to 75th percentile) as a measure of spread. Figure F- 3 shows two examples of box plots and how to interpret their data distribution. Where sufficient data were available, box plot data were plotted longitudinally to highlight potential differences that may be associated with land use, tributaries, or point sources along a stream.

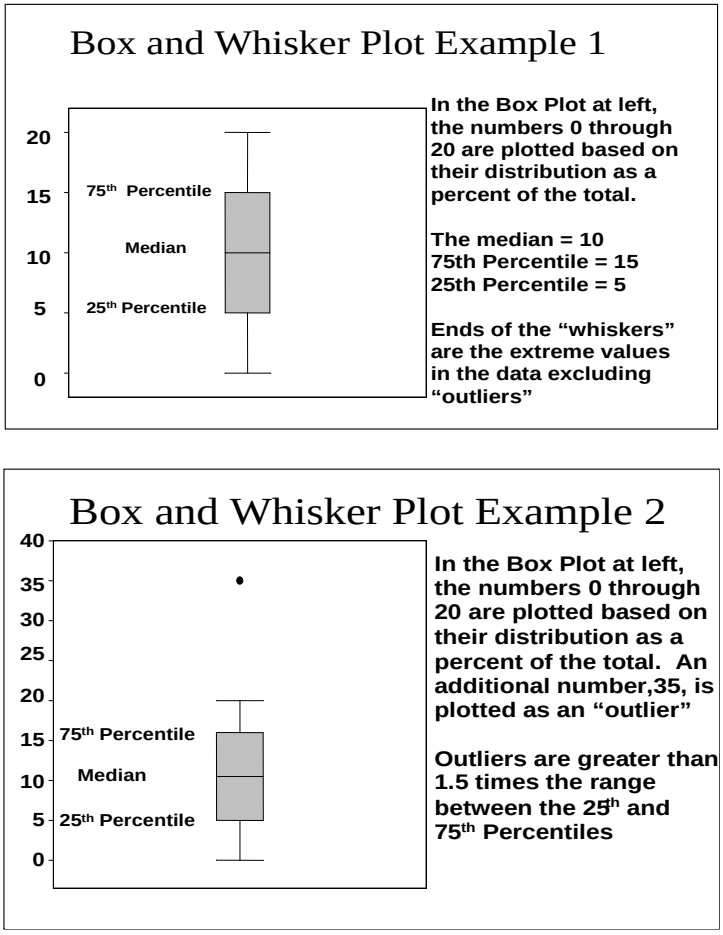

Figure F- 3: Two box and Whisker Plot examples.

LOAD DURATION CURVES

DEQ used another analytical approach, a load duration curve, to examine data from sites where daily flow data were available or could be calculated based on a relationship with flow measured at another site. DEQ chose the load duration curve approach because it illustrates bacteria loading under various flow conditions and can be used in targeting appropriate water quality restoration efforts (Cleland, 2002). Load duration curves are a method of determining a flow based loading capacity, assessing current conditions, and calculating the necessary reductions to comply with water quality criteria. Figure 3 illustrates an example load duration curve. Bacterial loads (in *E. coli* organisms/day) are calculated by multiplying the bacteria concentration in a 100 mL sample by a daily average stream flow. Bacterial loads are plotted in relation to the likelihood that a given flow rate will occur (exceedance probability) based on historical flow data. Low flows have a high exceedance probability, while high flows have a low exceedance probability. For example, an exceedance probability of 99% could indicate a drought and an exceedance probability of 5% could indicate a flood. The two curves in Figure F- 4 represent the two bacteria criteria (126 counts/100 mL and 406 count/100 mL) in terms of bacterial load as a function of flow. The graph in Figure 3 also classifies flow regimes into five categories. Exceedances, defined as bacteria loads above either of the two criteria curves, on the right side of the graph occur during low flows, not associated with runoff. Those on the left side of the graph occur during high flows generally associated with rainfall and runoff events. Exceedances at higher flow may be due to bank collapse or field runoff, while exceedances at lower flows might be attributable to small steady inputs such as point sources or faulty septic systems.

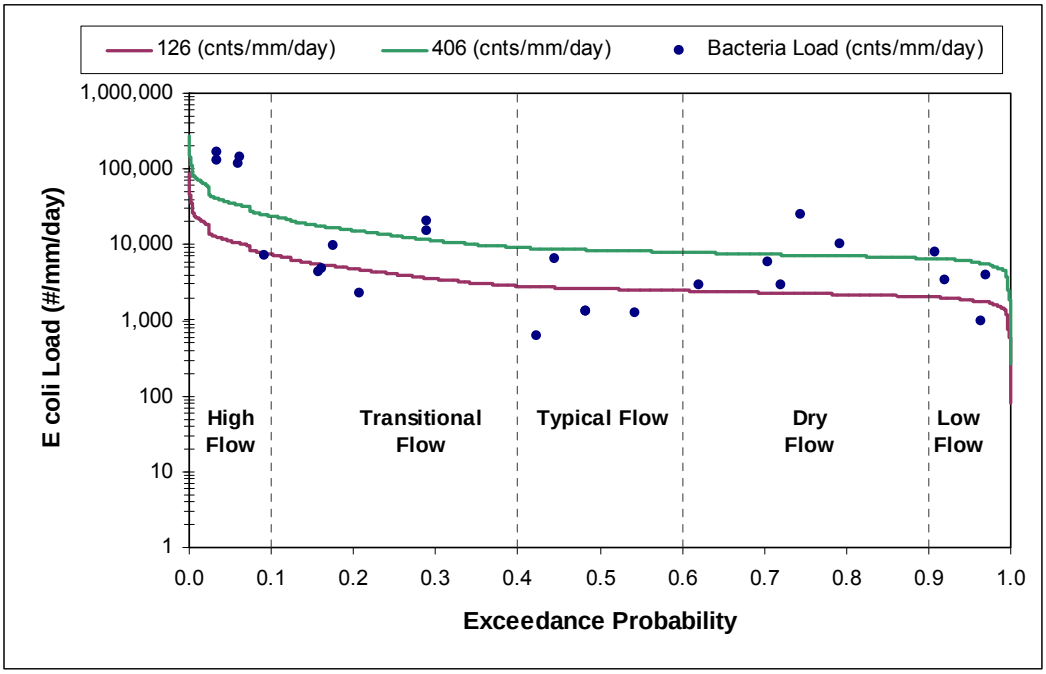

Figure F- 4: Example Load Duration Curve showing the loading capacity and calculated event loads.

Constructing a load duration curve requires a continuous flow record and associated bacterial count data from a site. Load duration curves normally reflect the annual distribution of stream discharge, so data should be from multiple full year data sets, with each year having the same start and end date. Four sites in the subbasin had sufficient data for this analysis: Molalla River at Knight's Bridge, Zollner Creek at Monitor-McKee Road, and Pudding River at Highways 211 and 214.

In this analysis, some sites had numerous bacteria measurements, but incomplete overlap between bacteria data collection events and measured stream flows. Where gaps existed, DEQ estimated stream flows at one site from those measured at another site as long as the stream flow records from the two sites correlated sufficiently (coefficient of determination, r^2 >0.80). The locations from which stream flows were measured or estimated are presented in Table F- 2. DEQ did not have a large data set at Silver Creek sites, so constructed a load duration curve for Silver Creek by combining *E. coli* and fecal coliform bacteria data collected by DEQ (1969 – 1993 and 2005 – 2006) and *E. coli* data collected by the Marion SWCD (2002 – 2006).

Table F- 2: Gages used for stream flow measurements to construct load duration curves. Load duration curves constructed for the sites that are shaded in the table made use of flow estimates calculated from flow at secondary gauges.

Site (s)	USGS Gage Number	USGS Gage Location	Begin Date	End Date	Secondary Gauge
Molalla River at Knights Bridge Rd.	14200000	MOLALLA RIVER NEAR CANBY, OR	8/1/1928	9/30/2006	
Silver Creek (3 sites at and d/s Silverton)	14200300	SILVER CREEK AT SILVERTON, OREG.	10/1/1963	9/30/1979	14198500 r^2=0.84
Pudding River at Saratoga Road	14201000	PUDDING RIVER NEAR MOUNT ANGEL, OREG.	10/1/1939	3/31/1966	14200000 r^2=0.87 14202000 r^2=0.91 14198500 r^2=0.74
Zollner Creek at Monitor McKee Road	14201300	ZOLLNER CREEK NEAR MT ANGEL, OR	7/1/1993	9/30/2005	
Pudding River at Hwy. 214 and 211	14201340	PUDDING RIVER NEAR WOODBURN, OR	10/1/1997	9/30/2005	
Pudding River at Hwy. 99E (Aurora)	14202000	PUDDING RIVER AT AURORA, OR	10/1/1928	12/3/2006	14201340 r^2=0.99

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