Translator Development for Bacterial Indicator TMDLs

Prepared for CDM Smith

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McLellan Lab *E. coli* Translator Development

This analysis and results memorandum has been prepared for CDM Smith and the Milwaukee River Basin TMDL development team for development team review and use in Total Maximum Daily Load (TMDL) analysis for bacteria. The water quality standard for bacterial indicator organisms in the Kinnickinnic (KK), Menomonee (MN) and Milwaukee (MKE) River watersheds is based on fecal coliform concentrations, whereas the water quality standard for the Milwaukee Harbor Estuary (EST) and nearshore Lake Michigan is based on *Escherichia coli* (*E. coli*) concentrations. Milwaukee Metropolitan Sewerage District (MMSD) monitoring programs measure fecal coliforms in the rivers and the HSPF/LSPC models output used for TMDL development is based on fecal coliforms. A "translator" is necessary to convert fecal coliform loadings to *E. coli* loadings for TMDL development in the Milwaukee Harbor Estuary. Fecal coliforms encompass several different organisms, with *E. coli* being one of these. However, the proportion of *E. coli* in the fecal coliform measurement is not consistent and depends on the source and age of the fecal pollution. Across many samples (n=497), there was a wide range of *E. coli* to fecal coliform ratios (Figure 1). We examined the influence of different contributing rivers, hydrological conditions and level of fecal pollution on the relationship between fecal coliforms and *E. coli* to determine if there were more reliable correlations seen under specific conditions—in which case multiple conditional translators would be necessary. Alternatively, we examined what ratio could be used that could be reliably applied to encompass the variability seen among the different conditions.

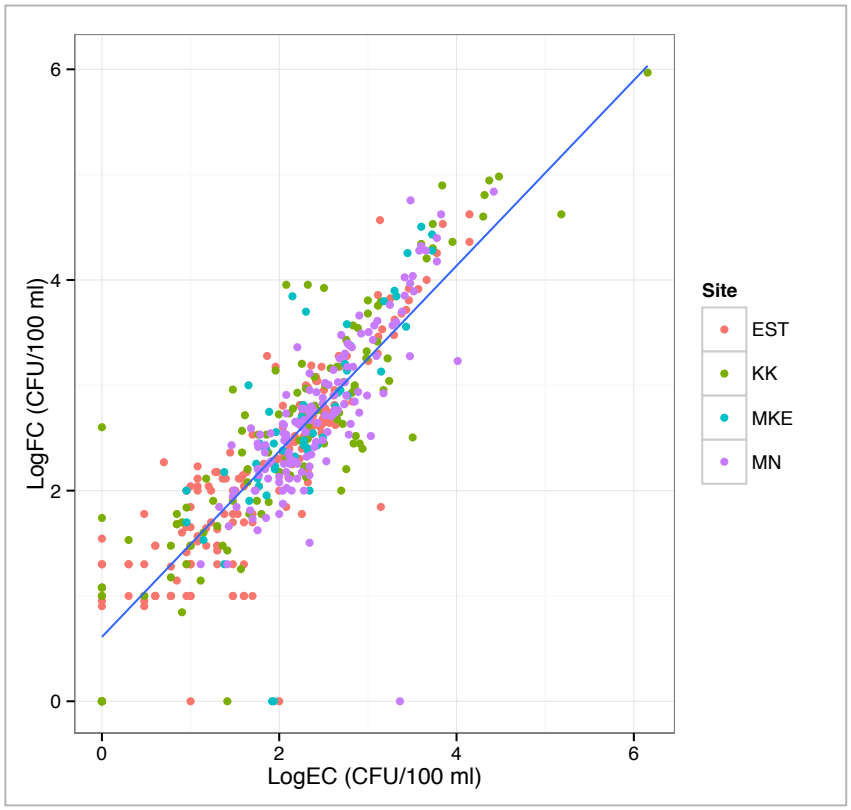


Figure 1. *Scatter plot of E. coli versus fecal coliforms from Milwaukee rivers and harbor estuary. Bacteria were measured by plate count in colony forming units (CFU). Linear regression of all data produced an R^2 of 0.74.*

Methods

Samples

We made use of existing data available through Milwaukee Metropolitan Sewerage District routine monitoring programs and the McLellan laboratory. Site locations are mapped in Figure 2, and Table 1 reports the number of samples collected and processed from each site. For MMSD samples, *E. coli* by Colilert® data was available for rivers, but is not comparable. The Colilert® method is based on recovery and growth of *E. coli* in liquid media, which recovers injured or stressed organisms more readily than membrane filtration and growth on solid media, which is the most common method used for quantifying fecal coliforms. Therefore, we used split samples from MMSD water quality program to evaluate *E. coli* (EC) and fecal coliform (FC) concentrations using the membrane filtration plate count method. River samples are listed in Table 1. In addition, samples were collected in the Milwaukee harbor estuary by the McLellan lab using an automated sampler (ISCO). Located at the Jones Island station, the discreet ISCO sampler collects 250 ml samples at programmed time intervals through an intake tube extending 6-7 feet into the channel. The ISCO samples complemented McLellan lab river samples as they were generally collected during the same time frame. Overall, we assessed data points from 497 samples to develop the translator.

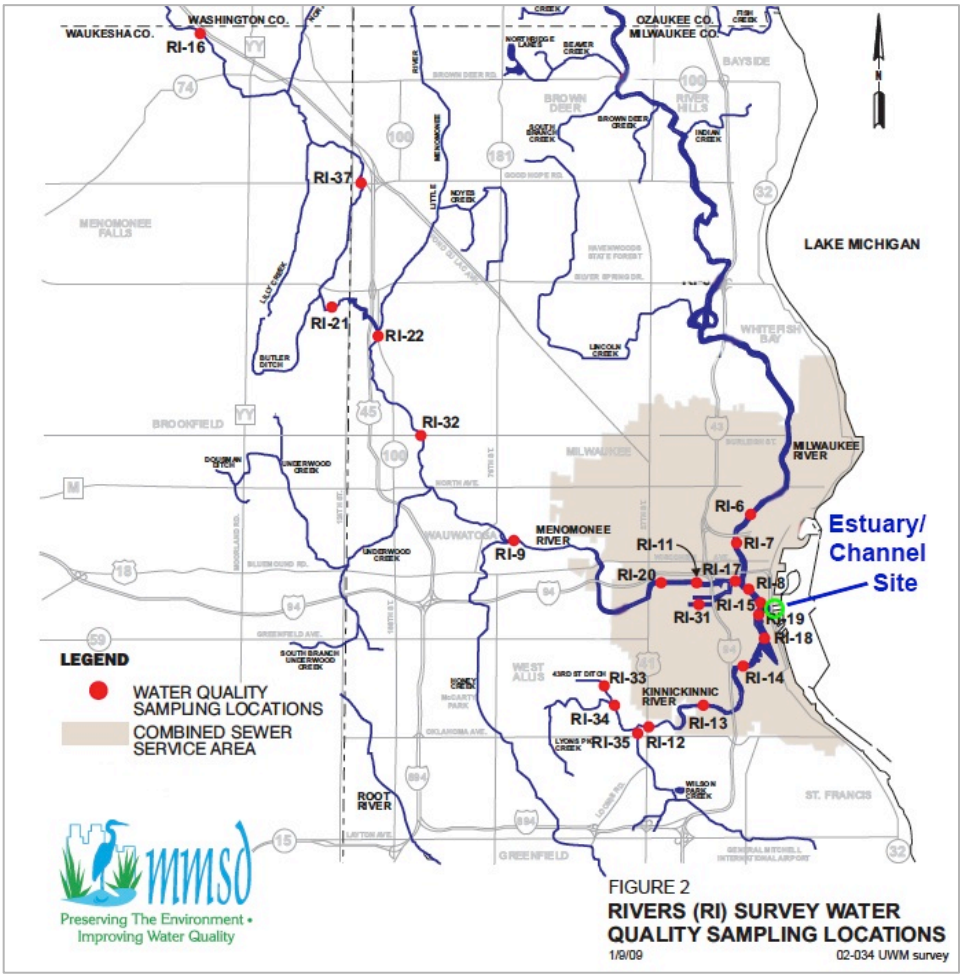


Figure 2*. Sampling sites used for E. coli and fecal coliform plate count assays. Modifications were made to a base map provided courtesy of Milwaukee Metropolitan Sewerage District.*

Table 1. *Collection sites and number of samples collected. Rivers samples from March 2011 – January 2013. Estuary/Channel samples from March 2011 – July 2014.*

Watershed	Sites	Total Samples
Kinnickinnic	RI12	12
Kinnickinnic	RI13	13
Kinnickinnic	RI14	26
Kinnickinnic	RI18	11
Kinnickinnic	RI19	12
Kinnickinnic	RI33	12
Kinnickinnic	RI34	13
Kinnickinnic	RI35	13
Menomonee	RI09	13
Menomonee	RI11	12
Menomonee	RI16	12
Menomonee	RI17	27
Menomonee	RI20	13
Menomonee	RI21	13
Menomonee	RI22	13
Menomonee	RI31	13
Menomonee	RI32	12
Menomonee	RI36	13
Milwaukee	RI06	14
Milwaukee	RI07	13
Milwaukee	RI08	13
Milwaukee	RI15	12
Estuary	Channel	192

Statistical Analysis

We assessed samples to answer three questions:

1. *Do we need to develop a separate translator for each river that would be proportionally combined (i.e. based on flow) for the estuary calculation?*

> We answered this by calculating the EC/FC ratios for all samples from each river and performing student's t-tests to see if the rivers showed significant differences between their average EC/FC ratios.

2. *Do different flow regimes have any effect on the EC/FC ratios of the rivers and channel?*

> We answered this by using ANOVA analysis to check for significant differences between EC/FC averages for each river reach and for the channel under different flow regimes.

3. *Do FC levels (ranging from low to high) have any effect on the average EC/FC ratios.*

> We answered this by using ANOVA analysis to compare average EC/FC ratios in the rivers and estuary for samples that had low, medium, high, and very high FC levels.

Analysis of data

1. *Do we need to develop a separate translator for each river that would be proportionally combined for the estuary calculation?*

To answer this question we calculated the EC/FC ratio for all samples from each river (Table 2 and Figure 3). We then looked for differences between the rivers by performing t-tests for equal means on each set of rivers, comparing their average ratios. No significant difference in average EC/FC ratio was found for any of the rivers. Table 3 shows the p-value calculated for each river pair. After a Bonferroni correction for multiple t-tests, all p-values are greater than $\alpha_{adjusted} = 0.02$ which tells us there is not a significant difference between the rivers in average EC/FC ratios. We interpreted this result to mean that we do not need to create a different translator for each river.

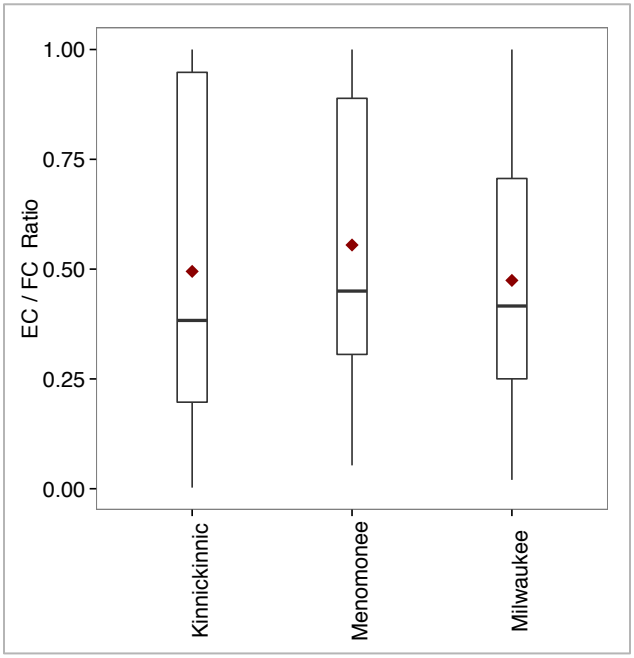


Figure 3. *Boxplots showing the range, median and average (red triangle) values for overall EC/FC ratios of each river.*

Table 2. *Average EC/FC ratios for sites on Milwaukee's rivers (± 1 standard deviation).*

River	KK (n=112)	MN (n=141)	MKE (n=52)
Average EC/FC	0.49 (±0.35)	.55 (±0.30)	.47 (±0.30)

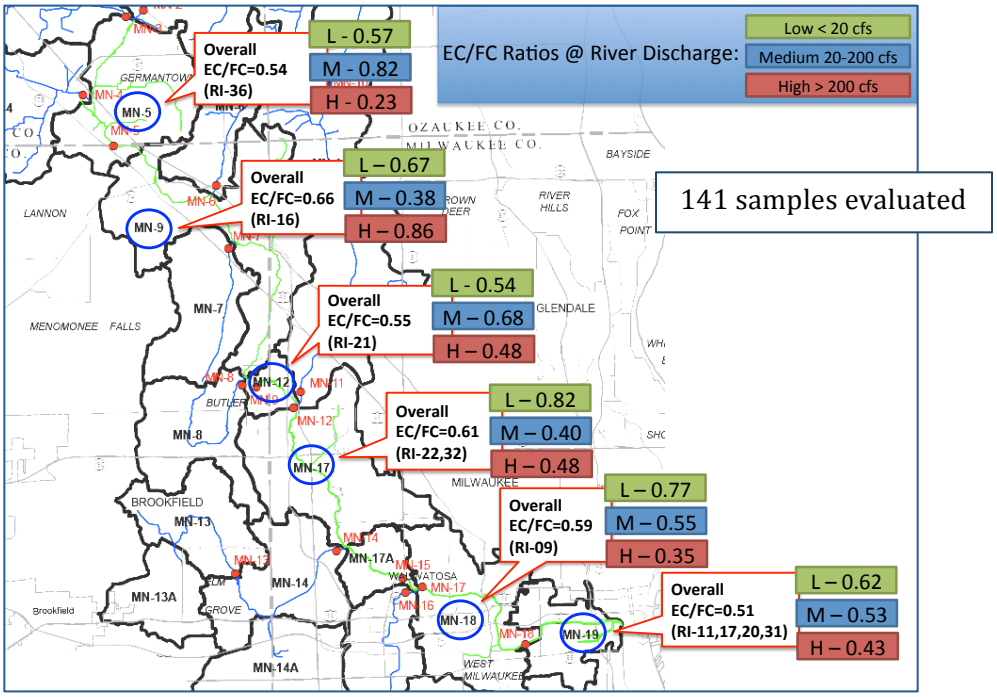
Table 3. *Results of two sample sets with unpaired t-tests comparing mean EC/FC ratios for each set of rivers. For multiple comparisons Bonferroni corrected* $\alpha = 0.02$.

T-Tests EC/FC Ratios	
RIVERS	**p-value**
MKE-MN	0.10
MKE-KK	0.70
KK-MN	0.15

2. *Do different flow regimes have any effect on the EC/FC ratios of the rivers and channel?*

To answer this question we calculated the EC/FC ratios for each river reach (Figure 4) at low, medium and high discharge rates. Because the rivers are different sizes, the discharge ranges included in each bin vary by river and are shown in Figure 4A, 4B and 4C. We then looked for differences between rivers in the EC/FC ratios within the binned discharge rates. Using ANOVA to test for significant differences ($\alpha = 0.05$), we found no significant difference in average EC/FC ratios between any of the rivers within the low, medium or high bins.

A.



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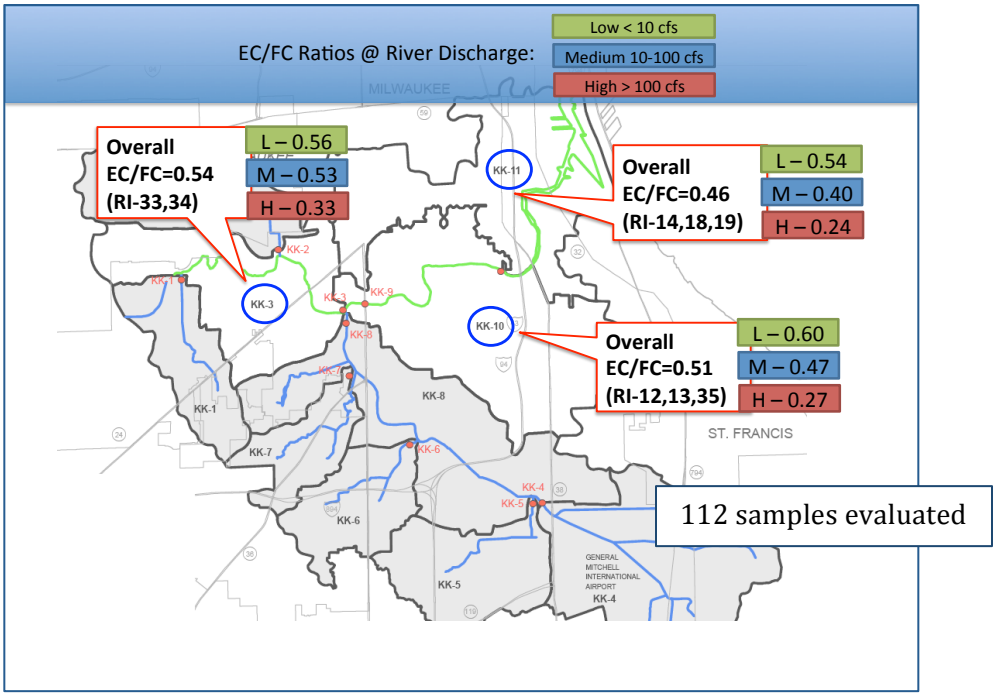

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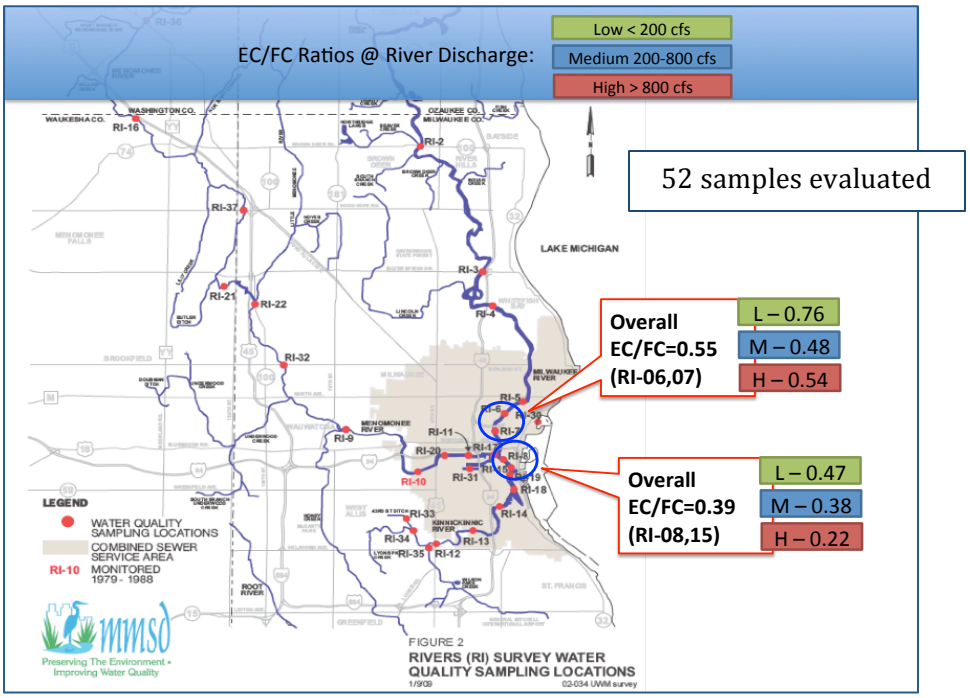

D.

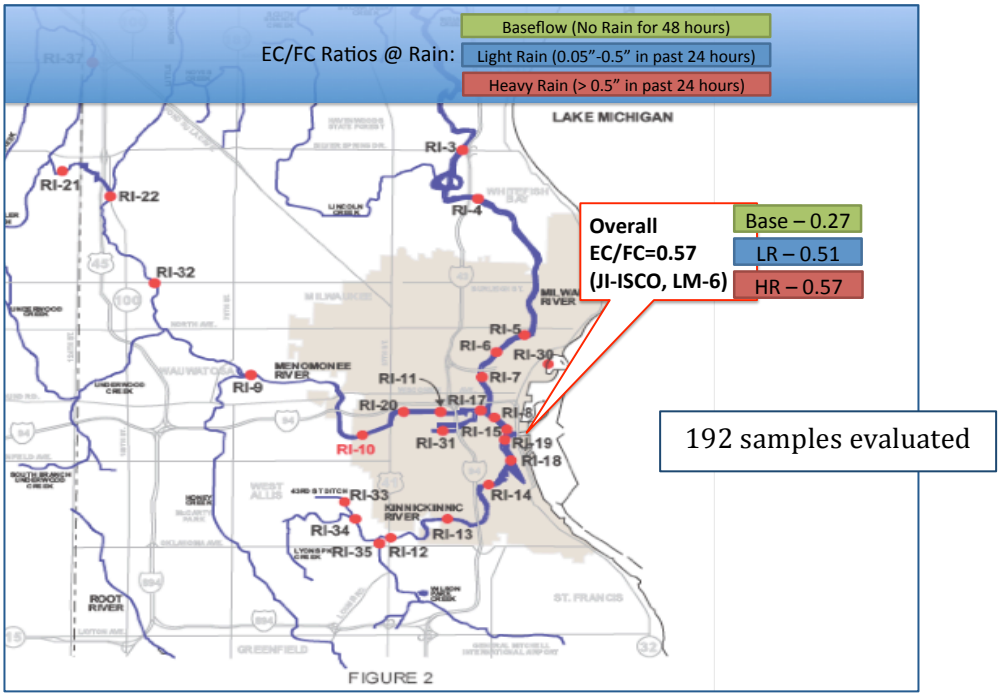

Figure 4. *Maps with sample locations for river reaches show EC/FC ratios for each reach from (**A**) Menomonee River, (**B**) Kinnickinnic River, and (**C**) Milwaukee River. The overall ratio for each reach is shown in the white box and ratio at low, medium and high discharge rates in cubic feet per second (cfs) in green, blue and red boxes respectively. The estuary/channel site (**D**) shows EC/FC ratios with overall weather conditions in the white box and baseflow, light rain, and heavy rain in green, blue, and red boxes respectively.*

Because there were no significant differences between the river EC/FC ratios, we combined river data for each discharge condition and recalculated mean EC/FC ratios at the various discharge rates for the composited data (Figure 5). For the composited dataset we found that at medium and high discharge rates, the mean ratio of *E. coli* to FC is significantly lower ($p < 0.001$) than the ratio at low discharge rates, meaning that at medium and high discharge rates the number of FC that are *E. coli* is smaller than at low discharge. These composite river datasets match the overall trend we found in individual river reaches as seen in Figure 4A-D.

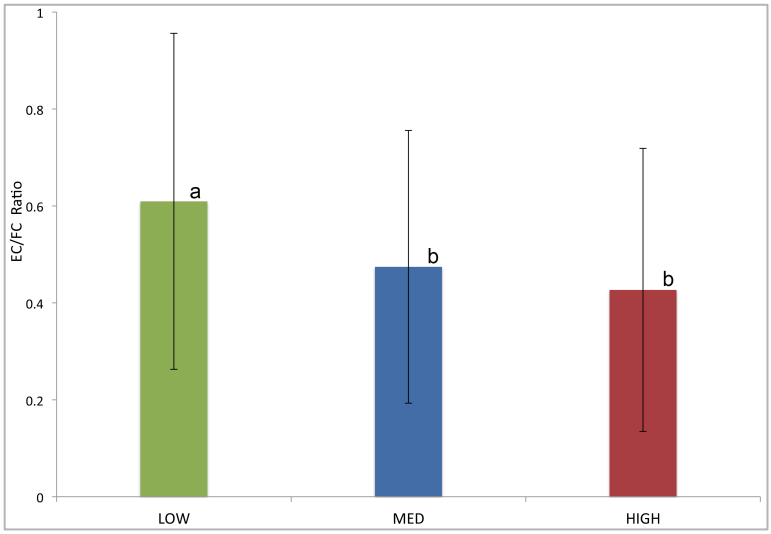

Figure 5. *Mean (± 1 SD) EC/FC ratio for low, medium and high river discharge rates using combined rivers in each discharge range. Letters above bars show significant differences.*

At the estuary/channel site the hydrodynamic effects of Lake Michigan cause bi-directional flow which is difficult to measure, therefore instead of binning by discharge rate we calculated the EC/FC ratios in baseflow, light rain and heavy rain conditions (Figure 4D). In contrast to the river dataset, the mean EC/FC ratio in baseflow conditions was significantly lower than in light and heavy rain conditions. Baseflow samples were collected when there had been no rainfall in the previous 48 hours, and would be comparable to low discharge rates from the rivers. In low discharge/baseflow conditions it is expected that a larger fraction of fecal pollution would be due to sewage from leaking infrastructure, as overland runoff carrying other fecal sources is minimal. Sewage has a higher fraction of *E. coli* than overland runoff, which is seen in the larger EC/FC ratios in the rivers during low discharge. However, at low discharge rates it takes longer for river fecal pollution to reach the estuary and *E. coli* dies at a faster pace than other fecal coliforms. Thus the difference in river and estuary EC/FC ratios can be explained by the pollution source and differential survival rates for various fecal coliforms.

3. *Do FC levels (ranging from low to very high) have any effect on the average EC/FC ratios.*

To answer this question, first we looked for differences in average FC counts and average *E. coli* counts between the rivers. We found there was not a significant difference in average FC count between any of the rivers by either ANOVA analysis (for log transformed counts) or by Kruskal-Wallis one-way analysis of variance (for raw counts). For *E. coli* counts, log transformed data (ANOVA, $F(2, 302) = 4.05$, $p = 0.02$) and raw data (Kruskal-Wallis, $x^2 = 6.175$, $p = 0.05$) showed a significant difference between the Kinnickinnic and Menomonee rivers. However, since the range of *E. coli* counts overlap substantially among the three rivers (Figure 6), we found it reasonable to combine river data to evaluate the effect of FC count ranges on EC/FC ratios.

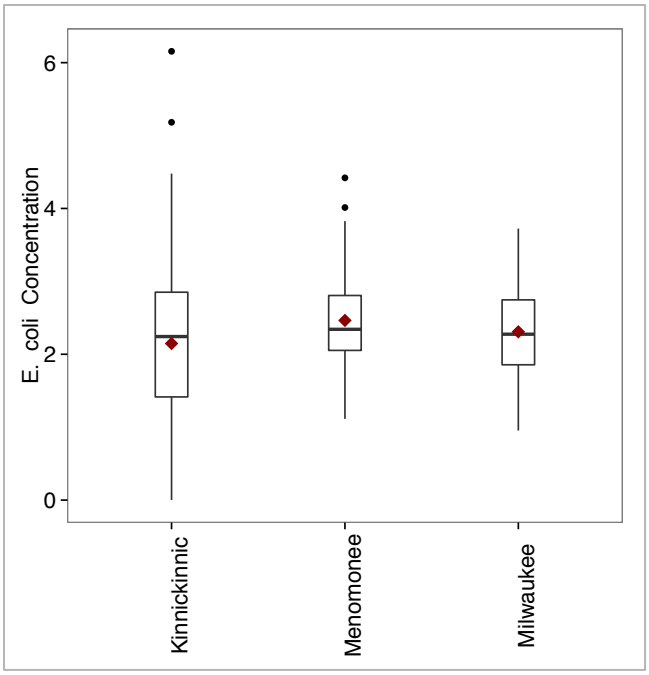

Figure 6. *Boxplots showing median and mean (red triangle) values for E. coli concentrations (CFU/100 ml) of each river.*

The composite river data was divided into low, medium and high FC count ranges and the associated EC/FC ratios were compared. As seen in the Table 4A, we found that at high FC counts the ratio of *E. coli* to FC is smaller than at medium and low FC counts. Meaning that, when FC counts are highest, the proportion of FC that are *E. coli* is smaller than when there are lower levels of FC present. This was true for the rivers and the channel (Table 4B). This is likely due to multiple factors including the age of pollution and the combination of sources.

Table 4. *Average EC/FC ratios for samples with low to very high fecal coliform ranges in (A) the combined rivers and (B) the estuary/channel.*

A.

Range	n	FC Count	Rivers EC/FC Mean	SD
Low	54	1 – 99	0.63	0.36
Medium	158	100 – 999	0.58	0.31
High	67	1,000 – 9,999	0.36	0.25
Very High	26	≥ 10,000	0.31	0.23

B.

Range	n	FC Count	Estuary EC/FC Mean	SD
Low	61	1 – 99	0.51	0.34
Medium	72	100 – 999	0.49	0.26
High	24	1,000 – 9,999	0.34	0.17
Very High	6	≥ 10,000	0.33	0.20

Translator Development

The data collected over a two–year period encompassed a wide range of *E. coli* to FC ratios (Figure 1). There was no significant difference between average EC/FC ratios when comparing river flow regime or FC count ranges to bin EC/FC ratios for each river. Further, averages and standard deviations for the *E. coli* to fecal coliform ratios found in the river and estuary/channel water samples are in a similar range as shown in Figure 4 and 5 under Analysis 2. The averages are summarized in Table 5. There was not a significant difference in average EC/FC ratio between any of the rivers. This meant that flow conditions or locations did not correspond to specific high or low values in the wide range of *E. coli* to FC ratios found in this system and the data could be considered as a single dataset.

Table 5. *Average EC/FC ratios for sites on Milwaukee rivers and estuary/channel (± 1 standard deviation)*

Site	KK (n=112)	MN (n=141)	MKE (n=52)	Estuary (n=192)
Average EC/FC	0.49 (±0.35)	.55 (±0.30)	.47 (±0.30)	.48 (±0.29)

For this study, we found that a translator range of 0.55 to 0.65 encompassed the majority of EC/FC ratios under different conditions. Including all river and channel samples, 69% of our data fall into or below the translator EC/FC range and 31% fall above it. However, if we just consider the ratio of the *E. coli* water quality limit (235 *E. coli* per 100 ml) and the FC standard water quality limit of 400 FC per 100 ml, the ratio is 0.5875. Since 400 FC per 100 ml needs to be met regardless of attempting to meet *E. coli* standards downstream, and the lower range of the translator should be 0.5875.

Additional confidence can be gained from the fact that samples with EC/FC ratios that fall above the translator range tend to have low FC counts. In our dataset, which is large and includes various hydrodynamic conditions, a large number of samples with EC/FC ratios greater than the 0.65 (high-end of the translator range) have FC counts low enough to meet the water quality standard of 400 CFU/100 ml, which would meet the *E. coli* standard of 235 CFU/100 ml if a translator of 0.5875 was used. Overall, 10% of the samples from the dataset would have been underestimated for *E. coli*. We also calculated the number of samples where *E. coli* would have been underestimated using 0.65, as a translator and the number of samples underestimated for *E. coli* was also 10%. Figure 7 shows the variability of fecal coliform counts in a number of EC/FC ranges.

In summary, we tested the translator using the data from our system (n=497 from many different conditions and locations) to determine how many of the samples would have resulted in underestimated *E. coli* using the FC levels and a range of translators from 0.5875 (the minimum based on water quality standards) and 0.65 (the most stringent translator). A total of 31% of the samples had a ratio of EC/FC higher than 0.65, meaning there was more *E. coli* than the translator calculation would suggest. However, only 51 of these samples (10% of the total dataset) were above 361 FC, which translates to 235 *E. coli*. We also calculated the number of samples that would be underestimated for *E. coli* using 0.5875 (the lower translator value in the range), and again found 10% of the samples would be underestimated. This means that the distribution of data did not include samples "on the bubble" (between the translators) that would not meet water quality criteria. It is not possible to be 100% sure of staying within water quality standards at all times unless the translator is 1. A translator of 1 would be an overly conservative approach to achieving reliable water quality and thus we recommend using our translator range to provide a confidence level of 90%.

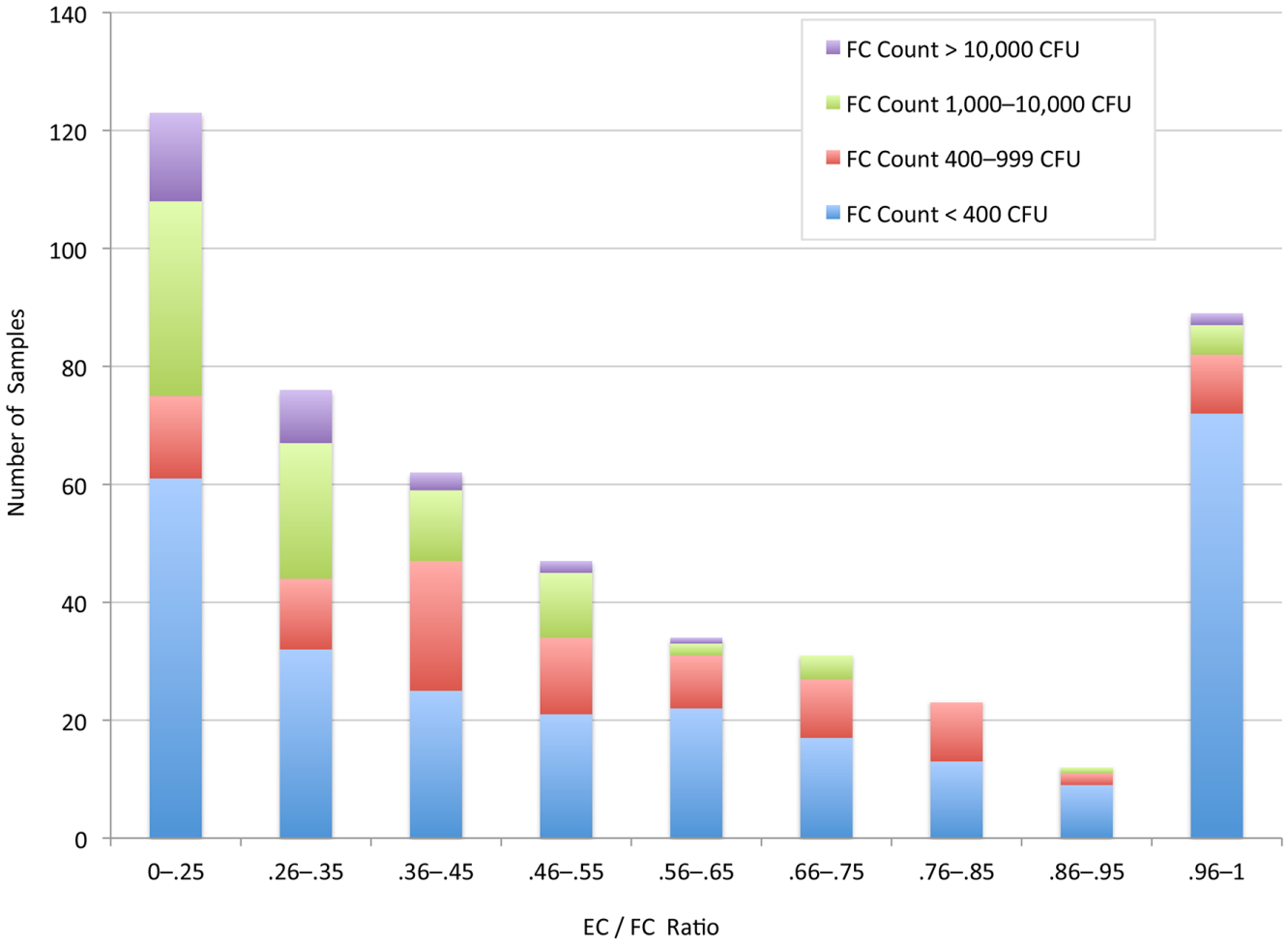

Figure 7. *Variability among the fecal coliform counts found in ranges of EC/FC ratios on Milwaukee rivers and the estuary/channel. Stacked bars represent the number of samples with various FC levels within each ratio.*

Conclusion

We recommend a translator of **0.5875 – 0.65** based on mean *E. coli* to FC ratios found in combined river datasets and in estuary/channel datasets. If a single number is used, we recommend 0.5875 for the translator. Data broken down by river, flow conditions and by range of FC counts gives us confidence in this translator range over variable conditions. Our results are consistent with the ratio of the recommended water criteria of 235 *E. coli*/100 ml for recreational waters and 400 FC/100 ml for rivers, which have a ratio of 0.5875.