This report addresses differences in results between concentrations of indicator bacteria groups commonly used to determine water quality suitability for water-contact recreation (REC-1). The two indicator groups are fecal coliform and *E. coli*. Simply put, fecal coliforms are a broad grouping of mammalian intestinal coliforms with *E. coli* as a subset of this larger group. As a subset, the expectation is that *E. coli* numbers will be somewhat less than those for fecal coliform in any given sample. In spite of this expectation, however, it is often assumed that the results will be identical and that *E. coli* concentrations can simply be substituted for those of fecal coliform in applicable standards. The confusion is compounded by the State of California's retention of the fecal coliform indicator as its basic freshwater standard in contrast with, and in opposition to, the EPA's adoption of *E. coli* as its standard. And the situation is further compounded in that most agencies doing their own testing are no longer analyzing for fecal coliforms, but are now measuring *E. coli* concentrations, having substituted a newer, faster and easier IDEXX methodology for the older tube-dilution procedure.

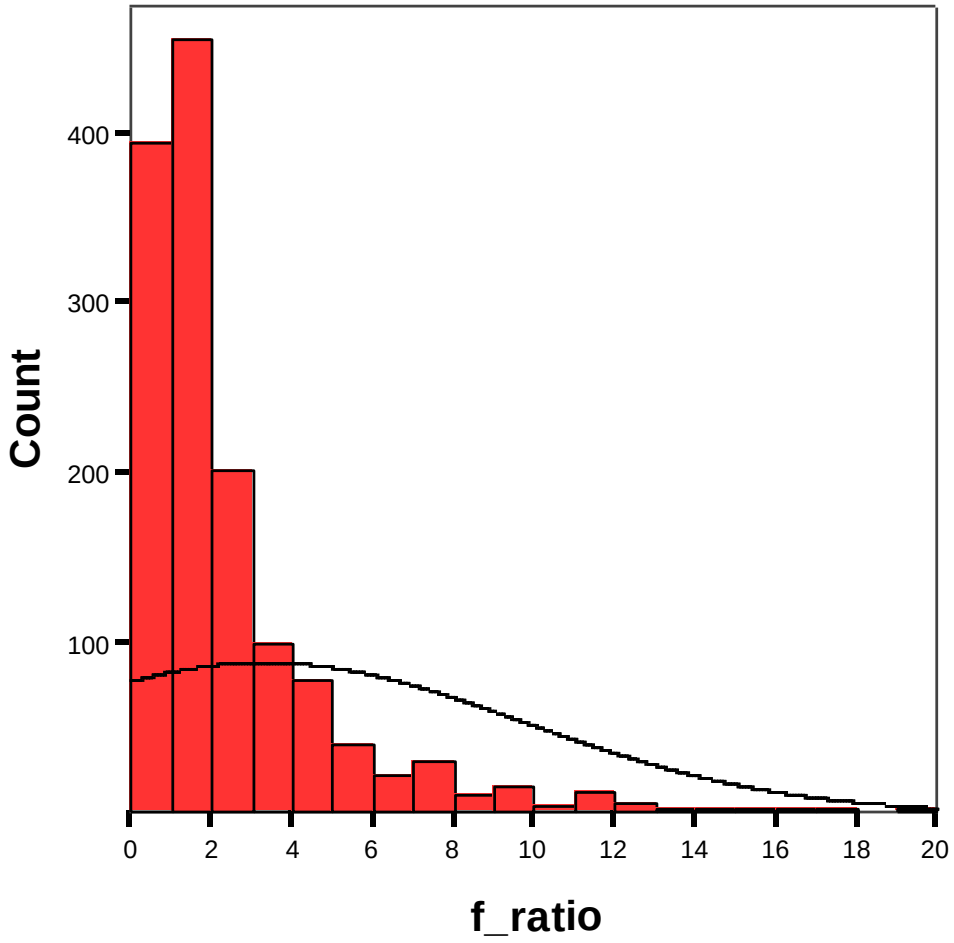
Having to meet a fecal coliform standard, but measuring only *E. coli*, *requires* an assumption as to the applicable ratio between concentrations of these two groups by any agency monitoring or regulating water quality. As stated above, by default this assumption is often 1:1. Besides being illogical, this has had the practical consequence of lowering acceptable standards; samples often regarded as acceptable were, in all probability, over the allowable fecal coliform limits. The situation is worsened by the State having *two* water quality standards utilizing fecal coliform concentrations: fecal coliform itself, and the fecal to total coliform ratio.

Since a number of water quality standards in present use state acceptable limits for *both* fecal coliform and *E. coli* an alternative to assuming a 1:1 relationship is to simply use the ratio implied by these dual standards. Typically, two standards are given: a single sample standard (not to exceed) of 400 fecal coliforms *or* 235 *E. coli*, and a 30-day geomean standard of 200 fecal coliforms *or* 126 *E. coli* (an example is the Central Coast RWQCB staff report on Resolution R3-2003-0031). (Concentrations are measured in the number of colony forming units (cfu) per 100 ml, more commonly expressed as most probable number in 100 ml (MPN/100 ml). Both the IDEXX and tube-dilution methodologies are statistical in nature and do not count actual bacteria, only their presence or absence.) Unfortunately, these two standards imply two different ratios: 400/235 or 1.70 and 200/126 or 1.59.

The third possibility is to actually use both methods of analysis on the same samples and derive a useable ratio applicable to a region. However, while simple in concept, it may not be all that simple in practice. The relationship may well vary between streams and with time of year. And, given that both test numbers are statistically derived, the probable error in any such analytically determined ratio may be extreme. But in furtherance of this aim, Mary Adams of the Central Board has been collecting these type of multiple test results and has graciously sent them to me. In a moment of weakness I volunteered to look them over and this report is the result.

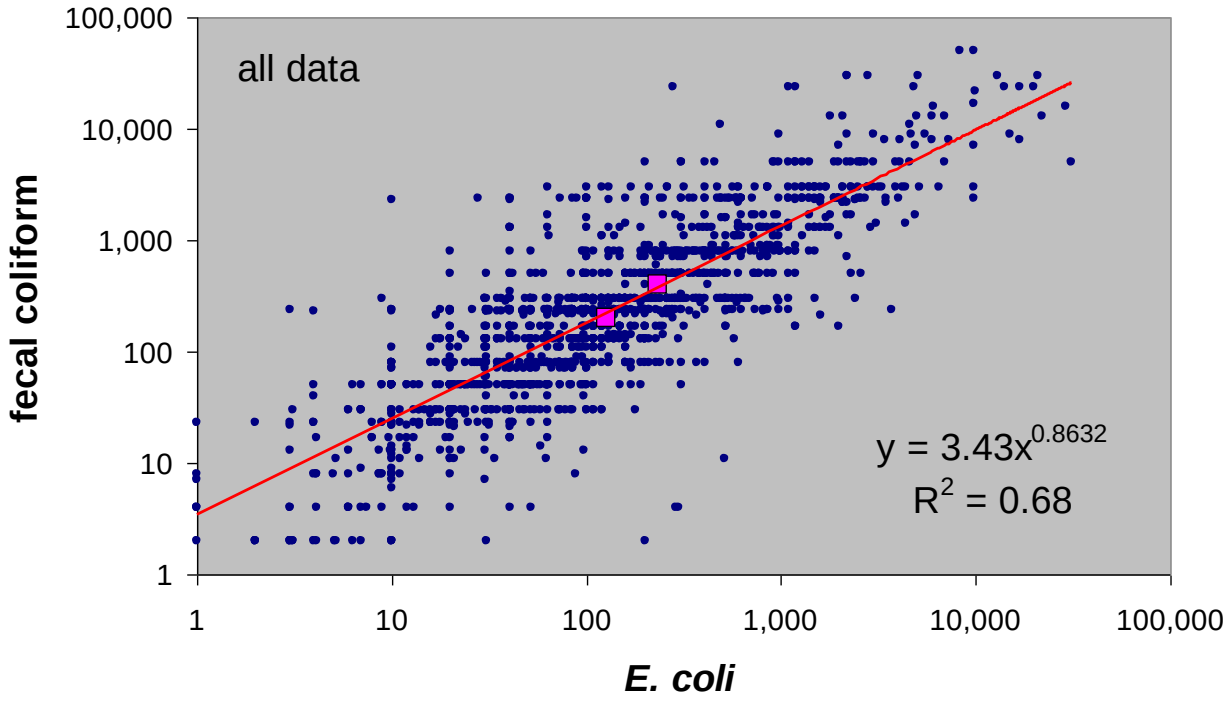
Mary's file contains sample results for total coliforms measured by both tube dilution and IDEXX, fecal coliform measured by tube-dilution and *E. coli* measured by IDEXX. There are 1455 individual samples from 125 locations in total; some locations have only 1 sample while the maximum number of samples from a single location is 44. Three of the total coliform, 19 of the fecal coliform and 15 of the *E. coli* results have negative values; these represent a total of 33 individual samples (some samples had more than one negative result). Not knowing what a

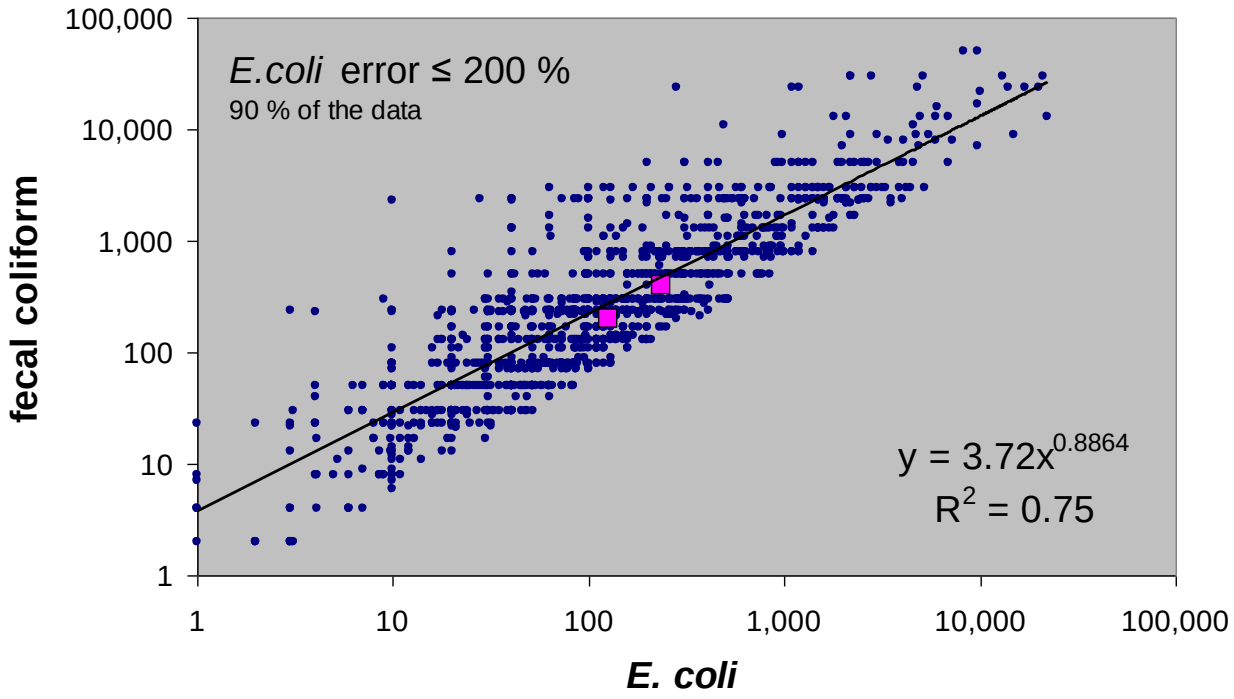
"negative" result might imply (and being too lazy to ask), and wanting to use logarithmic scales on any graphs, I simply eliminated these samples leaving a total of 1422. The fecal coliform to *E. coli* ratios for 1421 of these samples varied from 0.01 to 85.71 (eliminating one sample with a ratio of 230). A histogram of these ratios is shown in the histogram below (33 samples with ratios above 20 are not included).



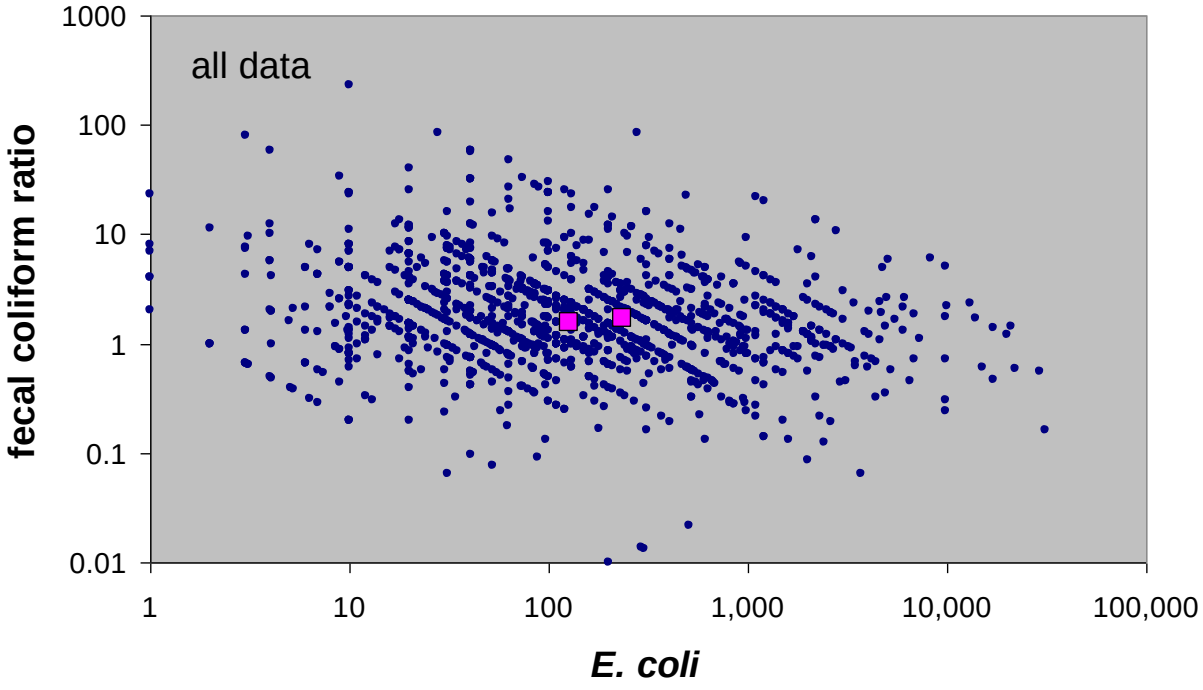
The distribution is highly skewed (the black line shows what a normal distribution of this data would look like) with a mean of 3.29 and a median of 1.65. Interestingly, the median value falls between the implied standard's ratios of 1.59 and 1.70 mentioned earlier. Eliminating all values less than 0.1 or greater than 10 reduced the mean and median to 2.19 and 1.55, respectively.

Plotting sample fecal coliform against *E. coli* concentrations yields the cloud of data points shown in the next figure. However, the power function shown on the graph does explain 68 % of the variation and reasonably coincides with implied standard's ratios (200 vs. 126 and 400 vs. 235; shown as large squares on the graph). I tried numerous ways of filtering these results, the third graph is one example: the % error between the actual fecal coliform concentration and a concentration estimated by multiplying the *E. coli* count by 1.7 (the implied 400/235 ratio) was calculated and any sample with >200 % error eliminated.

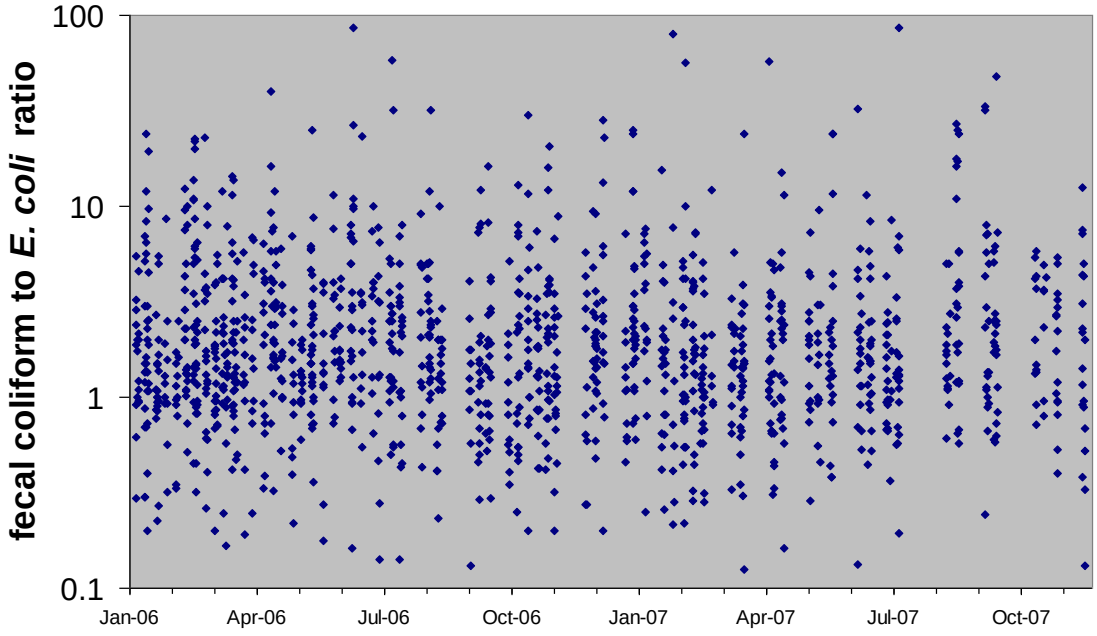


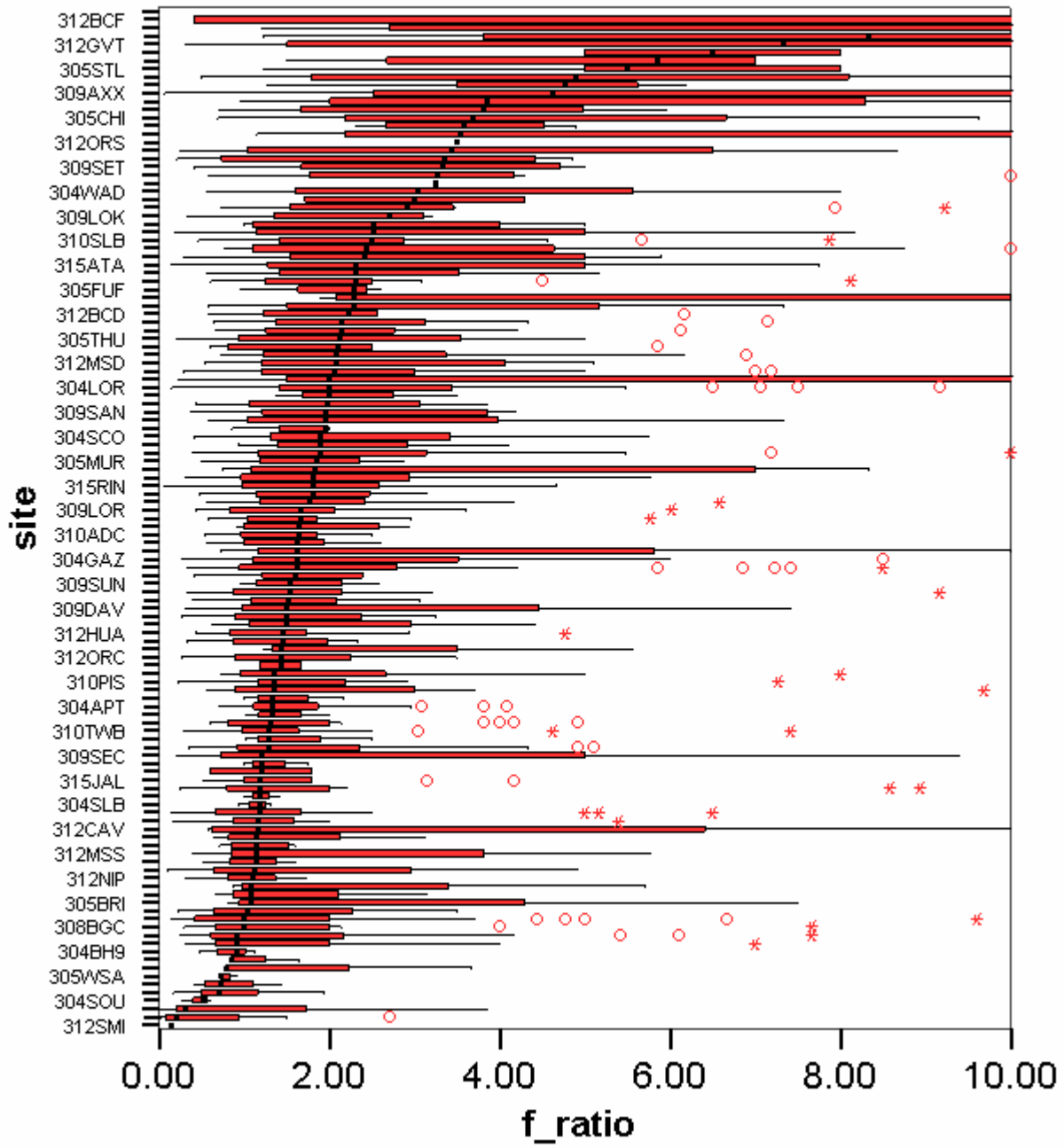


This filter eliminated 10 % of the data and slightly improved the power function regression line, but it also somewhat increased the predicted ratios: a 235 *E. coli* count being now equal to 470 fecal coliforms in contrast with 381 from the all-data equation. Other filters, such as setting a maximum fecal coliform concentration (e.g., < 500), and various other combinations changed the applicable regression equation and r-square values, but didn't affect the overall picture.



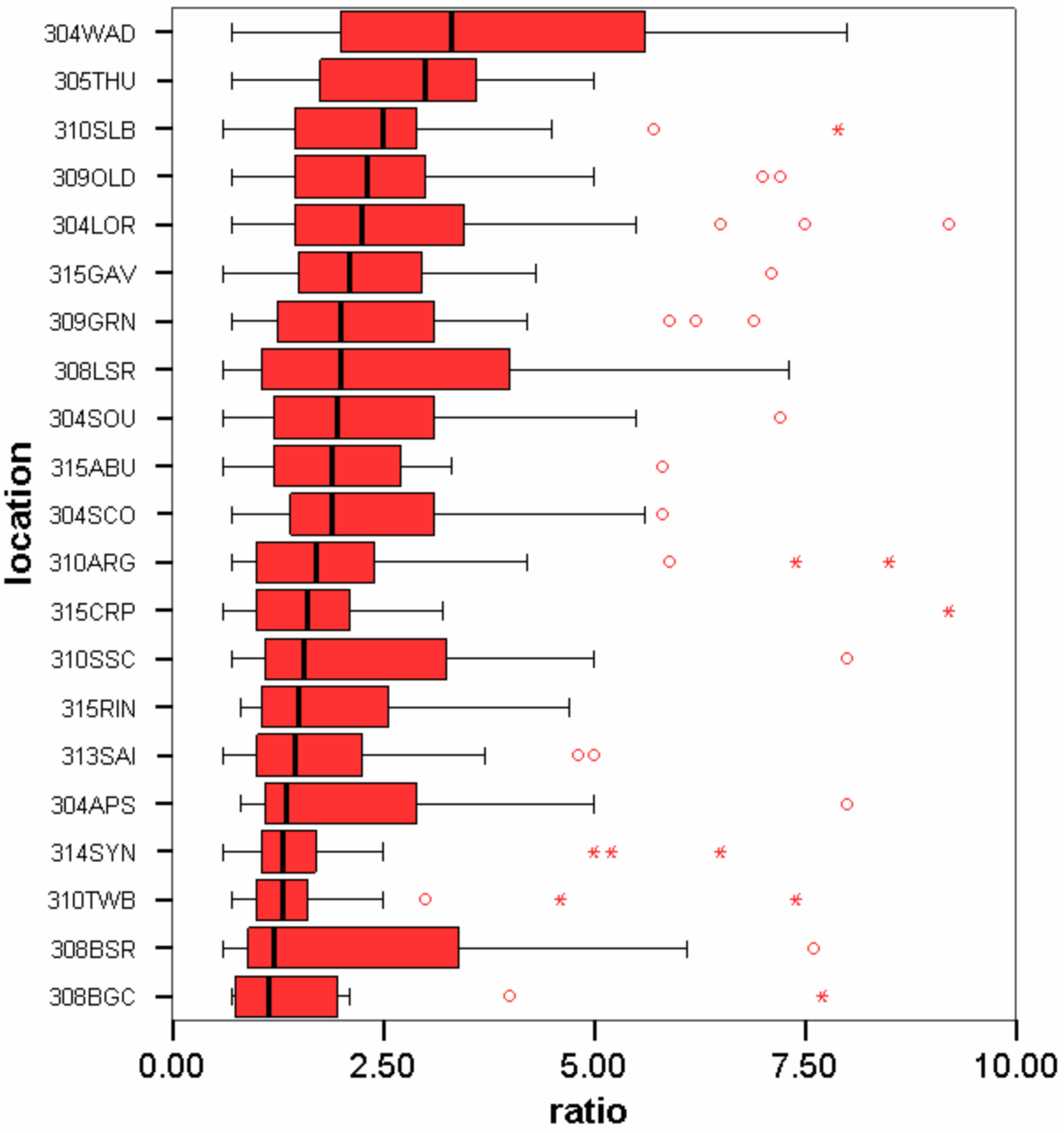
I also plotted sample fecal to *E. coli* ratios directly against *E. coli* concentrations with the result shown above. As in the other graphs, the implied standard's ratios are shown as large squares. That actual sample ratios vary over 3 orders-of-magnitude in the vicinity of the regulatory limits doesn't exactly help us reach any easily defensible conclusions. Before moving on I attempted to see if there were any obvious seasonal variations in the all-data data set, but gave it up as a lost cause (see graph below).



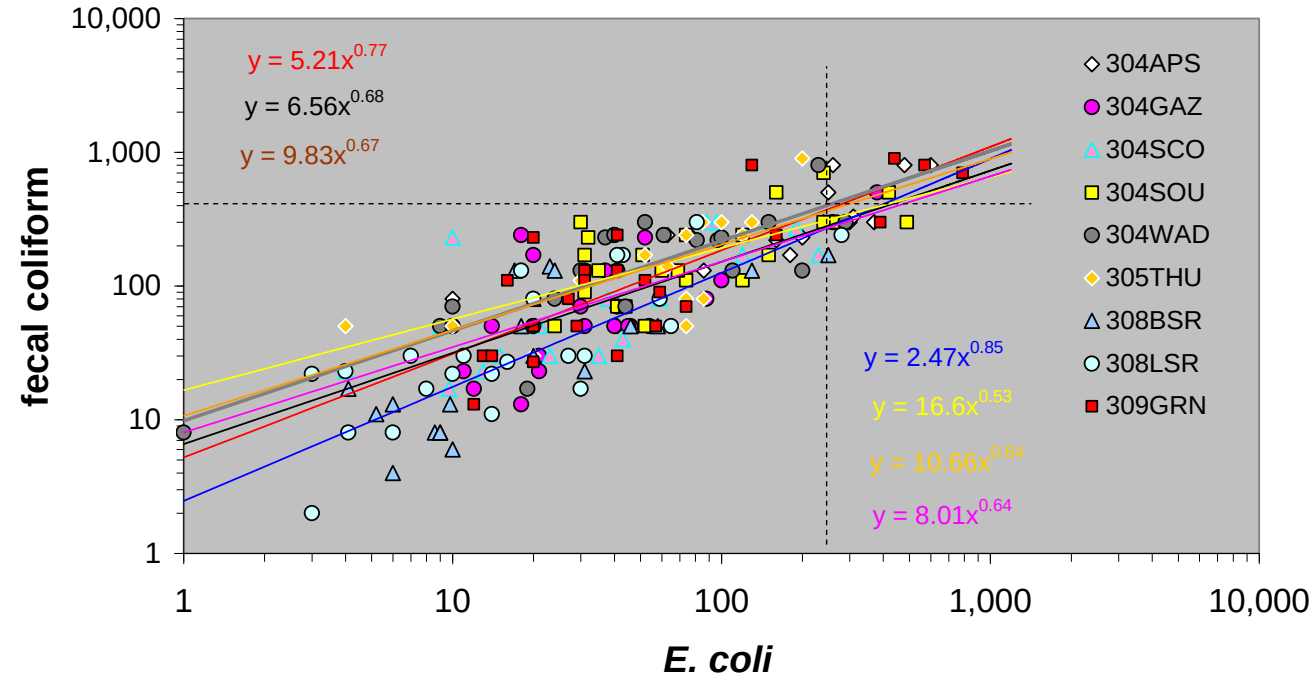


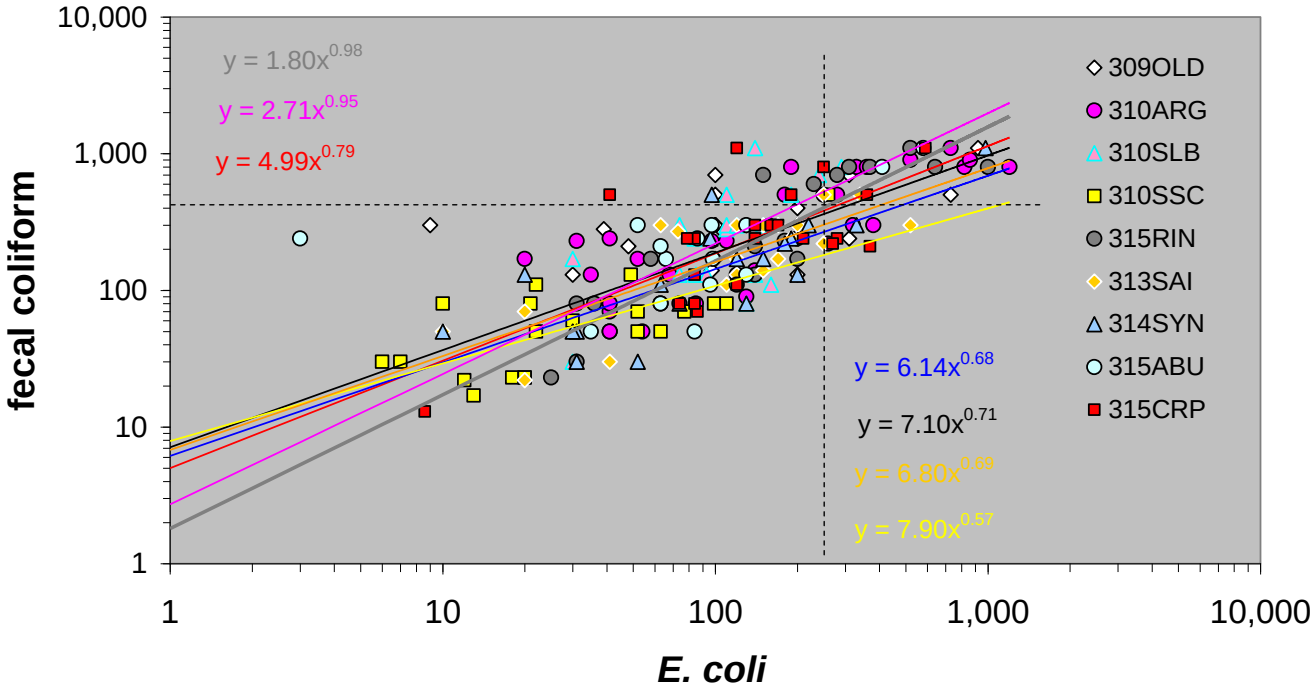
Moving on, I next looked at individual sampling sites. The above figure shows box plots of the fecal coliform to *E. coli* ratios for every location in the dataset. The ends of each box indicate the quartile points, i.e., each box covers the range of the middle 50 % of the data, and the whiskers extend to the highest and lowest values, excluding outliers (circles; values exceeding the inter-quartile range by 150 to 300 %) and extreme values (stars; values exceeding the interquartile range by more than 300 %). The heavy line inside each box indicates the median and I've arranged the various locations in order of increasing median values. Not every location is individually identified (only every third site). My purpose is not to provide a definitive look at each site where samples were collected, but an overall impression of the dataset.

That impression, unfortunately, is the same one given by the initial histogram and coordinate plots: there is an extremely wide variation in ratios and site to site variations are almost as extreme as those between individual samples. In order to reduce some of this complexity I eliminated any location with less than 15 samples; these are shown in the box plots below.



I have again arranged locations in order of increasing median values; the other box plot parameters are as before (including an arbitrary cutoff of any ratio values above 10); median values range from 1.17 (308BGC) to 2.31 (309OLD). The average median value for these 21 locations is 1.80; I find it comforting that this is close to the implied standard's 1.70 ratio.

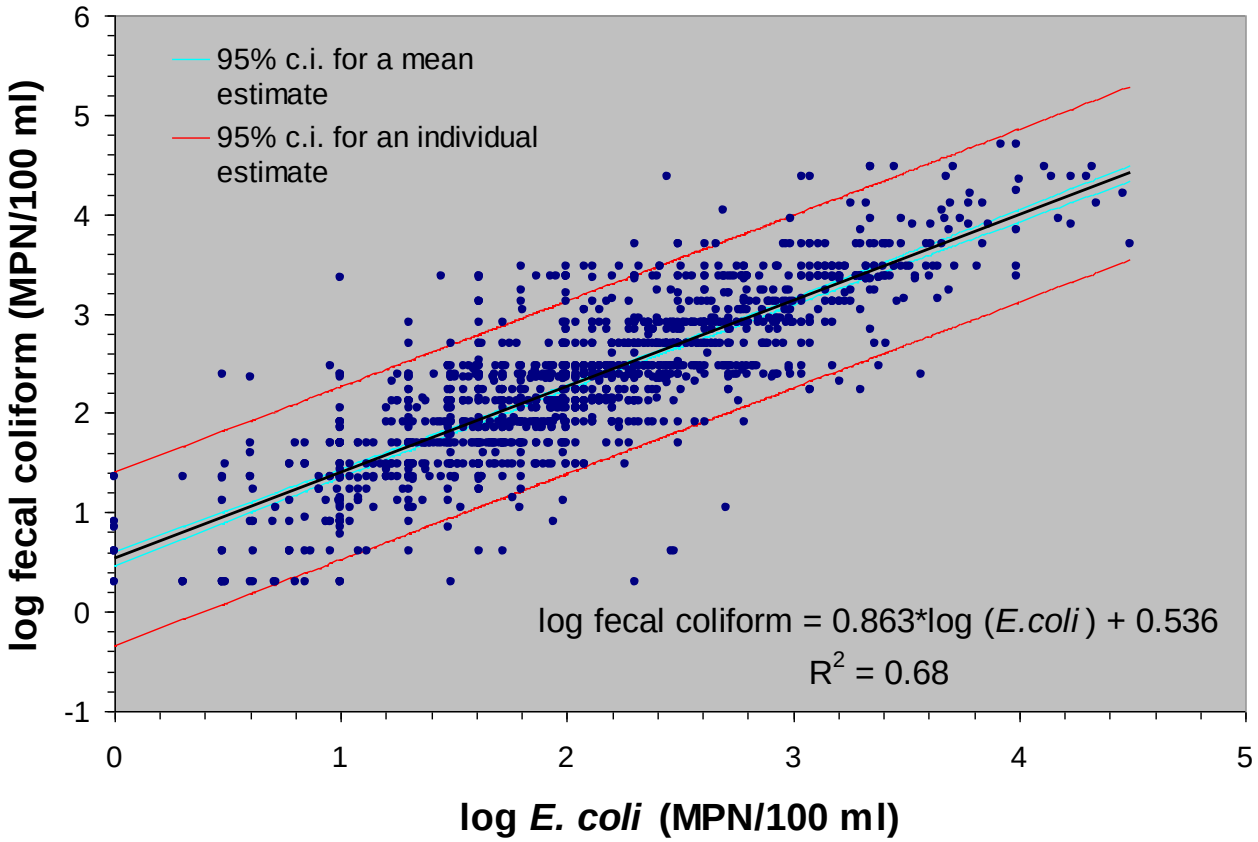




In the above graphs I've plotted data from 18 of these locations on log-log plots (the choice was arbitrary – the graphs were hard enough to interpret without trying to stuff in additional data). To provide a frame of reference, I've added dashed lines at the intersection of 400 fecal vs. 235 *E. coli*, the equivalence given in one of the implied standards.

I've shown power function regression lines for each of these sets of data; the regression equation for any particular line is shown in a similar color. Overall, the impression is probably more interesting than the details: almost all of the lines are similar and none differs greatly from the all-data equation (in the lower graph, r-squared values range from 0.40 to 0.85 and coefficients and exponents bracket those of the all-data relationship). If anything, the data in these graphs appears more tightly grouped, suggesting that sites with much fewer data are providing many of the stranger results in the all-data set. The spread amongst all the plotted points in the range of highest interest (say from 50 to 500) is generally less than an order-of –magnitude, i.e., more narrowly defined than in the all-data plot. However, combining data from all these points produces almost the same regression equation and r-square value as the larger overall dataset.

My feeling, given the similarities between each of these sites and between these individual sampling locations and the all-data dataset, is that there is very little to be gained concerning our major question by any further site by site examination.

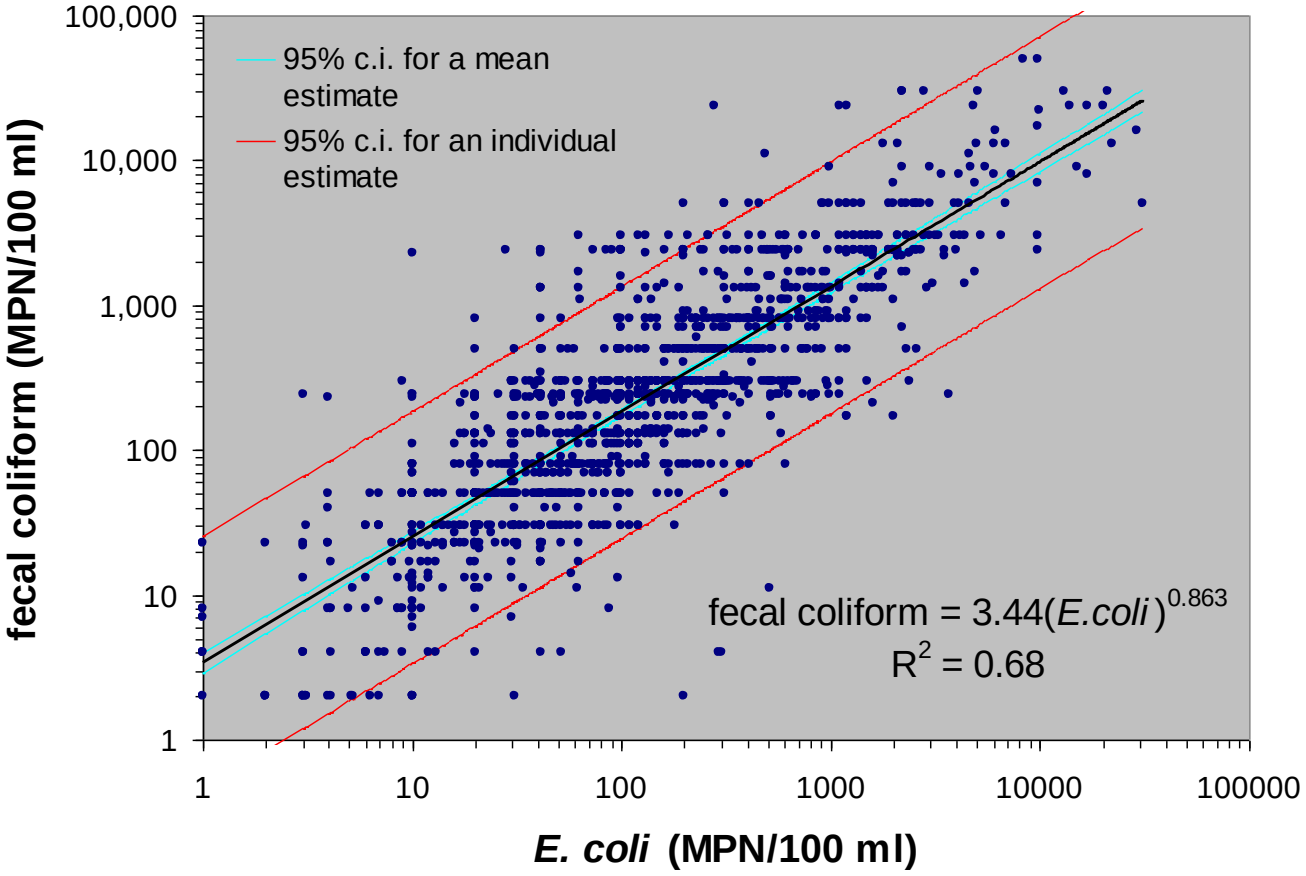


As a final step I want to return to the all-data regression equation. This is actually a very good regression relationship. It has a high r-square value (i.e., the equation can predict 68 % of the variability in the data) and a p-value of <0.0005 (a less than 5 in 10,000 chance that this relationship has occurred by accident); a log transformation of the data meets the required conditions of normality and the standardized residuals of the predictions are normally distributed and evenly dispersed. The log-transformed data, and the resulting linear equation, are shown above. I've also shown the 95 % confidence intervals (c.i.) for both a mean predictive and individual predictive response. Note that the bounds for these two types of predictions are vastly different. In other words, if we have a series of similar *E. coli* results we can enter this equation with their mean value and determine the expected mean fecal coliform concentration of these samples with a high degree of confidence. For example, if a series of *E. coli* samples are grouped around 235 MPN the equation will give us an equivalent fecal coliform concentration of 383; and we can be 95 % confident that the true value will lie between 362 and 405. If, however, we have a single *E. coli* sample with an MPN of 235 the equation will predict the same 383 fecal coliform concentration, but the 95 % confidence interval now extends from 52 to 2830.

With a lot of *E. coli* data we can make a good prediction as to what the average fecal coliform concentration was, but determining what is the fecal coliform concentration in a single *E. coli* sample – which is the very point in question here – well, we might be better off using a dart board. Therefore, my recommendation is that any indicator bacteria standards adopted by the Regional Board *should* include both fecal coliform and *E. coli* as separate alternative requirements.

Samples should meet *either* a fecal coliform standard *or* the *E. coli* standard.

And since the median ratios, howsoever we look at Mary's data, come very close to the 1.7 implied by numerous multiple standards already in use, I would further recommend that this be the adopted value. It should be applied not only in developing any *E. coli* standard (I'm thinking here of a possible relaxed "less than full body contact" or "occasional body contact" recreational standard, an REC-2 or REC-3 standard, if you will), but in calculating the fecal to total coliform ratio if it is retained in the new regulations (and I expect it to be retained). Put more plainly, any use of *E. coli* concentrations in calculating the fecal to total coliform ratio should first require multiplication by 1.7, i.e., the ratio should be calculated by *either* dividing fecal by total coliforms *or* by dividing 1.7 times the *E. coli* concentration by total coliforms.



And finally, this figure shows the 95 % mean and individual regression equation prediction confidence intervals on my original log-log plot.

Mary's data also includes sample total coliform concentrations analyzed by both tube-dilution and IDEXX methods. I analyzed those data but will reserve my observations for a separate report.