

THE AC/TC BACTERIAL RATIO: A TOOL FOR WATERSHED QUALITY MANAGEMENT

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ABSTRACT

An inexpensive tool for indicating microbial quality in watersheds is presented that utilizes the conventional total coliform test, comparing the relative concentrations of different colonies that form on a membrane filter fed by m-Endo media. These bacterial colonies can be classified into 3 types; typical (TC), atypical (AC), and background. The ratio of the concentrations of AC colonies to those of TC is related to water quality, fecal loadings, and fecal age. The AC/TC ratio relies upon shifts in populations between indigenous and introduced bacteria, with the indigenous bacteria providing a baseline against which the concentrations of the introduced are evaluated. When the AC/TC ratio is low (<5), fresh fecal material is in the water and pathogen risk can be expected to be higher. As time passes, the AC/TC ratio increases (>20) and can be related to healthier water quality conditions. Different types of runoff have different AC/TC values with human sewage at the lowest end of the spectrum with a value of 1.5 under normal conditions.

KEYWORDS:

Indicators; water quality; runoff; coliforms; atypicals

INTRODUCTION

In order to effectively manage a watershed impacted by nonpoint source fecal pollution, and reduce the risk of waterborne illness to recreational and other watershed users, one must have an easily applied tool, or analytical yardstick, to indicate problem areas, design effective interventions, and to measure intervention success. However, many of the analytical techniques developed for microbial water quality are expensive, meticulous, and difficult for the average engineer to apply. The ratio presented here is simple, uses well-known and easily obtained technology. The AC/TC ratio has proven to be an invaluable tool for initial

watershed screening by microbiologists, engineers, water plant operators, and even high school students. The ratio relies upon comparing a heterogeneous group of generally autochthonous bacteria that are stimulated to grow by nutrient enrichment (AC), to gram negative, fast-lactose fermenting, bacteria (TC) considered to be indicative of potential fecal contamination. Brion and Mao (2000) first published observations on the differences in the ratios of AC/TC obtained from several years of sampling runoff in urban and agricultural streams and ponds impacted by different fecal sources. Since then the AC/TC ratio has been used in several other follow-up studies and has been shown to be related not just to the predominant source of fecal material (Brion and Lingireddy, 1999; Brion et al., 2002; Booth and Brion, in press), but to the age of fecal material (Neiman and Brion, 2003; Booth and Brion, in press), and even to indicate general microbial watershed quality (Neiman and Brion, 2003, Booth and Brion, in press). Yet unpublished studies have shown the AC/TC ratio to be constant throughout conventional sewage treatment, although impacted by rain events, and to behave similarly in animal compost heaps as it does in the watershed environment. This paper will present a concise summary of these findings and provide an outline of how to apply this new bacterial ratio and interpret the results for watershed management of nonpoint sources of fecal pollution.

METHODS

Microbial analysis for total and fecal coliform and fecal streptococci used membrane filtration techniques as described in Standard Methods (APHA, 1992). Growth media were M-Endo broth, M-FC broth, and KF Streptococcus agar obtained from Difco and prepared according to directions for total coliform, fecal coliform, and fecal streptococci respectively. Each bacterial test was incubated at their specified temperature and counted at the specified time with respects to phenotypic colony appearance. On the total coliform plates, total (TC), background (BC), and atypical colonies (AC) were counted consistent with the criteria specified in Standard Methods (APHA, 1992). At least 3 dilutions of each sample, with 2 replicates per dilution, were analyzed to provide final counts. Statistical analysis of data was done utilizing SigmaStat, a program available from Systat Software Inc.

RESULTS

Identification of predominant fecal sources in surface water

In a multiyear study of a small watershed used as a supplemental potable water supply for the city of Lexington, Kentucky, USA, distinct differences in the AC/TC ratio from areas with different land-use associated fecal material types were noted. Samples were taken from numerous sites throughout the watershed and classified as suburban or agriculturally impacted, with suburban sites split further into impounded or free-flowing categories. Prior bacteriophage typing studies had not shown human associated, F-specific RNA phage to be present at the agricultural or suburban sites (Brion, Meschke, and Sobsey, 2002) so the predominate fecal sources were the animals observed to be associated with the land-use. Microbial samples were taken 1-2 times a week at these sites for a one year period and the information evaluated for insights into fecal source by the conventional fecal coliform/fecal streptococci ratio (FC/FS), and the new AC/TC ratio. Table 1 presents the average values for the AC/TC and FC/FS ratios, irrespective of weather conditions, at these different types of fecally impacted sites for the year studied.

As can be seen, the AC/TC ratios for surface waters impacted by different fecal types associated with observed land use is quite different, ranging from 103 for impounded suburban runoff to <5 for sewage. The geometric mean AC concentrations between the surface water sites were not found to be significantly different (average all sites = $10^{4.9}$ AC cfu/100mL), so the difference in the AC/TC ratios is due primarily to the differences in fecal coliform concentrations as referenced against the atypicals. These average AC/TC ratio values were found to be significantly different by Kruskal-Wallis one-way ANOVA ($H = 76.557$ with 3 degrees of freedom, $P = <0.001$) and between all pairwise comparisons, with the exception of flowing agricultural and sewage (Dunn's Method, $P > 0.05$). So, the AC/TC ratio was successful in identifying the predominant fecal source with significance with the exception of distinguishing between sewage and flowing agricultural runoff. When stratifying the data to exclude days when rainfall was impacting the quality of the influent sewage, the AC/TC ratio for sewage was 1.6, a value close to that previously reported from earlier studies by Brion and Mao (2002) of 1.5 and distinguishable from agricultural impacted surface water. The influx of aged fecal material from stormwater scour increased the AC/TC ratio from an average of 1.6 to 5.9, and decreased the FC/FS ratio from 3.8 to 2.8; both ratios moving towards values not representative of human sewage.

Table 1. Average bacterial ratios in surface water runoff sorted by land-use associated fecal sources

<i>Site Classification</i>	<i>Average AC/TC Ratio (n)</i>	<i>Average FC/FS Ratio (n)</i>
Impounded Suburban	102.6 (75)	3.7 (76)
Flowing Suburban	18.9 (134)	5.2 (124)
Flowing Agricultural	10.0 (59)	5.0 (45)
Raw Human Sewage	3.9 (11)	3.3 (11)

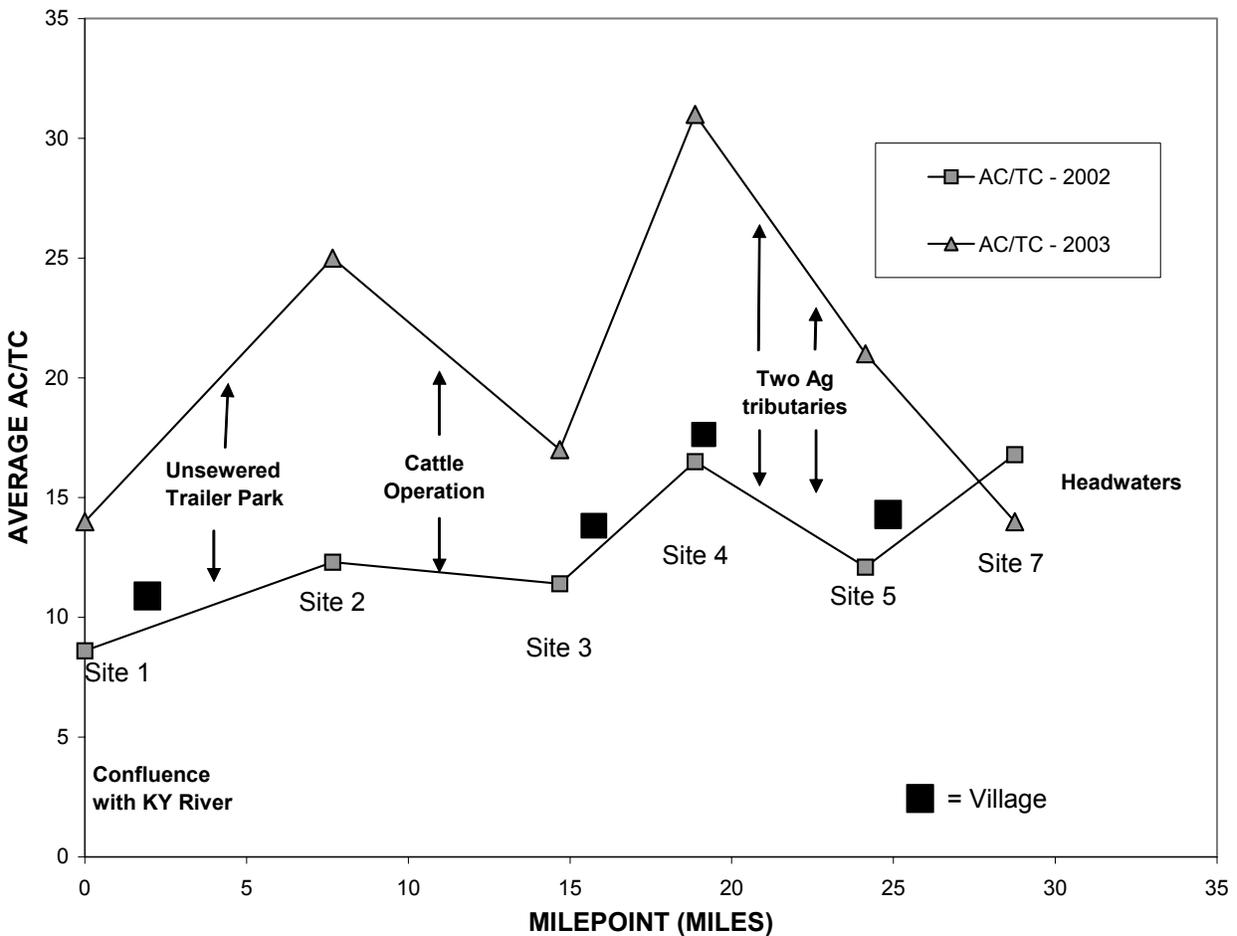
The FC/FS ratio was of little value in classifying the predominate fecal sources in this study. The differences in the average FC/FS values were not found to be significant by all pairwise multiple comparison procedures, except for comparing flowing agricultural stream quality with that of flowing suburban (Dunn's Method, $P < 0.05$ for significance), and these average values would indicate human sewage ($FC/FS \leq 4$) for sites impacted primarily by cattle feces.

Identification of hot-spots in watersheds

As reported by Booth and Brion (in press), significant drops in the average value of the AC/TC ratio were able to pinpoint inputs of known sources of untreated domestic sewage into a small creek before (2002) and after (2003) some of these communities were provided with a forced main sewer line. As can be seen in Figure 1, from the agricultural headwaters of this creek (Site 7) where the AC/TC ratio is >10 , and well

within the range of flowing surface water values cited above, the AC/TC ratio steadily declines to <10 with sharp decreases seen as it passes through inadequately sewered towns that add untreated human sewage to the agriculturally impacted creek. In the next year, after partial sewage service had been provided via a large forced main, the AC/TC ratios are overall higher along the creek. Areas that did not fully connect to the sewer main still showed significant declines in the AC/TC ratio after the creek absorbed their untreated sewage input. Average concentrations of indicator bacteria were not able to pinpoint the impacts of inputs of human sewage for either year as the concentrations at each site were statistically indeterminate, but the AC/TC ratio was able to pinpoint the human sewage impacted hot-spots along this creek with ease.

Figure 1. Changes in the AC/TC ratio along Eagle Creek



Fecal loading at Site 7 was mildly increased in 2003 as compared to 2002 and the AC/TC ratio slightly depressed from last years average as a result. This is thought to be due to a much wetter summer in 2003. The previous summer had long periods (weeks) of no precipitation, whereas the summer of 2003 had rainfall nearly every week providing fresh fecal inputs from land scour, reflected in a slightly depressed average AC/TC ratio at site 7 (16.8 for 2002 vs. 14 for 2003) and an increase overall in headwater fecal coliform (FC) concentrations. FC concentrations were not found to be statistically different between the sites based on the

geometric mean and in 2002 were 101 cfu/100mL at the headwaters rising to 149 cfu/100mL at the confluence with the Kentucky River. Overall, in 2003, FC levels fall along the entire length of the creek from a geometric mean of 140 cfu/100mL at the headwaters to 90 cfu/100mL at the confluence, showing the effectiveness of the sewer project, but this trend is not statistically significant. Indeed, T-tests do not indicate the FC levels in 2003 are significantly different from those of 2002 ($P < 0.01$), nor when compared between sites by ANOVA. As is the case examining FC values for both years along the length of the creek, the FC concentrations would not index any particular source as cause for the elevated FC levels as all geometric mean values are statistically indeterminate. Yet, the AC/TC ratio shows improvement in the creek.

Prediction of relative fecal age

It has been reported by Neiman and Brion (2003) that in both bench scale, batch laboratory studies on river water, and in the Kentucky River from which the water was taken, that the AC/TC ratio falls after an influx of fresh fecal material and then rises over time. Also, it has been observed that AC/TC ratios invariably drop in surface water after rain events as fresher fecal material is washed into the system (Brion et al., 2002; Neiman and Brion, 2003). Reviewing data from the Kentucky River, it was seen that after an intense period of rain, where the AC/TC ratio dropped below 3, it rose slowly to a value of 10 by the third clear day after the storms stopped, rising to a high of 79 on day 7, before dropping with the next storm event back into the upper teens. The only known exception to the drop in AC/TC ratio with rain is for domestic sewage as noted above where influx of aged material caused a rise. The drop in the AC/TC ratio in surface waters is due to a combination of factors, the die off of fecal coliforms and the growth of the atypical group in response to nutrient influx into the system. Both of these factors would cause a rise in the observed AC/TC ratio. In support of these published findings are presented the unpublished results of an animal manure study that shows the same rise in AC/TC ratios over time in two types of aging animal manure (Table 2). Briefly, fresh manure was obtained from nearby farms and put on a platform for two weeks. At the very beginning and at the end 5 replicate samples of leachate solution with varying amounts of manure were prepared and analyzed for bacterial content with the concentrations averaged.

Table 2. Changes in bacterial ratios from manure leachate over time

<i>Manure type</i>	<i>Fresh AC/TC</i>		<i>Aged</i>	
	AC/TC (n)	FC/FS (n)	AC/TC (n)	FC/FS (n)
Horse	0.2 (33)	0.4 (32)	11.8 (40)	24.4 (40)
Cow	0.5 (13)	0.4 (13)	2.9 (37)	143.5 (39)

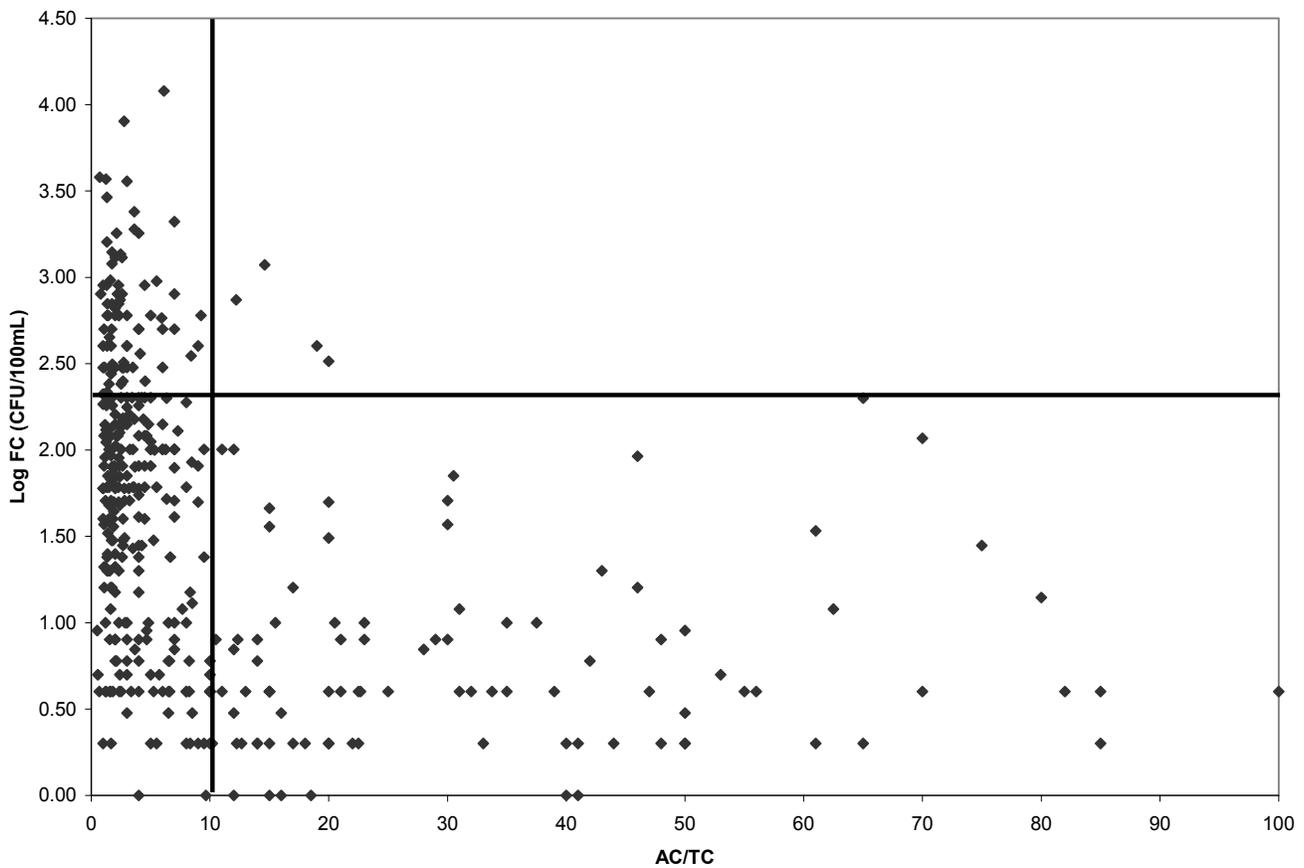
As is readily apparent, AC/TC and FC/FS ratios in fresh manure both start at values < 1 and rise over time. The average AC/TC values for both fresh and aged horse versus cow manure leachate are statistically different by Mann-Whitney Rank Sum test ($P < 0.001$), but the FC/FS are not. The rise in the AC/TC ratios seen in the controlled studies is expected to occur in the environment as it did in these pile studies, but this

finding has not yet been confirmed. This would confirm observations of higher AC/TC ratios from empty cattle feedlots during rain events. The aged fecal material gives off a higher AC/TC ratio than for fresh fecal material indicating the passage of time.

Using the AC/TC ratio for watershed quality control

It was proposed by Booth and Brion (in press) that the AC/TC ratio could serve as a surface water control standard for use in the Eagle Creek watershed presented in Figure 1, and that improvements in water quality by implementation of best management practices could be tracked by the relationship between increasing average AC/TC ratios and decreasing FC concentrations.

Figure 2. Relationship between the AC/TC ratio and FC loadings with respects to a 200 cfu/100mL limit in the Kentucky River



In the Eagle Creek study, the water was often considered hazardous for recreational contact due to FC and enterococci concentrations. The geometric mean value for FC concentrations for this creek were high, 151 cfu/100mL. During the time of study, 22 of 98 water quality samples from Eagle Creek had FC concentrations >200 cfu/100mL when the AC/TC ratios were greater than 10. Compare this to the results presented in Figure 2 above from analysis of the multiyear database from the Kentucky River (Neiman and Brion, 2003), with average FC concentrations of only 25 cfu/100mL. Figure 2 shows that only 4 of 408

water samples had FC concentrations greater than 200 cfu/100 mL when the AC/TC ratio was greater than 10. The figure also shows how the majority of high FC concentrations occur when the AC/TC ratio is below 10. Clearly, higher AC/TC ratios trend with lower FC loads, and lower AC/TC ratios occur with higher FC loads and as presented prior, fresher fecal contamination. Therefore, it should be a goal to increase the overall AC/TC status of a surface water source in conjunction with lowering the FC loadings. By aiming for a stable and higher AC/TC ratio in a watershed, not only will the total numbers of fecal indicators decrease, but the age of fecal material present will increase, reducing the numbers of potential pathogens.

CONCLUSIONS

The AC/TC ratio provides an easy to apply method to quantify and qualify the impacts of fecal materials into our surface waters. Measurements of the AC/TC ratio along creeks and rivers can help identify fresh inputs of human sewage and the impact of best management practices. This simple, easy, and economical approach to understanding and controlling watershed quality is within the grasp of engineers and public health agents and should be applied more frequently to validate its use in waters other than the eastern United States.

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