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INDICATOR BACTERIA CONCENTRATIONS OF TWO NORTHWEST ARKANSAS STREAMS IN RELATION TO FLOW AND SEASON

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ABSTRACT. Concentrations of indicator bacteria such as fecal coliform (FC) and fecal streptococcus (FS) are often used to assess the suitability of waters for their intended use(s) and to allocate resources for water quality improvement measures. There is evidence, however, that concentrations of FC and FS can be influenced by variables such as season and flow rate during sampling, which could lead to biased results. The objective of this study was to assess the impacts of season and flow rate on concentrations of FC and FS. Fecal indicator bacteria concentrations were measured for approximately three years at five sites on two Northwest Arkansas streams. Flow data were collected at two of the five sites. Land use in the basins draining the streams was primarily pasture (57-90%) and forest (6-40%). Significant seasonal influences on FC and FS concentrations were detected for all sampling sites, with the highest concentrations occurring in summer. On the two sites with flow data, flow rate generally had a significant effect on FC and FS concentrations during all seasons, with FC and FS concentrations increasing with flow rate. Ratios of FC and FS, which have been used in the past to differentiate between animal and human sources of fecal pollution, did not appear to reliably indicate the major sources of fecal indicator bacteria. The findings of this study suggest a potential for fixed sampling intervals to contribute to biased results. The issue of biased results might be avoided by sampling during times of year and flow conditions that support the intended use(s) of the waters.

Keywords. Stream flow, Water quality, Fecal coliform, Fecal streptococcus.

Indicator bacteria concentrations are often used to assess the quality of waters used for primary (e.g., swimming) and secondary (wading, fishing, etc.) contact. The most common measure of this type is the concentration of fecal coliform (FC) in the water. Fecal coliforms are enteric to warm-blooded animals, and their presence in water indicates the potential presence of pathogenic organisms. Typical FC standards for waters designated for primary and secondary contact use are 200 and 1,000 colony forming units (cfu)/100 mL, respectively (e.g., Arkansas Dept. of Pollution Control and Ecology, 1992). Fecal streptococcus (FS) analyses are also performed to assess water quality. Fecal streptococcus is predominately an enteric organism but is not as reliable an indicator of fecal pollution as FC, because non-enteric FS species are also native to insects, soil, and vegetation (Geldreich, 1976; Hunt et al., 1979; Kibbey et al., 1978).

Fecal coliform and streptococcus analyses are used not only to determine the general suitability of waters for their designated uses, but also to help prioritize the use of available resources to improve water quality. State

agencies, for example, routinely use FC analysis results to define focal points for both voluntary and regulatory water quality improvement programs. Unfortunately, FC and/or FS analysis results can sometimes give rise to more questions than answers. It is difficult, for example, to distinguish among the contributions of human versus animal sources, point versus nonpoint sources, and background versus above-background sources on the basis of FC and/or FS data. If these issues are unresolved, programs implemented to improve water quality could be misdirected, impractical or unnecessary.

Geldreich et al. (1968) proposed using the ratio of FC to FS concentrations (FC/FS) to help determine the source of fecal water pollution provided certain sampling conditions were present (Geldreich, 1967, 1970; Greenberg et al., 1985). In general, FC/FS values of less than 0.7 were taken as indicating pollution primarily from animal sources, while values of 4 and greater suggested primarily human sources. The availability of such a tool for reliably identifying pollution sources would aid immensely in determining where effort should be focused to best control fecal contamination. While some researchers have reported success in using FC/FS ratios to discriminate between human and animal sources (Doran and Linn, 1979; Doran et al.; 1981 Tiedemann et al., 1988), use of FC/FS values is not currently recommended due to variable die-off rates among FS species as well as other factors (Greenberg et al., 1992).

Nonpoint sources of fecal contamination can have a significant (even dominant) role in terms of microbiological quality of water, particularly in non-urban watersheds. The transport of FC and FS in runoff from source areas such as pasture, rangeland, and forest has been amply documented in reviews on the subject by Crane et

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al. (1983), Bohn and Buckhouse (1985), and Baxter-Potter and Gilliland (1988). The contribution of nonpoint sources to FC and FS pollution depends on a host of variables. The influential variables can be categorized as manure application/deposition variables (e.g., type of manure, concentration of bacteria in the manure, and rate of application/deposition), microbial survival variables (temperature, soil/manure physical and chemical conditions, sunlight, pH, presence of toxic substances, competition and organic matter; Gerba et al., 1975) and transport variables (rainfall, runoff, proximity to water body, etc.). Unfortunately, the effects of these variables and their interactions have been difficult to quantify in a general sense. Published studies relating stream FC and FS concentrations to cattle grazing within the drainage basin, for example, contain apparently inconsistent conclusions regarding the significance of the role of cattle (Doran and Linn, 1979; Doran et al., 1981; Jawson et al., 1982; Stephenson and Street, 1978; Skinner et al., 1974; Tiedemann et al., 1988; Gary et al., 1983; Milne, 1976). Therefore, while nonpoint sources can play a significant role with respect to microbiological water quality, it is difficult to predict the magnitude of this role relative to those of other sources.

The issue of differentiating between background and above-background FC and FS sources is similarly problematic. It appears that even background FC and FS concentrations in streams can also exceed primary contact standards, as reported by Hollon et al. (1982) for an ungrazed area in Tennessee and Blevins et al. (1995) for agricultural regions in central and western Kentucky. Niemi and Niemi (1991) also reported that streams draining pristine areas in Finland sometimes did not meet microbiological quality standards for good swimming water. The question of background FC concentrations is further complicated by evidence that indicator bacteria persist in stream sediments (e.g., Hendricks and Morrison, 1967; Sherer et al., 1992).

Due in part to the practical difficulties in assessing the controllable proportions of microbiological pollution, some researchers have questioned whether the microbiological quality standards, which were originally developed in the context of point source pollutants, are realistic for nonpoint sources in general (e.g., Jawson et al., 1982; Hunt et al., 1979; Doran and Linn, 1979; Doran et al., 1981; Bohn and Buckhouse, 1985). Jawson et al. (1982) suggested that testing for specific pathogens might provide a better indication of potential health hazards than simply using FC concentrations. There is also evidence that sampling protocols can bias microbiological quality assessments. Studies reported by Doran and Linn (1979), Doran et al. (1981), Howell et al. (1995), Jawson et al. (1982), and Skinner et al. (1974) indicate that FC and FS concentrations are generally higher in the warmer months than in the cooler months. Stephenson and Street (1978) and Tiedemann et al. (1988) found that stream FC and FS concentrations were also higher during periods of high flow than during periods of low flow. Within some limits, therefore, it appears that FC and FS analysis results may be influenced simply by when and under what flow conditions water samples are collected. The implications of this are significant. If, for example, sufficient sampling is conducted during high flow conditions (presumably when

nonpoint sources dominate), then the stream could be categorized as "impaired". Significant resources might then be devoted to improving the microbiological quality of the stream even though the "impairment" does not actually occur except at times when uses such as swimming and fishing are impractical.

The objectives of this study were to determine the effects of (a) stream flow rate, (b) time of year on FC and FS concentrations, and (c) to assess the usefulness of FC/FS ratios in discriminating between human and animal pollution sources. The findings of this study can help better define the role of sampling protocols with regard to microbiological quality and ultimately be helpful in allocating resources to improve microbiological water quality.

METHODS AND MATERIALS

STUDY AREA

Water from two streams (Moores Creek and Beatty Branch) was sampled from September 1991 to April 1994. These streams merge at Lincoln Lake, which is located in Northwest Arkansas north of the city of Lincoln (36° 00' N, 94° 25' W). The climate is humid with a mean annual temperature of 14.1°C and annual rainfall of 1120 mm (National Climatic Data Center, 1994). The two stream basins consist of approximately 3240 ha, 2120 ha of which is drained by Moores Creek, and the remainder (1120 ha) by Beatty Branch. Elevations within the basins range from approximately 365 to 487 m with a mean elevation of 429 m. Steep slopes exist at the northernmost and southernmost regions of the basins as well as near streams in the lower one-third of the basins. Both streams have stony (approximately 7 to 15 cm diameter) beds in the lower reaches with some formation of small pools occurring during low flow periods. Flow occurs in the streams during most of the year, but the streams have been observed to be completely dry during extended periods of low rainfall.

Fifteen soil series are represented in the basins, with the Captina, Enders, Enders-Allegheny, Hector-Mountainburg, and Linker series covering nearly 70% of the total area. The Captina and Enders series are characterized as having moderately good drainage; the Allegheny and Linker series have good drainage, and the Hector-Mountainburg complex has good to somewhat excessive drainage (Harper et al., 1969).

The major land uses within the basins are pasture (56% overall) and deciduous forest (34% overall). The primary agricultural enterprises in the basin include beef cattle and confined animal (predominately poultry) production. Apple orchards, dairy facilities, and other agricultural operations also exist within the basins, but the quantity and areal extent of these operations are insignificant in comparison to grazing and confined animal production.

WATER QUALITY MONITORING

Five stream flow sampling sites (fig. 1) were monitored from September 1991 to April 1994. Three of the sites (MA, MB, and MC) were on Moores Creek, and the remaining sites (BA and BB) were on Beatty Branch. The sub-basin area associated with the MA site was approximately 1800 ha, or 85% of the total area drained by Moores Creek. Approximately 800 ha, or 71% of the total Beatty Branch drainage area, drained past site BA. The

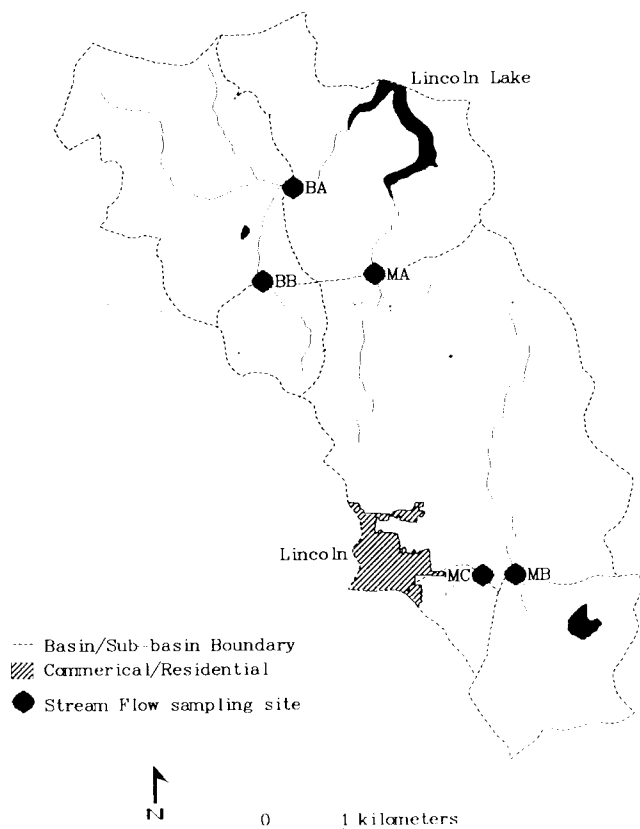


Figure 1—Sampling sites and associated sub-basins.

upstream sites MB, MC, and BB were associated with sub-basin areas of approximately 370, 90, and 150 ha, respectively. Land use was determined for the sub-basin associated with each monitoring site as given in table 1. The more upstream basins (MB, MC, and BB) had the highest proportions of pasture land use. Most of the residential land use occurred in the MA and MC basins. It should also be noted that there are several confined animal operations (especially poultry) in the basins, and the manure from these operations is usually land-applied to nearby pastures.

Stream flow samples (1 L sample size) were collected at each monitoring site on a two-week sampling interval. These “grab” samples generally represented base flow conditions, although the timing of sampling occasionally coincided with storm flow. Two sites (MA and BA) were also instrumented to record flow depths and to

Table 1. Land uses within sampling site drainage basins

Category	Monitoring Site				
	MA	MB	MC	BA	BB
	Areal Coverage (%)				
Pasture	61.8	73.2	81.0	56.5	90.2
Orchards	2.5	7.4	0.0	0.5	2.1
Poultry houses	1.6	0.5	0.0	0.4	2.0
Other agricultural	0.1	0.0	0.0	1.4	0.0
Residential	5.4	0.0	4.2	0.0	0.1
Commercial	1.7	0.0	0.0	0.0	0.0
Water	0.0	3.7	0.0	0.0	0.0
Forest	25.9	15.2	14.8	39.5	5.6
Other	1.0	0.0	0.0	1.7	0.0

automatically sample flow during storm events. A pressure transducer (model PCD950, Druck, Inc.) was secured to a concrete flagstone and placed in the stream bed at these sites to measure flow depth. The output from the pressure transducers was measured and recorded at 5-min intervals by data loggers (model CR10 measurement and control modules, Campbell Scientific, Inc.) and was also used to initiate water sampling during storm events. The intakes of the automatic water samplers (model 800SL portable liquid sampler, American Sigma) were secured to trees at the edges of the stream beds in the immediate vicinity of the pressure transducers. The sampler tubing and pressure transducer wiring were shielded with plastic conduit and buried from the stream beds to the instrument shelters. The pressure transducers and water samplers were interfaced so that sampling (1-L sample volume) initiated upon detection of a storm event and continued at 2-h intervals until the storm event had ended. All instruments were powered by batteries and were operational on a near-continuous basis throughout the project duration.

Rating curves for the MA and BA sampling sites were developed by measuring discharge at a range of stages using procedures described by U.S. Geological Survey (1969). The rating curves were then constructed according to techniques recommended by U.S. Geological Survey (1984). The slope-conveyance method was used to extend the rating curve for stages above which discharge measurements were available.

Storm flow samples were collected as soon as possible (<24 h) following each runoff event and transported to the Arkansas Water Resources Center Water Quality Laboratory for FC and FS analysis. All FC and FS analyses were conducted by the membrane filtration method (Greenberg et al., 1992) within 24 h of sample delivery. The automated sampler collection bottles were thoroughly washed and acid-rinsed between storm events. Data from the FS and FC analyses were used with the observed runoff rates to compute flow-weighted concentrations of these indicator bacteria for all storm events sampled. Storm event FC/FS ratios were computed based on flow-weighted mean runoff concentrations of FC and FS.

The effect of flow rate on FC and FS concentrations was assessed by regressing the natural logarithms of the respective concentrations against the natural logarithms of flow rates (mean flow rate for storm event samples, instantaneous flow rate for grab samples). The effects of time of year on FC and FS concentrations were evaluated by first grouping the observations according to the “season” in which they occurred. Four, three-month seasons were used: January through March (winter); April through June (spring); July through September (summer); and October through December (fall). One-way analysis of variance (ANOVA) was then performed using natural logarithms of FC and FS concentrations to test the significance of season as an explanatory treatment. The studentized Newman-Keuls method was used to separate means whenever ANOVA indicated a significant season effect.

RESULTS AND DISCUSSION

Selected statistics of FC, FS and FC/FS ratio are given in table 2. There were no significant differences in mean FC or FS concentrations among sampling sites. Average FC

Table 2. Selected fecal coliform (FC), fecal streptococcus (FS) and FC/FS ratio statistics

Parameter	Site				
	MA	MB	MC	BA	BB
FC (cfu/100 mL)					
Mean*	1.8×10^2	2.0×10^2	1.9×10^2	2.5×10^2	3.1×10^2
Maximum	7.1×10^4	5.9×10^4	7.1×10^4	1.6×10^4	7.5×10^4
FS (cfu/100 mL)					
Mean*	3.6×10^2	1.5×10^2	2.6×10^2	2.9×10^2	1.9×10^2
Maximum	9.2×10^4	7.9×10^4	5.1×10^4	2.3×10^4	6.1×10^4
FC/FS					
Mean†	2.2	3.8	4.4	2.1	4.9
Maximum	90.0	55.7	57.5	20.0	42.7

* Geometric mean.
 † Arithmetic mean.

concentrations were very near the primary contact limit for all sites. Both means and maxima of FC and FS concentrations were approximately two orders of magnitude lower than reported (Edwards, 1996) in runoff from small (up to 1.4 ha) grazed fields in headwaters (approximately 3 km distant from the nearest stream sampling sites) of the Moores Creek and Beatty Branch basins. This finding of relatively low stream FC and FS concentrations suggests that field runoff FC and FS concentrations decreased due to dilution of field runoff by relatively clean runoff from other sources, sedimentation, die-off and/or predation.

SEASONAL EFFECTS

Concentrations of FC and FS were significantly ($p < 0.05$) affected by the season during which the sample was collected for all sampling sites (table 3). Except for the MA site, the highest concentrations of FC and FS always occurred during the summer, although these concentrations were sometimes not significantly different from those observed during the

Table 3. Seasonal mean* fecal coliform (FC) and fecal streptococcus (FS) concentrations for the MB, MC, and BB sampling sites

Site	Season			
	Winter	Spring	Summer	Fall
(cfu/100 mL)				
MA				
FC	2.8×10^1 b ²	7.3×10^2 a	2.4×10^2 a	1.8×10^2 a
FS	5.4×10^1 b	8.7×10^2 a	1.2×10^3 a	3.5×10^2 ab
FC/FS	0.5	0.8	0.2	0.5
MB				
FC	3.8×10^1 b	9.6×10^2 a	3.4×10^3 a	1.0×10^2 b
FS	3.5×10^1 c	2.4×10^2 b	3.6×10^3 a	1.6×10^2 bc
FC/FS	1.1	4.0	0.9	0.6
MC				
FC	5.7×10^1 b	4.1×10^2 b	7.2×10^3 a	5.4×10^1 b
FS	1.1×10^2 b	4.4×10^2 ab	3.5×10^3 a	1.2×10^2 b
FC/FS	0.5	0.9	2.1	0.5
BA				
FC	3.6×10^1 b	5.4×10^2 a	6.2×10^2 a	2.9×10^2 a
FS	4.1×10^1 b	5.3×10^2 a	7.1×10^2 a	4.4×10^2 a
FC/FS	0.9	1.0	0.9	0.7
BB				
FC	5.9×10^1 c	9.4×10^2 b	1.4×10^4 a	2.2×10^2 bc
FS	7.1×10^1 b	2.0×10^2 b	5.8×10^3 a	1.8×10^2 b
FC/FS	0.8	4.7	2.4	1.2

* Geometric mean.
 † Within-row means followed by the same letter are not significantly ($p > 0.05$) different.

spring and/or fall. The lowest FC and FS concentrations occurred during the winter and fall, and there were usually no significant differences in mean FC and FS concentrations between those two seasons. These findings corroborate the earlier results of Doran and Linn (1979), Doran et al. (1981), Howell (1996), Jawson et al. (1982), and Skinner et al. (1974), who also reported that indicator bacteria concentrations were generally higher during warmer months than in cooler months. Ratios of FC to FS concentrations demonstrated no significant dependence on timing of sample collection.

The relationship between FC and FS concentrations and season of sample collection has significant implications from the standpoint of water quality assessment. The data of table 3 (particularly for the MB, MC, and BB sites, for which samples generally represented base flow conditions) suggest that "background" concentrations of FC and FS are higher during warmer months than during cooler months. If dates of sample collection are weighted in favor of warmer months, then the sample analysis results can be biased in favor of a poor water quality assessment. Conversely, if sampling times are weighted in favor of cooler months, then the results can underestimate the concentrations of indicator bacteria present during warmer months (when uses such as swimming and fishing are most likely to occur). The effects of season on water quality assessment are demonstrated in figures 2 and 3. The data of figures 2 and 3 were determined by fitting a lognormal probability curve to the seasonally grouped FC data and then finding the cumulative probability corresponding to a primary contact standard of 200 cfu/100 mL and a secondary contact standard of 1,000 cfu/1000 mL. If stream flow at the BB site, for example, were sampled only during summer, then the stream would meet primary and secondary contact standards less than 10% of the time. Alternatively, if only winter sampling was conducted, then the stream would meet primary and secondary contact standards approximately 65 and 85% of the time, respectively.

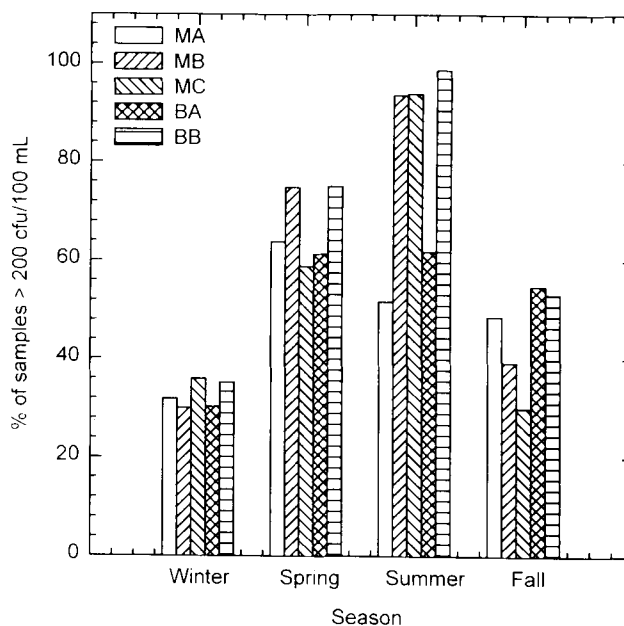


Figure 2—Proportions of stream samples not meeting primary contact standards (200 cfu/100 mL) for fecal coliform.

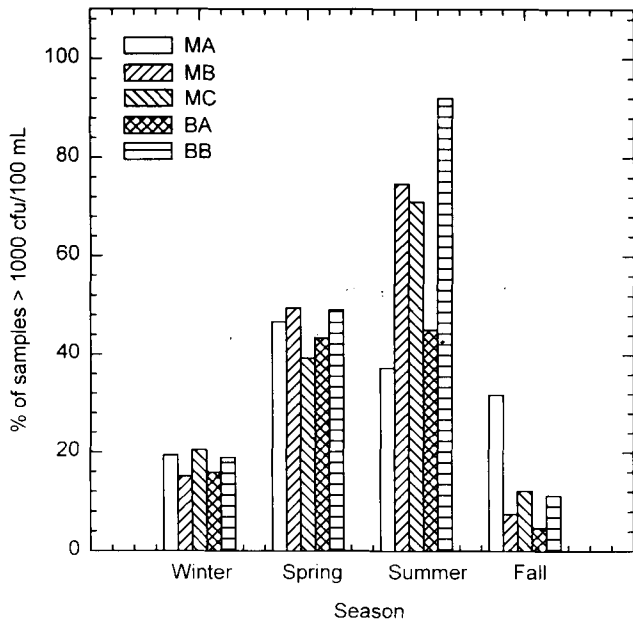


Figure 3—Proportions of stream samples not meeting secondary contact standards (1,000 cfu/100 mL) for fecal coliform.

FLOW RATE EFFECTS

Both FC and FS concentrations were significantly ($p < 0.05$) affected by flow rate for all seasons at sampling site MA. A typical relationship between flow rate and concentrations of FC and FS is given in figure 4, in which both FC and FS concentrations may be seen to demonstrate close correspondence with flow rate. Flow rate was significantly related ($p < 0.10$) to FS during all seasons and to FC concentration during the spring and fall seasons for the BA sampling site, although relationships between flow rate and FC and FS concentrations were generally weaker for the BA site than for the MA site. Ratios of FC/FS were

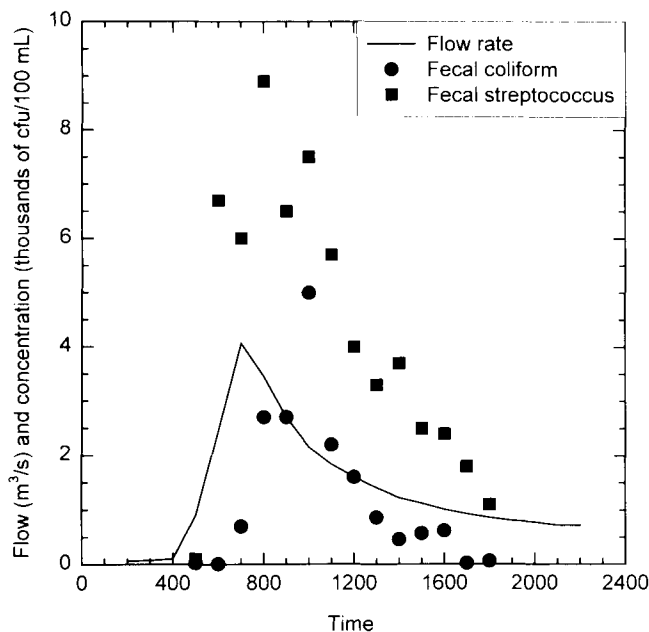


Figure 4—Flow and concentrations of fecal coliform and streptococcus for the storm event of 15 November 1991.

not related to stream flow rate. The equations relating FC and FS concentrations to stream flow are given in table 4 along with respective coefficients of determination.

As noted in the previously cited studies, both FC and FS concentrations increased with increasing flow rate (except for FC at site BA during spring and fall). This general relationship reflects significant FC and FS inputs to the streams from nonpoint sources and might indicate the resuspension of FC and FS persisting in stream sediments (Sherer, 1988). Table 4 indicates, however, that the role of flow with respect to FC and FS concentrations differs depending on the season. The exponents on stream flow are generally highest in winter, followed by summer. These data suggest that the role of stream flow, and thus the contributions of nonpoint sources and sediment resuspension to FC and FS concentrations, is greatest in the winter. The coefficients of the equations given in table 4 help to quantify seasonal background FC and FS concentrations; in general, the magnitudes of these coefficients reflect the trends detected while analyzing seasonal effects (table 3).

The dependence of FC and FS concentrations on flow rate gives rise to the possibility of inaccurately assessing microbiological water quality due to the timing of sample collection. In other words, sampling during periods of high flow can result in relatively high FC and FS concentrations, even though the flow conditions at sampling do not support one or more of the stream's intended uses.

FC TO FS RATIOS

Ratios of FC to FS concentrations were not reliable indicators of the source of the FC and FS in the stream samples. The MB, BA and BB sampling sites had limited residential land use in the respective basins (table 1), yet the mean FC/FS ratios were considerably higher than would have been expected from contamination by animals (≤ 0.7) and were even greater than the ratios that would have been expected for pollution by human sources (≤ 4). Similar conclusions were reached by Edwards et al. (1996) in a study of runoff from pasture fields (with no human pollution FC or FS sources) draining into Moores Creek and Beatty Branch. In that study, FC/FS values were generally lower than in this one, but there were several storm events in that study during which FC/FS values of >100 were recorded. Taken together, the data from the studies suggest the occurrence of higher die off rates of FS than FC, a phenomenon that tended to increase FC/FS values as the water flowed downstream. The initial FC/FS ratios in water may quickly change due to the rapid mortality of indicator FS such as *Streptococcus bovis*

Table 4. Equations relating fecal coliform (FC) and fecal streptococcus (FS) concentrations to stream flow rate (Q) for the MA and BA sampling sites. FC and FS have units of cfu/100 mL, Q has units of m^3/s

Site	FC	r^2	FS	r^2	Range of Q (m^3/s)
MA					
Winter	$FC = 1.3 \times 10^4 Q^{2.6}$	0.60	$FS = 1.6 \times 10^4 Q^{2.3}$	0.61	0.04-5.18
Spring	$FC = 3.1 \times 10^3 Q^{0.9}$	0.16	$FS = 4.2 \times 10^3 Q^{0.9}$	0.18	0.01-6.20
Summer	$FC = 1.9 \times 10^4 Q^{1.4}$	0.53	$FS = 3.3 \times 10^4 Q^{1.1}$	0.50	0.00-7.06
Fall	$FC = 7.8 \times 10^2 Q^{1.0}$	0.25	$FS = 2.2 \times 10^3 Q^{1.2}$	0.34	0.00-5.50
BA					
Winter	$FC = 2.8 \times 10^3 Q^{2.2}$	0.56	$FS = 1.6 \times 10^3 Q^{1.8}$	0.53	0.02-1.78
Spring	NS*		$FS = 4.9 \times 10^3 Q^{1.1}$	0.13	0.03-2.49
Summer	$FC = 7.3 \times 10^4 Q^{1.8}$	0.35	$FS = 1.1 \times 10^5 Q^{1.8}$	0.31	0.01-3.14
Fall	NS		$FS = 5.1 \times 10^3 Q^{1.3}$	0.21	0.03-2.49

* No significant relationship ($p > 0.10$) between FC and Q.

(Geldreich, 1976) as well as the rapid growth of FC (*Escherichia coli*) in sediments when conditions are favorable (warm, neutral water with abundant organic matter) (Howell, 1996). It should not be surprising, then, that FC/FS ratios in non-urban settings often bear little relationship to the source and that this relationship deteriorates downstream of the source, particularly in warmer conditions.

SUMMARY AND CONCLUSIONS

Stream flow was sampled for nearly three years at five sites and analyzed for fecal coliform (FC) and fecal streptococcus (FS). Both grab and storm samples were collected from two sampling sites, while only grab samples were collected at the remaining three sites. Land use within the respective basins varied but consisted primarily of pasture (57 to 90%) and forest (6 to 40%).

Both FC and FS concentrations were significantly ($p < 0.05$) affected by the time of year during which samples were collected. The highest concentrations were observed during the summer months. Concentrations of FC and FS were also generally affected by flow rate, with both concentrations increasing with increasing flow rate. Ratios of FC to FS concentrations were higher than would have been expected for primarily animal sources. Given the very small proportion of residential land use in some of the sampling site basins, however, it appears unlikely that humans were the primary source. Use of the FC/FS ratio thus did not appear to reliably indicate the source of the FC and FS in the stream flow samples.

The dependence of FC and FS concentrations on time of year and flow conditions can potentially lead to a biased assessment of stream quality. This bias can be best avoided by sampling during the time(s) of year and flow conditions under which the intended use(s) is practical, rather than sampling according to an arbitrarily fixed schedule.

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