USE OF BACTERIAL INDICATORS IN ASSESSMENT OF WATER QUALITY OF THE EAST VERDE RIVER

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INTRODUCTION

A limited water quality survey of the East Verde River during the summer of 1979 (Sommerfeld et al., 1979) indicated that although the River was generally of good physicochemical quality, the upper reach contained large numbers of fecal coliform and streptococci bacteria, with the former frequently exceeding water quality standards (AWQCC, 1979). Both groups of bacteria are characteristic of the feces of warm-blooded animals (Geldreich et al., 1962a,b; Geldreich et al., 1964; Geldreich and Kenner, 1979). Although these organisms are not of great pathogenic significance in the natural environment, their presence in water is an indication of fecal contamination and the possibility of bacterial and viral pathogens.

This study was undertaken in an attempt to (1) determine whether the bacteriological water quality problem previously observed in the East Verde River persists beyond the heavy use summer period and to (2) identify the source of bacterial contamination found in the River.

DESCRIPTION OF STUDY AREA

The East Verde River originates about 24 km northwest of Payson, Arizona in the escarpment of the Mogollon Rim and flows in a southwesterly direction for approximately 80 km before its confluence with the Verde River. Average annual streamflow is about 850 ac-ft (104 ha-m) near the head and about 13,500 ac-ft (1665 ha-m) near its mouth at the Verde River. Streamflow near the head averages slightly over 1.0 cfs during non-flood periods. Streamflow in the East Verde River is augmented by the addition of about 25 to 30 cfs by pipeline from Blue Ridge Reservoir located about 32 km northeast of Pine on East Clear Creek. The water supplement to the East Verde results from a water trade made by the Salt River Project and Phelps Dodge Corporation involving Gila River Basin and Colorado River Basin waters.

The upper East Verde River is the major attraction in a heavily-used recreational area. Primary streamside activities are camping, hiking, picnicing, fishing, wading and swimming. Much of the recreational use is dispersed since campgrounds are undeveloped. Two campgrounds do exist in the study area. The largest is un-named and located just above the bridge crossing of the East Verde River. The other, Waterwheel Campground, is located below the confluence of Ellison Creek with the East Verde. In addition to the campgrounds just mentioned, several areas of primary and secondary homes have been developed on private land in close proximity to the River. They include Washington Park, Rim Trail, Verde Glenn, and Whispering Pines Subdivisions. The latter three directly border the River.

METHODS

SAMPLING LOCATIONS

Sampling sites on the upper East Verde River were selected to isolate portions of the River with respect to type of streamside activity. Five sites were sampled as follows: (1) just below the confluence of the East Verde River and pipeline aquaduct, (2) at the abandoned Gaging Station below Rim Trail and Verde Glenn Subdivisions, (3)

at bridge crossing below campground but above Whispering Pines Subdivision, (4) at river crossing just below Whispering Pines Subdivision, and (5) at Waterwheel Campground (Fig. 1).

SAMPLING DATES

The above sites were sampled six times between August and December, 1980. Each of the sampling periods consisted of daily samples at each site for three consecutive days over a weekend period (Friday - Sunday). Actual sampling dates were August 8-10 and 22-24, September 5-7, October 10-12, November 7-9 and December 5-7.

BACTERIOLOGY

East Verde River water was collected in sterile 50 ml Swinnex syringes and filtered through 0.45 µm sterile membrane filters contained in sterile Swinnex filter holders. After filtration of 20, 50 and 100 ml of river water, filters were placed on selective media and incubated. In fecal coliform enumeration, filters were placed on m-FC Agar and incubated for 24 hr at 44.5°C. Viable fecal coliform bacteria produced colonies that could be recognized by their blue coloration. For fecal streptococci enumeration, filters were placed on KF Streptococcus Agar and incubated for 48 hr at 35°C. Viable fecal streptococci produced pink to dark red colonies. In both cases, colony counts were made using a Bausch and Lomb stereomicroscope.

For speciation of the fecal streptococci, colonies were isolated from KF plates obtained during the first and second sampling days. At least 20% of the fecal streptococcus colonies that developed in the KF plates or a minimum of 10 colonies (where 20% was less than 10) were isolated. In a few instances more or less than this percentage was used because of very high or low counts. At least 100 colonies were isolated for each sampling trip. The isolated colonies were placed into Brain Heart Infusion (BHI) broth and agar for subsequent confirmation and speciation according to the standard protocol (USEPA, 1978). A brief description of the protocol follows.

After incubation for 24 hr at 35°C on BHI agar, each isolate was tested for catalase activity. Catalase negative organisms were again inoculated into duplicate BHI broth tubes and incubated at 45°C and 10°C. Growth was evaluated after two and five days, respectively. Growth at both temperatures indicates a potential enterococcus or Group Q streptococci. Growth at 45°C only suggests the Streptococcus bovis or S. equinus group. A positive starch hydrolysis test is confirmation of these bacteria. Lactose fermentation is used to differentiate between the two. Streptococcus bovis gives a positive acid reaction, whereas S. equinus gives no reaction. A third organism that may be included in this group is S. salivarius which in addition to not growing at 10°C does not hydrolyze starch (Buchanan and Gibbons, 1974). Those isolates demonstrating growth at both temperatures are confirmed as enterococci by their growth in 6.5% NaCl-BHI broth (35°C), BHI broth, pH 9.6 and reduction of 0.1% methylene blue in skim milk. Confirmed enterococci were evaluated for their ability to reduce potassium tellurite and tetrazolium and to ferment D-sorbitol and glycerol. Negative reactions are suggestive of Streptococcus faecium which is confirmed by the fermentation of L-arabinose. Positive reactions in the above tests indicate S. faecalis and its subspecies. The ability to hydrolyze gelatin and blood is used to distinguish subspecies. Gelatin hydrolysis indicates S. faecalis subsp. liquefaciens. Those isolates which do not hydrolyze gelatin are placed in Blood Agar and evaluated for hemolysis. Beta-hemolysis is characteristic of S. faecalis subsp. zymogenes. No hemolysis (alpha or gamma) is indicative of S. faecalis subsp. faecalis.

RESULTS AND DISCUSSION

In the East Verde River, fecal coliform densities were variable depending on sampling trip, site and day. On a sampling trip basis, fecal coliform numbers declined from the first to last trip, reflecting the decreased human activity in the watershed and/or decreased viability of the bacteria with decreasing temperatures and onset of winter (Fig. 2). On a daily basis, coliform numbers varied considerably more than was observed during the previous study (Sommerfeld et al., 1979). The much greater variability may be, in part, due to the greater variability in River discharge that occurred during the present study. Because of the large daily variability in coliform numbers, differences between sites were not statistically significant although site means differed noticeably (Fig. 3). Site 1 showed the least variability and had the lowest number of coliforms. This is expected since this site is subject to the least streamside activity. Daily means at this site typically ranged from non-detectable to 25/100 ml. The only exception to this occurred on the third day of the second sampling trip, when a rainstorm occurred just prior to sampling. Fecal coliform numbers were

exceptionally high at all sites on that date, ranging from 900 to 3,355/100 ml. This suggests that fecal coliforms do exist in high numbers in the soil or sediment of the watershed and are carried by runoff into the river.

Water quality standards for fecal coliforms were exceeded on several occasions during the first two sampling trips. Based on geometric means of a five sample minimum, only one sample from the East Verde River (site 4) was excessive on trip 1. However, during precipitation runoff (trip 2, day 3) all samples exceeded the single sample maximum of 800 fecal coliform per 100 ml.

Fecal streptococcus densities also decreased from first to last sampling trip, paralleling fecal coliform numbers and the drop in recreational use. Streptococcus numbers were lowest at site 1 and increased on a downstream basis to site 4 (Fig. 4). Site 5 had higher streptococcus numbers than sites 1, 2 and 3 but slightly lower densities than recorded at site 4. On a daily basis there was considerable variability in streptococcus numbers. Days 1 and 3 had the highest mean but they were not significantly different than that observed during day 2. Similar observations were made during the previous study on this watershed (Sommerfeld et al., 1979). The actual number of fecal streptococci per 100 ml compares closely with that reported in Oak Creek (Story, 1976). The effect of precipitation on streptococcus densities was readily apparent, as numbers increased 10 to 100 times over those detected prior and subsequent days to precipitation. According to Clausen et al. (1977), fecal streptococci are generally less numberous in rivers and lakes than fecal coliforms. Data collected in this study and previously in this and other streams in Arizona indicate the opposite to be the case, namely, that streptococci dominate (Story and Christensen, 1976; Sommerfeld et al., 1979).

Two techniques have been used to attempt to identify the source of fecal pollution in the East Verde. The simplest is the ratio of fecal coliforms to fecal streptococci (Geldreich and Kenner, 1969). In human feces, the quotient is 4.0 or greater, whereas in the feces of animals or waste waters so contaminated, the quotient is less than 0.7. Ratios in the East Verde varied from 0.1 to 5.0, with only one being 4.0 or greater and only 33% of the ratios equalling or exceeding 0.7 (Fig. 5). This would seem to suggest that the source of most of the bacterial contamination is from non-human sources. Ratios that exceeded 0.7 occurred sporadically, without regard to day of sampling or sampling trip. However, sites 2, 3 and 4 accounted for 84% of the ratios above 0.7, suggesting increasing human impact in this stretch of the River. While it is important to know the source of fecal contamination in the River, concern should not be limited to organisms of human origin since human pathogens can be harbored in non-human sources (Butler and Busbee, 1967; Geldreich, 1970).

The second technique used to identify the source of fecal pollution was the speciation of the fecal streptococci. Several species are reportedly unique to human feces, including Streptococcus faecalis subsp. faecalis, S. salivarius, S. durans (= S. faecium), and S. mitis (Cooper and Ramadan, 1955; Bartley and Slanetz, 1960; Kenner et al., 1960). Feces from livestock can be distinguished from humans by the presence of significant proportions of S. bovis and S. equinus (Geldreich and Kenner, 1969; Kenner et al., 1960). Species of enterococci such as S. faecalis subsp. liquefaciens, on the other hand, are more ubiquitous and are associated with humans, livestock and insects (Mallman and Seligmann, 1950; Geldreich et al., 1964). Other streptococci such as S. faecium subsp. casselflavis and S. avium have been found in association with plants, insects and birds.

Of the total number of fecal streptococci isolated from the upper East Verde, 21% were identifiable as S. faecalis subsp. faecalis. This percentage compares closely with the 22% reported for Oak Creek (Rumery, 1976) and is higher than the 15% reported in the Nile River (Saleh, 1980), both areas of heavy human activity. There was considerable variation in the percentage of S. faecalis subsp. faecalis from sampling trip to sampling trip. The percentage varied from about 20 to 52% for the first three trips and decreased to around 10% or less during the latter three trips. The distribution of this indicator species on a site basis is given in Table 1. Site 1, the site of the least human activity, had the lowest S. faecalis subsp. faecalis percentage of all the sites. From site 1 to site 5, the percentage of S. faecalis subsp. faecalis increased gradually from 9% to 33%, suggesting that some human fecal pollution occurs throughout the entire study area (Table 1). According to Cooper and Ramadan (1955), S. faecalis subsp. faecalis comprises about 40% of the streptococcus population in human feces. If this percentage is valid, one may conclude that in the lower portion of the study area, most of the contamination is of human origin.

In addition to the presence of S. faecalis subsp. faecalis, other isolates of human origin were detected. Two species, S. salivarius and S. faecium accounted for 13% and 10% of the total isolates (Table 1). The former species is characteristic of the human buccal tract and enters the digestive tract and feces, while the latter

(often considered analogous to S. durans) generally occurs in human feces as a small proportion of the total streptococcus population (Clausen et al., 1977). There is some disagreement about the source of S. faecium. Stuart et al. (1976) consider this species to be distinct from S. durans and not characteristic of humans but of other warm-blooded animals.

Two species, S. bovis and S. equinus, considered to be specific indicators of animal feces were rare in the East Verde River. Only one isolate of S. equinus and none of S. bovis was detected throughout the entire study. These species apparently die off rapidly (Geldreich et al., 1968; Geldreich and Kenner, 1969) and their isolation should reflect recent fecal contamination. Rumery (1976), similarly found these species in very low numbers in Oak Creek, Arizona.

The other S. faecalis subspecies, S. faecalis subsp. liquefaciens and subsp. zymogenes, considered to be non-specific with respect to host organism represented 29% and 1% of the total streptococcus isolates. Group Q organisms represented 26% of the total isolates (Table 1).

The results obtained from speciation of the isolated streptococci therefore suggests that fecal contamination at the lower sites in the East Verde is primarily of human origin. This is in contrast to the fecal coliform to fecal streptococcus ratios which are usually less than 0.7 and suggestive of contamination from warm-blooded animals. Similar paradoxical results were reported from Oak Creek (Rumery, 1976). Such results may be due to the fact that fecal streptococci survive longer in the soil and sediments or in association with plants and insects than do fecal coliforms (Geldreich et al., 1968; Geldreich and Kenner, 1969). This residual streptococcus population may enter the aquatic environment during recreational and other streamside disturbances. A residual streptococcus population could conceal the significance of fecal coliform input and produce low fecal coliform to fecal streptococcus ratios. These results appear to provide further proof that fecal coliform to fecal streptococci ratios are useful only in situations where fecal or sewage contamination is continual or recent as previously suggested (Geldreich and Kenner, 1969).

CONCLUSIONS

Fecal coliform and streptococcus numbers were considerably lower in the fall-winter period than reported in the summer, indicating that bacterial problems in the watershed are seasonal. Bacterial numbers increased gradually on a downstream basis indicating that non-point source contamination occurred on both private and public lands.

Fecal coliform to fecal streptococcus ratios were typically less than 0.7 and suggestive that animals are the major source of fecal contamination in the East Verde River. Since ratios are considered useful only when applied to recent contamination and indicators of recent contamination were rare, the validity of the ratios are questionable.

Streptococcus faecalis subsp. faecalis, an enterococcus unique to human feces, was isolated in increasing abundance (9% to 33%) with distance downstream in the River. The presence of significant numbers of this indicator species suggests that humans are a major source of bacterial contamination in the East Verde River.

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LITERATURE CITED

Arizona Water Quality Control Council. 1979. Water quality standards for surface waters. 43 p.

Bartley, C.H. and L.W. Slanetz. 1960. Types and sanitary significance of fecal streptococci isolated from feces, sewage and water. Am. J. Pub. Health 50: 1545-1552.

Buchanan, R.E. and N.E. Gibbons. 1974. Bergey's Manual of Determinative Bacteriology. Williams and Wilkens Co., Baltimore, Md.

Butler, C.E. and C.E. Busbee. 1967. Human enteric pathogens in dogs in Fairbanks, Alaska. Pub. Health Reports 82: 465-470.

Clausen, E.M., B.L. Green and W. Litsky. 1977. Fecal streptococci: indicators of pollution, pages 247-264. IN A.W. Hoadley and B.J. Dutka (eds.). Bacterial Indicators/Health Hazards Associated with Water. ASTM, Philadelphia, Pa.

Cooper, K.E. and F.M. Ramadan. 1955. Studies in the differentiation between human and animal pollution by means of fecal streptococci. J. gen. Microbiol. 12: 180-190.

Geldreich, E.E. 1970. Applying bacteriological parameters to recreational water quality. J. Am. Water Works Ass. 62: 113.

Geldreich, E.E. and B.A. Kenner. 1969. Concepts of fecal streptococci in stream pollution. J. Water Poll. Contr. Fed. 41: R 336.

Geldreich, E.E., B.A. Kenner and P.W. Kabler. 1964. Occurrence of coliform, fecal coliforms and streptococci on vegetation and insects. Appl. Microbiol. 12: 63.

Geldreich, E.E., C.B. Huff, R.H. Bordner, D.W. Kabler and H.F. Clark. 1962a. The fecal coli-aerogenes flora of soils from various geographical areas. J. Appl. Bacteriol. 25: 87.

Geldreich, E.E., L.C. Best, B.A. Kenner and D.J. Van Donsel. 1968. The bacteriological aspects of stormwater pollution. J. Water Pollut. Control Fed. 40: 1861-1872.

Geldreich, E.E., R.H. Bordner, C.B. Huff, H.F. Clark and R.W. Kabler. 1962b. Type distribution of coliform bacteria in feces of warm-blooded animals. J. Water Poll. Contr. Fed. 34: 295.

Kenner, B.A., H.F. Clark and P.W. Kabler. 1960. Fecal streptococci. II. Quantification of streptococci in feces. Am. J. Pub. Health 50: 1553-1559.

Mallman, W.L. and E.B. Seligman. 1950. A comparative study of media for the detection of streptococci in water and sewage. Am. J. Pub. Health 40: 286-289.

Rumery, J.K. 1976. Bacterial water quality studies in Oak Creek Canyon. Unpublished Report to the Coconino National Forest, U.S. Forest Service.

Saleh, F.A. 1980. Isolation and enumeration of faecal streptococci from Nile water (1975-1976). Water Res. 14: 1669-1678.

Sommerfeld, M.R., P.V. Athey and B.C. Mueller. 1979. Impact of recreation on water qaulity of the East Verde River. Final Report to the Central Arizona Association of Government, Florence, Arizona. 99 p.

Story, M. and R. Christensen. 1976. Water quality survey - Slide Rock in Oak Creek Canyon, May-September 1976. Unpublished report. 35 p.

Stuart, S.A., G.A. McFeters, J.W. Schillinger and D.G. Stuart. 1976. Aquatic indicator bacteria in the high alpine zone. Appl. Environ. Microbiol. 31: 163-167.

U.S. Environmental Protection Agency. 1978. Microbiological methods for monitoring the environment. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

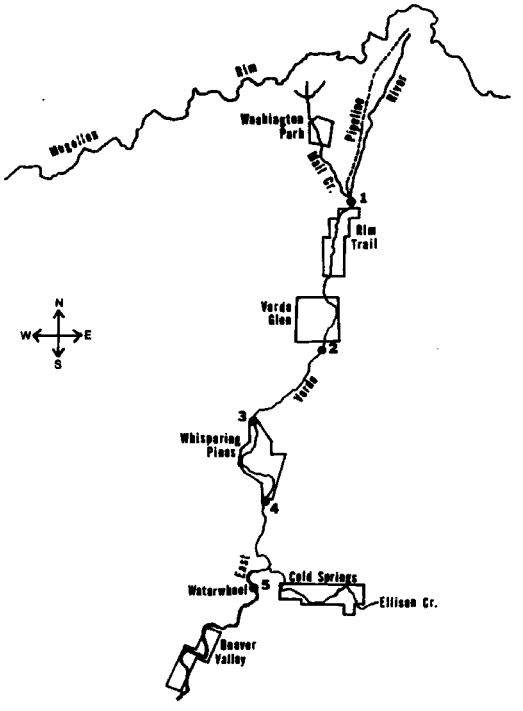

Figure 1. Locations of sampling sites on the East Verde River. Dots and numbers represent approximate site locations.

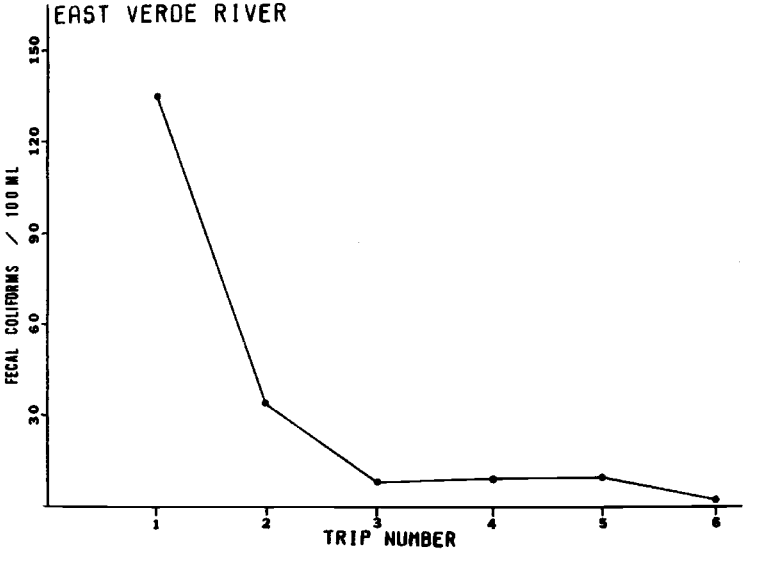

Figure 2. Mean fecal coliform numbers in the East Verde River during each trip of the study period.

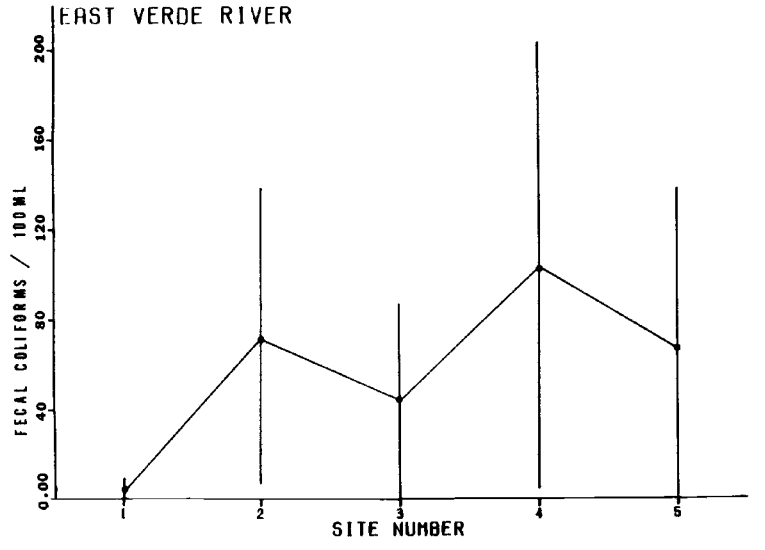

Figure 3. Mean and 95% confidence intervals for fecal coliform numbers at sites on the East Verde River.

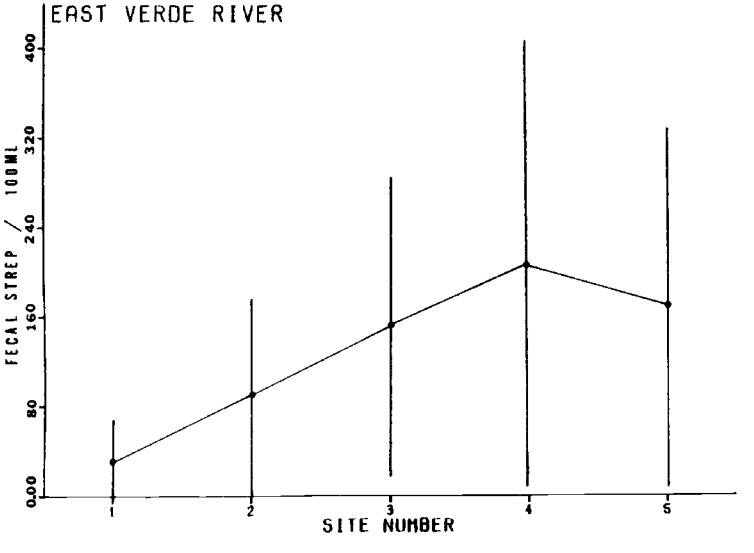

Figure 4. Mean and 95% confidence intervals for fecal streptococcus numbers at sites in the East Verde River.

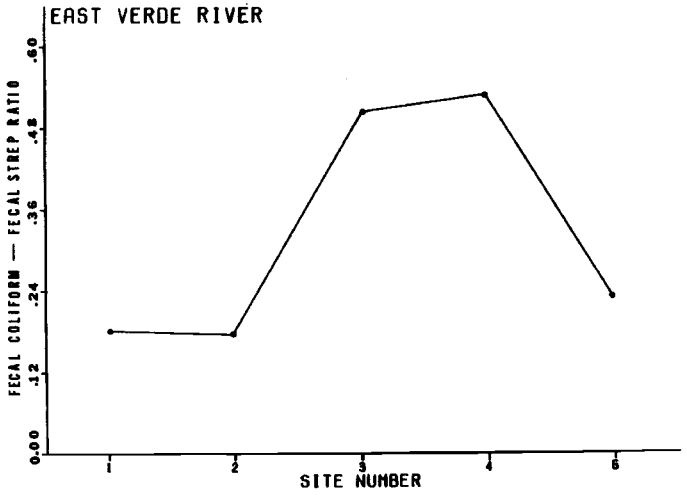

Figure 5. Fecal coliform to fecal streptococcus ratios for sites on the East Verde River. Data points represent mean values for the six sampling trips.

Table 1. Distribution of species of fecal streptococci as a percent of the total fecal streptococci isolated from sites in the East Verde River.

	Sites					Total for All Sites
	1	2	3	4	5	
S. bovis	0	0	0	0	0	0
S. equinus	1	0	0	0	0	0
S. salivarius	10	14	12	15	13	13
S. faecium	9	13	6	16	5	10
"Group Q"	55	32	15	24	22	26
S. faecalis						
subsp. faecalis	9	15	19	23	33	21
subsp. liquifaciens	17	25	48	21	27	29
subsp. zymogenes	0	1	0	1	0	1
Totals	100	100	100	100	100	100