A COMPARISON OF MACROSCOPIC AND MICROSCOPIC INDICATORS OF POLLUTION

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The most efficient parameter to assess pollution in streams receiving organic waste material has been shown to be the benthic macroinvertebrate stream community. Benthic organisms are more fixed in habitat than are diatoms. Both groups of organisms can adequately express the state of a stream with respect to pollution. Diatoms are sensitive to waste and are immediately affected by it. Benthic organisms reveal both present and past environmental conditions, whereas diatoms reveal only present environmental conditions. Equations to express community diversity in the stream have been evaluated. The simple biomass test was satisfactory in representing changes in stream conditions. The similarity index performed better than other indexes examined and, although somewhat difficult to produce, it may be the best way to compare stations on different streams because it can correlate the number of groups common to any two stations.

With increasing numbers of complex environmental problems, there is need for a simple and meaningful method by which one can assess the consequences of pollution and express the results numerically. Various tests have been devised to recognize the presence of pollution and to detect the effects of past exposure to wastes (1-4). Chemical tests are easy to run, but are not very revealing because pollutants are seldom discharged continuously, and their presence may be missed by sampling at an inappropriate time. It is apparent that a biological group, normally present in significant numbers and easy to sample is needed. Benthic macroinvertebrates and diatoms have frequently been used to evaluate stream productivity and should, therefore, be useful in determining the degree and extent of pollution. Since 1950, a number of methods have used changes in the aquatic community as a means of assessing pollution. To evaluate data, there have been many expressions used to compare conditions from one location to another. Probably, the most popular method for comparing samples is the species diversity index per individual ($\bar{D}$). There are other more sophisticated methods, such as Duncan's new multiple range test. Each index is a valuable step toward interpreting the various segments of a stream survey. These methods can be applied to part or to the entire aquatic community.

The purposes of this study are to relate major and minor changes in the aquatic community of Caney Creek to contaminants from industrial or domestic wastes, and to determine whether changes in the microscopic or macroscopic community are the more meaningful indicators of pollution.

DESCRIPTION OF SURVEY AREA

The Caney Creek experimental stream facilities are located in Adair and Cherokee counties, Oklahoma. As shown in Figure 1, the main channel originates just south of Stilwell, flows 17 miles westward, and empties into the Illinois River at Lake Tenkiller. The bottom of the stream consists mostly of limestone and chert pebbles usually less than five inches in diameter. The principal source of water is mountain springs located throughout the Caney Creek drainage basin. Four stations were selected along the eastern section of the stream for the collection of microscopic periphyton and benthic macroinvertibrates. Physical and chemical data, as well as visual biological analysis, were surveyed at 11 stations located along the stream. Similar stream environments were selected for each sampling site. Basic parameters used for site selections were substrate, light, stream velocity, and depth. Station 2 is located one-tenth mile above the point of discharge from a trickling filter system used to treat domestic sewage.

Proc. Okla. Acad. Sci. 50: 49-56 (1970)

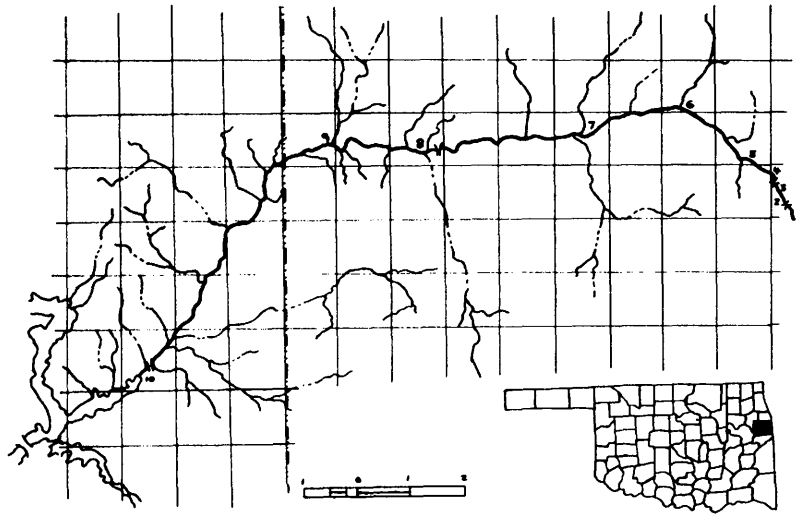

FIGURE 1. Caney Creek drainage basin.

Station 3 is located one-tenth mile below this outfall and one-tenth mile above the point of discharge from a two-stage aerobic treatment system used to treat waste from a large cannery. Station 4 is located one-tenth mile below the industrial outfall. Station 5 is located one and one-tenth mile below the industrial outfall. Other stations are shown in Figure 1. At approximately four and one-half miles below the industrial outfall, Caney Creek begins to flow underground. It remains underground, except for occasional pools, for 4 miles. From this point the stream flows above ground for nearly eight miles before emptying into Lake Tenkiller.

METHODS

At each of four principal stations, biological analysis consisted of sampling the benthic macroinvertebrates and the microscopic periphyton, the diatoms. Sampling continued from May, 1969 to October, 1969. Macroscopic benthos samples were collected using a standard Surber sampler, sorted in a No. 30 U.S. Standard Sieve. Organisms were preserved in a mixture of formalin and methyl alcohol. When large numbers of organisms were present, they were removed by sugar solution flotation (5). The samples were sorted and individuals were identified and counted (6, 7).

Microscopic samples of the diatoms were surveyed by using spring clips to suspend microscope slides in Caney Creek. The slide surfaces were placed parallel to the stream flow. The slides were removed at biweekly intervals, placed in coplin jars, and examined (8). Following microscopic examination, permanent mounts were prepared using a methyl alcohol procedure.

Several expressions were used to summarize the large amount of information about numbers and kinds of organisms. In these expressions maximum diversity exists if each individual belongs to a different species, and minimum diversity exists if all individuals belong to the same species. The separation of many individuals into several species produces a distribution which gives an intermediate diversity. It is the handling of this information that enables diversity indices to compare stream communities.

Total number of individuals (N), number of individuals per species (n_i), and number of species in a unit area (s) were used to calculate the species diversity indices (d), Simpson's (9) community diversity (D_c), total community species diversity (D), and diversity per individual ($\bar{D}$). These indices are as follows:

$$d = \frac{s-1}{\ln N} \text{ - - - - - - - - - - } \text{Eq. 1}$$

$$d = \frac{s-1}{N^2} \text{ - - - - - - - - - } \text{Eq. 2}$$

$$d = \frac{s-1}{N} \text{ - - - - - - - - - - } \text{Eq. 3}$$

$$D_c = \sum \frac{n_i(n_i-1)}{N(N-1)} \text{ - - - - - - } \text{Eq. 4}$$

$$D = \sum n_i \ln \frac{n_i}{N} \text{ - - - - - } \text{Eq. 5}$$

$$\bar{D} = \sum \frac{n_i}{N} \ln \frac{n_i}{N} \text{ - - - - - } \text{Eq. 6}$$

$$C = \frac{2(\Sigma P_1)}{\Sigma P_i + \Sigma P_j} \text{ - - - - - } \text{Eq. 7}$$

Equation 7 is the expression for the coefficient of similarity between two stations based on the prominence values. It is a statistical method developed by Burlington (10). The prominence value (P) was obtained for each group at each station by multiplying the density of the group at that station by the square root of the frequency of this group at all stations. P_i is the sum of the prominence values of all groups at station i, P_j is the sum of the values at station j, and P_1 is the sum of the lower of the two prominence values that the two stations have in common for the group. This forms a correlation between the number of groups in common between two stations and the coefficient of similarity.

From the benthic macroinvertebrate sample, biomass data were obtained as the weight of debris and organisms remaining after drying at 110 C for several hours minus the weight of the debris remaining after incineration at 600 C for one hour. The results are reported in grams.

Data for physical and chemical analyses included stream velocity and stream flow, dissolved oxygen, chemical oxygen demand, alkalinity and turbidity (Jackson Units), pH, total dissolved solids, and various metals, including the phosphate complex.

RESULTS AND DISCUSSION

The numbers of various pollution intolerant and pollution tolerant macroinvertebrates collected during the sampling period is presented in Table 1. Pollution tolerant oligochaetes and dipterans were found in small numbers at station 2, although they became more numerous following the outfall from the trickling filter at station 3. Numbers remained high throughout this area and into the area of station 4, below the industrial outfall. While the number of these tolerant organisms increased, the number of pollution intolerant groups, i.e., stoneflies, mayflies, caddis flies, and the groups isopoda and neuroptera, decreased. As the flow carried these tolerant organisms from station 2, through the polluted stations 3 and 4, and into the area of station 5, there was a gradual increase in their numbers. Increased numbers of the pollution tolerant oligochaetes and dipterans, along with decreased numbers of the pollution intolerant organisms, indicate there was an important alteration in the biological stream community. This alteration was probably due to the organic waste being deposited in the stream. While this effect appeared to be quite important at stations 3 and 4, a definite recovery was noticed at station 5. This showed that, at a distance of one mile below the outfalls, the stream diluted or utilized the major constituents of the waste so that the organic material had less effect on the stream population.

The number of leeches (hirudinea), a pollution tolerant group, found at all stations indicated there may be an effect at the clean water station 2 from the waste outfalls downstream. The numbers of coleop-

TABLE 1. Numbers of benthic macroinvertebrates per ft² (seasonal).

STATION	2						3						4						5					
WEEK MONTH	2 JUN	1 JUL	3 JUL	2 AUG	1 SEP	— X	2 JUN	1 JUL	3 JUL	2 AUG	1 SEP	— X	2 JUN	1 JUL	3 JUL	2 AUG	1 SEP	— X	2 JUN	1 JUL	3 JUL	2 AUG	1 SEP	— X
OLIGOCHAETA																								
Limnodrilus sp.	1	1	1	2	1	1	29	33	2	11	3	16	46	63	—	26	6	28	29	4	1	1	—	7
Tubifex sp.	—	—	—	—	—	—	1	3	—	—	—	1	38	7	3	—	—	11	—	—	2	—	—	—
Bdellodrilus Sp.	5	—	—	2	—	1	—	—	—	2	—	—	—	1	—	7	—	2	8	1	—	—	—	2
HIRUDINEA																								
Helobdella nepheloidea	—	3	—	1	—	1	—	—	—	1	—	—	—	—	—	—	6	1	—	—	1	—	7	2
Glossiphonia sp.	—	—	2	1	—	1	—	2	—	1	—	1	—	—	1	1	—	—	—	3	1	5	—	2
ISOPODA																								
Asellus militaris	3	1	7	4	6	4	—	1	—	2	2	1	—	2	5	1	—	2	12	16	25	31	17	21
EPHEMEROPTERA																								
Baetis sp.	—	1	36	13	7	11	—	—	—	—	—	—	—	—	1	—	—	—	7	22	—	2	1	6
Caenis sp.	—	—	2	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Ameletus sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
ODONATA																								
Macromia sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	1	—	—	—	—	—	—
Nehallenia sp.	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PLECOPTERA																								
Isoperla sp.	—	1	8	5	4	4	—	—	1	—	—	—	—	—	—	—	—	—	2	3	1	1	—	1
NEUROPTERA																								
Sialis sp.	—	—	3	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	13	—	—	—	—	3
TRICOPTERA																								
Hydropsyche sp.	—	—	7	1	3	2	—	1	—	—	—	—	—	—	1	2	—	1	—	—	—	—	1	—
COLEOPTERA																								
Peltodytes littoralis	—	—	—	3	2	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	3	2	1
Haliplus sp.	—	—	2	1	—	1	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
Hydrophilus sp.	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—	—	—	—	—	2	—	—	—
DIPTERA																								
Tendipes sp.	4	2	6	1	—	3	25	298	548	367	332	314	183	530	752	257	956	536	46	29	4	12	14	21
Psychoda sp.	—	—	—	—	—	—	426	262	40	11	16	151	69	47	8	5	7	27	8	3	—	—	—	2
Pentaneura sp.	—	—	7	—	—	1	39	8	—	2	1	10	37	74	6	—	—	23	—	—	—	8	—	2
Simulium sp.	—	—	1	1	1	1	—	—	—	—	—	—	—	—	3	—	3	1	—	—	—	—	—	—
Blepharicera sp.	—	—	—	—	—	—	—	—	4	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—
Hemerodromia sp.	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dixa sp.	—	—	—	—	—	—	—	—	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—

tera and odonata are not significant because of the rapid flow. These organisms are associated with quiet, slow moving streams.

The large number of *Psychoda* in the polluted zone in June was probably due to ineffective operation of the trickling filter. As the filter began to operate more efficiently, the number of these organisms decreased, but concurrently the number of *Tendipes* increased. The dominance of this organism at stations 3 and 4 was due to a large amount of sludge deposited in the stream bottom. Sludge appeared to be an important factor in alteration of the stream population. Further work may reveal that more efficient removal of sedimented solids may significantly improve the stream population.

Thus, the benthic macroinvertebrates present at each station suggest that station 2 is a clean water zone, that stations 3 and 4 are both polluted zones, and that station 5 is in the recovery zone.

The numbers and variety of organisms obtained from the microscopic samples were quite different from benthic samples. In the laboratory analysis of diatoms found on the submerged microscope slides, many different species and numbers of individuals were observed. In nearly every case, *Navicula* was the dominant genus. There were usually many *Fragilaria* and *Diatoma* at each station. Occasionally, *Cocconeis* was the dominant genus. Results sometimes showed a wide distribution both in species and in number of individuals at the same station in successive samples. It appears these organisms are quite sensitive to changes in stream quality and are, therefore, useful indicators of present pollution but doubtful indicators of past pollution.

Results from Tables 2 and 3 describe various indices used to evaluate the stream population data. As seen in Table 2, in evaluating the ratio of number of macroscopic species to number of macroscopic individuals,

TABLE 2. *Macroscopic organisms. Number of species (s), numbers of individuals (N), diversity indices, and biomass by station and date.*

STATION	DATE	s	N	$\frac{s}{N}$	$\frac{s-1}{\ln N}$	$\frac{s-1}{N^2}(10^3)$	$\frac{s-1}{N}$	$D_c(10^3)$	$D(10^2)$	$\bar{D}$	BIOMASS
2	2 JUN	4	13	0.31	1.17	17.75	1.11	0.4	2.43	18.7	0.058
	1 JUL	6	9	0.67	2.28	61.73	2.00	0.1	1.68	18.7	0.074
	3 JUL	13	84	0.15	2.71	10.42	1.42	14.7	15.72	18.7	0.037
	2 AUG	14	37	0.38	3.60	1.70	2.30	1.9	6.92	18.7	0.081
	1 SEP	7	24	0.29	1.89	9.50	1.43	0.8	4.49	18.7	0.062
	MEAN			0.36	2.33	20.22	1.65	3.6	6.25	18.7	0.062
3	2 JUN	5	520	0.01	0.64	0.02	0.22	1835.9	97.32	18.7	0.286
	1 JUL	8	608	0.01	1.09	0.02	0.32	1577.5	113.79	18.7	0.495
	3 JUL	9	603	0.01	1.25	0.02	0.37	3008.8	112.30	18.6	0.769
	2 AUG	7	396	0.02	1.00	0.04	0.35	1342.1	74.12	18.7	0.243
	1 SEP	5	354	0.01	0.68	0.03	0.27	1098.3	66.25	18.7	0.343
	MEAN			0.01	0.93	0.03	0.31	1772.4	92.81	18.7	0.427
4	2 JUN	5	373	0.01	0.68	0.03	0.26	426.9	69.81	18.7	0.206
	1 JUL	7	724	0.01	0.91	0.01	0.26	2914.8	135.50	18.7	0.264
	3 JUL	13	785	0.02	1.80	0.02	0.46	5641.5	146.92	18.7	0.677
	2 AUG	8	300	0.03	1.23	0.08	0.46	662.8	56.15	18.7	0.400
	1 SEP	7	981	0.01	0.87	0.01	0.22	9121.6	183.60	18.7	0.609
	MEAN			0.02	1.10	0.03	0.33	3753.5	118.40	18.7	0.431
5	2 JUN	8	129	0.06	1.44	0.42	0.70	34.1	24.14	18.7	0.165
	1 JUL	8	90	0.09	1.56	0.86	0.84	18.8	16.84	18.7	0.186
	3 JUL	9	44	0.20	2.11	4.13	1.35	9.2	8.24	18.7	0.478
	2 AUG	8	49	0.16	1.80	2.92	1.14	4.7	9.17	18.7	0.117
	1 SEP	7	40	0.17	1.62	3.75	1.11	4.0	7.49	18.7	0.131
	MEAN			0.14	1.71	2.42	1.03	14.2	13.18	18.7	0.215

(S/N, p/N), the mean value at station 2 (0.36) is decreased to 0.01 at station 3 and to 0.02 at station 4. The increased value of 0.14 obtained at station 5 indicates the stream is approaching the condition existing before the waste outfall at station 2. Another index, expression 1, behaves in a similar manner with a wide distribution between values at each station. Menhinick (3) used this expression and expression 3. He found the first index unsatisfactory because of wide variation with sample size. He suggested that index 3 could be used to compare samples of different sizes. In this study there was variation in sample sizes, yet both indices apparently worked quite well. Expression 2 gives an unusually large difference between numbers at stations 2 and 3. If this index is accurate, the small value of the index at station 5 indicates the stream is only gradually cleaning up. In the community diversity index, expression 4, there is a wide distribution between the clean water stations and the polluted stations. In this case, the value at station 5 indicates the stream has effectively recovered. In the expression for total community species diversity, expression 5, the results give a small distribution. There is still a change between station 2 and stations 3 and 4, followed by a return toward a clean water zone at station 5. The diversity per individual index, expression 6, gives no distribution and indicates the condition of the stream as a static one. Since this is a dimensionless expression, there is apparently a community structure problem in sample numbers.

In applying these indices to microscopic organisms each index was less satisfactory than when applied to macroscopic organisms. As shown in Table 3, there are increases and decreases from one station to

TABLE 3. *Microscopic organisms. Numbers of species (s), numbers of individuals (N), and diversity indices by station and date.*

STATION	DATE	s	N	$\frac{s}{N}$	$\frac{s-1}{\ln N}$	$\frac{s-1}{N^2}(10^3)$	$\frac{s-1}{N}$	$D_c(10^3)$	$D(10^2)$	$\bar{D}$
2	2 JUN	5	51	0.10	1.02	1.54	0.70	8.8	9.54	18.7
	1 JUL	8	1579	0.01	0.95	0.01	0.20	8868.1	295.53	18.7
	3 JUL	9	807	0.01	1.20	0.01	0.32	1397.9	151.04	18.7
	2 AUG	10	890	0.01	1.33	0.01	0.34	1862.1	166.57	18.7
	1 SEP	13	1222	0.08	1.69	0.01	0.38	3275.4	228.71	18.7
	MEAN			0.06	1.27	1.01	0.52	2195.7	128.42	18.7
3	2 JUN	6	63	0.10	1.21	1.26	0.76	8.3	11.79	18.7
	1 JUL	7	190	0.04	1.14	0.17	0.51	83.9	35.56	18.7
	3 JUL	6	82	0.07	1.13	0.74	0.66	26.6	15.35	18.7
	2 AUG	8	176	0.05	1.35	0.22	0.60	103.0	32.94	18.7
	1 SEP	9	424	0.02	1.32	0.14	0.44	401.7	79.36	18.7
	MEAN			0.05	1.30	0.39	0.59	144.3	37.54	18.7
4	2 JUN	5	74	0.07	0.93	0.73	0.58	16.3	13.85	18.7
	1 JUL	5	521	0.01	0.64	0.02	0.22	831.9	97.51	18.7
	3 JUL	3	27	0.11	0.61	2.74	0.58	2.3	5.05	18.7
	2 AUG	9	775	0.09	1.20	1.37	0.32	1343.6	145.05	18.7
	1 SEP	3	10	0.30	0.87	20.00	0.95	0.3	1.87	18.7
	MEAN			0.11	0.81	4.40	0.56	313.5	39.41	18.7
5	2 JUN	6	51	0.12	1.27	1.92	0.84	6.8	9.54	18.7
	1 JUL	4	448	0.01	0.49	0.02	0.19	870.4	83.85	18.7
	3 JUL	5	210	0.02	0.76	0.10	0.35	153.1	37.61	18.7
	2 AUG	5	33	0.15	1.14	3.67	0.87	3.2	6.17	18.7
	1 SEP	4	288	0.01	0.53	0.04	0.24	598.4	53.90	18.7
	MEAN			0.09	1.15	2.31	0.65	281.3	37.35	18.7

another. The community diversity and the total community species diversity indices (expressions 4 and 5, respectively) work most successfully. They indicate changes in the population moving from station 2 to 3, but show no improvement in stream conditions at station 5.

Most of the indices in Tables 2 and 3 show that conditions at station 4 are nearly identical or slightly improved over conditions at station 3. If this is the case, the amount of organic material contributing to the stream's altered community structure may be attributed to the domestic trickling filter and not to the two-stage aeration system. Thus, the organic matter from the aeration system does not have a significant effect on the localized stream community. Proceeding farther downstream, most of the indices show an improvement in the stream's condition from station 4 to station 5. If this is the case, and it visually appears to be, the waste must be utilized or removed relatively close to its source. Conditions farther downstream indicate there is no delayed pollutional effect from the industrial waste.

Biomass data given in Table 2 resembles data derived from the diversity indices in that conditions at station 2 are different from those at stations 3 and 4, and in that the area at station 5 resembles the clean water station rather than the polluted stations. Similarity indices given in Tables 4 and 5 show, for macroscopic samples, a high similarity between stations 2 and 5 and between stations 3 and 4. The highest similarity between stations 3 and 4 indicates that both of these stations are polluted to nearly the same extent; each is dissimilar or has a low similarity to the clean water station 2. The great dissimilarity between stations 4 and 5 shows that station 5 is in an improved condition. From microscopic results, the indices show a limited difference between stations. It does show that stations 2 and 5 are more similar than are stations 2 and 3 or 2 and 4. Unlike the macroscopic index, this index gives the greatest similarity between stations 3 and 5. The result does not appear to be reflected by the other parameters.

TABLE 4. *Macroscopic coefficients of similarity between stations*

STATION NO.	STATION NO. 5	4	3	2
5	—			
4	0.1034	—		
3	0.1307	0.6727	—	
2	0.3887	0.0278	0.0281	—

TABLE 5. *Microscopic coefficients of similarity between stations.*

STATION NO.	STATION NO. 5	4	3	2
5	—			
4	0.6601	—		
3	0.7662	0.6541	—	
2	0.4335	0.3896	0.4070	—

TABLE 6. *Physical and chemical conditions along Caney Creek (summer, 1969).*

STATION	DISTANCE (mi)	VELOCITY (fps)	FLOW (mgd)	D.O.	C.O.D.	ALKALINITY	TURBIDITY	pH	TDS	PO_4
2	0.000	0.292	0.20	7.9	4.7	130	7	7.2	150	0.27
OUTFALL 1	0.133	0.348	0.90	—	—	—	—	7.1	—	—
3	0.166	0.348	0.90	4.8	18.7	140	10	7.3	200	15.0
OUTFALL 2	0.240	0.348	0.90	—	—	—	—	7.2	—	—
4	0.373	1.000	2.37	4.6	23.4	160	10	7.5	220	7.0
5	1.373	0.645	3.25	6.1	14.0	150	8	7.5	200	4.0
6	3.090	0.477	3.42	6.5	—	—	—	7.4	—	4.0
7	4.730	—	3.58	7.1	—	—	—	7.4	—	1.0
8	8.980	—	6.52	—	9.4	—	—	—	—	0.2
9	10.13	—	12.70	—	—	—	—	—	—	0.2
10	16.43	—	15.80	7.6	—	—	—	—	—	—

ACKNOWLEDGMENT

The funds for this project were provided by a grant from DOI Contract No. 12060 DSB.

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