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Richard W. Tew

Samuel S. Egdorf

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WASTEWATER EFFLUENT-LADEN WATERS from Las Vegas Wash (LVW) form a density current that may be detected in Boulder Basin of Lake Mead at considerable distances from the wash estuary.[1] This led to the suspicion that water from the inflowing stream [40 mgd (1.5×10^5 cu m/day)] might not be rapidly diluted in the enormous volume of the lake [19 mil acre-ft (2.3×10^{10} cu m)], but might persist as a recognizable entity to the vicinity of the intake of a major water source for populous Clark County, Nev.

Because of the detection sensitivity implicit in the use of bacteria as tracers, the signal amplification factor inherent in their growth on media, and precedent in work on streams [2-4] and air,[5] it was decided to investigate the possibility that they might be used to study the practical problem under consideration.

Ideally, certain thermophilic spore-forming bacteria may be deliberately added as tracers.[6] The next best choice would be a mesophilic spore former (such as *Bacillus subtilis* var. *niger*), and a third alternative would be naturally occurring populations of bacteria indigenous to LVW. The last alternative was adopted because of the health hazards (though slight) inherent in adding large numbers of spores to the environment under investigation.

The development of an experimental plan for use of indigenous LVW bacteria as tracers was guided by four precepts:

1. The tracer should be indigenous to the wash but not to the lake.
2. It should be present only intermittently in the wash. For this reason, the use of coliforms as a group did not seem to be indicated, because prior experience showed their continuous presence.
3. The tracer should not be present in other water sources tributary to the lake. Fortunately, Boulder Basin receives water of other continuous, contributing streams (Colorado and Virgin Rivers) as a combined flow through Boulder Canyon, and Anderson and Pritchard's data [7] showed that the influence of this influx would be confined to certain regions of the waters of Boulder Basin, changing in a predictable way depending on the time of the year. Examination of their results indicated that concurrent sampling of Boulder Canyon and LVW might not be necessary.
4. The tracer (signal) should be easily recognizable and present in significant enough numbers to be detected in the lake bacterial population (noise) after dilution. Although a rough estimate of 10^4 to 10^5 bacteria/ml LVW water could be used to calculate numbers entering the lake per unit time, the influence of factors affecting dilution [lower limit of lake epilimnion at 10 m, cosedimentation with particulates, dissipation of the density current, lake dilution rate, organism survival (death or growth), and sampling frequency] made distribution estimates very tenuous and implied that empirical determination would be necessary.

Obviously, quantitative or even qualitative values for any of these factors could not be predetermined. This necessitated a factorial experimental plan in which their influence could be discerned in a matrix of organism distributions by sampling station location, depth, and time.

METHODS

Sampling stations were located (Figure 1) in LVW (Station 1), between the LVW estuary and the water system's inlet (Sta-

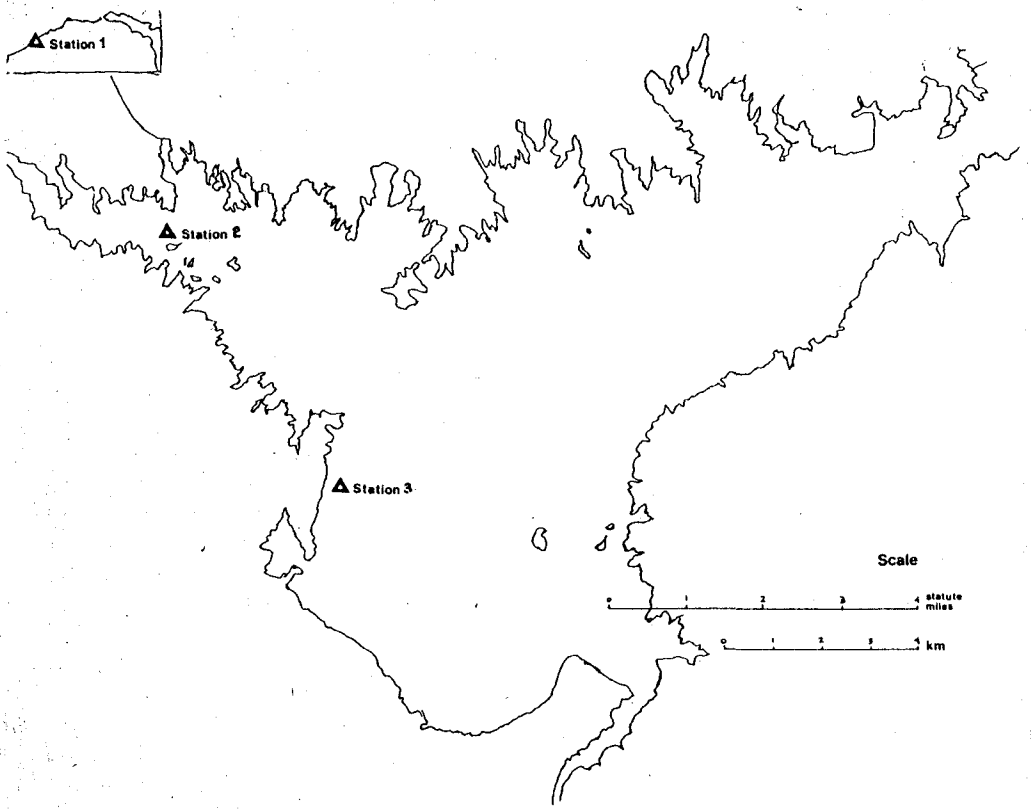

FIGURE 1.—Location of sampling stations in Las Vegas Wash and Boulder Basin, Lake Mead.

tion 2), and at the water system's inlet (Station 3). Statistically valid [8] samples were taken from the surface at Station 1 and at 10-m intervals at Stations 2 and 3 with a van Dorn bottle sanitized with 50:50 ethyl alcohol:acetone and removed to the laboratory, where initial steps in culturing were performed within 6 hr of collection. Sampling dates were May 24, June 10 and 24, and July 12, 1974.

Total counts were performed according to standard membrane filter procedures,[9] except that 0.1 percent peptone water was used as a diluent.[10] Enumeration of colonies and determination of percentage distributions of organisms according to colonial morphology and color were done after 5 days incubation at 25°C.

Colonies representing each population component were transferred to tryptone-glucose-extract slants, and an oxidase test was performed [11] to eliminate enterics. Oxidase-positive isolates and oxidase-negative yellow or orange chromogens were further differentiated by gram reaction and morphology and by their reactions in dextrose broth, litmus milk, and sulfide-indole-motility medium.* The strategy was to obtain the maximum number of descriptors from the fewest and simplest tests. Bona fide identification was not attempted. Isolates represented that fraction of the population actually counted; bacteria present in low numbers were thus selected against.

RESULTS

When the 215 isolates were screened for similar characteristics, 33 operational units with identical properties were discerned and designated by letter. The temporal

* All products of Difco, Detroit, Mich.

TABLE I.—Distribution of Bacterial Tracers Indigenous to Las Vegas Wash in Boulder Basin, Lake Mead

Las Vegas Wash (Station 1)				Boulder Basin (Stations 2 and 3)				
Isolate Qualifying as Tracer	Date Found (1974)	Tracer/ml	Tracer in Total Bacterial Count (%)	Date Found (1974)	Station No.	Depth (m)	Tracer/ml	Tracer in Total Bacterial Count (%)
H	May 24	45	1	May 24	2	20	30	3
J	May 24	454	10	May 24	2	30	870	67
				June 24	3	0	104	58
						40	354	46
N	June 10	110	5	June 10	2	10	<1	1
					3	0	<1	4
						20	2	4
P	June 10	110	5	June 24	2	20	59	16
						50	398	34
					3	30	124	10
AA	June 24	5,500	33	June 24	2	10	465	48
						20	89	24
						30	103	19
						40	423	42
						50	285	28
				July 12	2	0	12	29
						40	140	87
					3	30	210	70
						40	670	96

and spatial distribution matrix of these units was then examined for (*a*) presence at Station 1 on any, but not more than one, of the sampling dates and (*b*) presence at Station 1 before appearance at Station 3. Units fulfilling these conditions were assumed to be bacterial tracers indigenous to LVW.

The distribution of these tracers is given in Table I. The results suggest that water from LVW was recognizable mostly in the hypolimnion at the water systems inlet with a transit time of about 2 wk.

The percentage of the total number of bacteria found by plating or similar methods may be small and variable according to the environment sampled.[12] Also, it should be recalled that oxidase-negative nonchromogens were preselected against. Therefore, the data in Table II on positive descriptors for tracers or other operational units are not claimed to represent the properties of water bacteria present, but rather are given to indicate the relative utility of the tests used. Of these, pigment

TABLE II.—Characteristics of Tracer and Nontracer Bacterial Operational Units

	Tracers Having Characteristic	Number of OU Having Characteristic
Gram Negative Rods	H, J, P, AA	24
Gram Positive		
Rods	N	4
Rods with spores	—	3
Cocci	—	2
Yeast	—	1
Pigment		
None	H, J	14
Orange or yellow	N, P, AA	14
Other	—	5
Dextrose		
Acid	J, N, P	25
Gas	J	5
No acid or gas	H, AA	8
SIM		
H_2S	—	5
Indole	—	5
Motile	All	29
Litmus Milk		
Unchanged	P	7
Acid	J, N	14
Proteolyzed	J	14
Alkaline	H, AA	12

and reactions in litmus milk were the most useful and motility the least.

DISCUSSION AND CONCLUSIONS

The data indicate that bacteria appearing sporadically in an inflowing stream (already there) may be used to trace the distribution of the waters of the stream in those of a lake and that the use of a factorial sampling and assay experimental plan allows compliance with Precepts 1 and 2 given above.

Two additional measures would have greatly amplified the meaningfulness of the results, however. First, much more frequent sampling at Station 1 should have been performed to determine more adequately the numbers of tracers with time. Consider, for example, the data for N, P, and H. Numbers given for N and P may have represented the end of a surge, those for H the beginning of one. More thorough sampling may have allowed one to conclude, not just surmise, that LVW waters were carried rapidly to the vicinity of Station 2 by the density current, but from there more slowly to Station 3 by other mechanisms.

Second, cultures of tracers and filter-sterilized water samples should have been retained so that death or growth rates could be determined. The data imply that the tracers tended to appear at depth at Station 3, but perhaps they just survived longer in the colder water of the hypolimnion.

Generally, it was very fortunate that total counts of samples from Boulder Basin were so low; otherwise, tracers might not have been detectable amid more numerous bacteria native to the lake (Precept 4). Perhaps the results reflected and were greatly aided by the situation cited by Rodina,[13] who observed that plating reveals 100 times fewer total bacteria present in unpolluted than in polluted water. It has been known for many years [14] that low plate counts (1,000 to 2,000/ml) are indicative of oligotrophy. The viable counts were in this range; thus, Rodina's conclusion may have applied. With such low total numbers recorded for lake samples, and with significant percentages of the bacterial population in many lake samples consisting of tracers, it is difficult to recognize any organisms as indigenous to the lake by standard membrane filter methods. Boulder Canyon input was not sampled or assayed (Precept 3). Also, however, penetration of the tracers to Station 3 formed a recognizable pattern; when such a pattern exists it may be desirable but not absolutely necessary to assay all inflows.

The labor involved in describing population components could be reduced by additional preselection (for example, consideration of yellow or orange chromogens only) and by technical improvements such as replica plating, perhaps to one or two media designed to provide descriptors of greater individual value.

The advantage of the indigenous bacterial tracer technique over the use of fluorescent dyes is not in the relative sensitivity of the basic assays, but rather in the enormous amount of tracer already present in the inflow.

The matrical sampling and assay scheme may be arranged and managed to detect only entering bacterial surges of predetermined significant magnitude.

These advantages are particularly significant when determination of water distribution into a very large volume (8.2×10^9 cu m in Boulder Basin) is considered.

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Authors. Richard W. Tew, Samuel S. Egdorf, and James E. Deacon are associated with the Department of Biological Sciences, University of Nevada, Las Vegas.

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