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Patchiness in the Distribution of Planktonic Heterotrophic Bacteria in Lakes[1]

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By using conventional dilution plating procedures, the heterotrophic planktonic bacteria in Lake Washington were found to be distributed in small-scale (<1.0 ml) and large-scale (>1 m in horizontal distance) patches. Recommendations are made on sampling and enumeration procedures to minimize the effect of patchiness on obtaining an accurate estimate of bacterial numbers.

Direct light and scanning electron microscopic observations of filtered marine and freshwater samples have led some investigators to conclude that planktonic bacteria may commonly exist in aggregates attached to detrital particles (4, 6). If bacteria are aggregated in patches, then it is of interest to determine what volume of sample one must obtain from a planktonic habitat to accurately estimate the number of bacteria in the habitat. Obviously, if the sample volumes are as small as the volume of one detrital aggregate, those samples that include a detrital particle will have large numbers of organisms, whereas those with no particle will have few or none. On the other hand, if each sample contains numerous detrital aggregates or patches, one should be able to obtain a close estimate of numbers from the census of any one sample. Thus, in addition to knowing whether or not patchiness exists in the plankton, it is of importance to know the volume of the patches so that a proper sampling procedure can be devised. Because bacteriologists routinely use dilution plating as a means of enumerating planktonic bacteria, we decided to determine if patchiness in the heterotrophic bacterial community would be detectable by using this procedure and, if so, to propose modifications of the procedure that would enable a more accurate assessment of numbers.

Most of the literature on patchiness in the plankton is concerned with phytoplankton and zooplankton rather than bacteria. Patchiness of phytoplankton on various spatial scales has been reported by Bainbridge (1), Cassie (2, 3), Platt et al. (7), and Richerson et al. (8).

Duplicate vertical casts, separated by no more than 20 m horizontally, were obtained from Lake Washington by using aseptic Cobet samplers (Hydro Products, San Diego, Calif.). Samples were stored on ice for about 3 h prior to the inoculation of spread plates of CPS agar (sodium caseinate, 0.5 g; peptone, 0.5 g; soluble starch, 0.5 g; glycerol, 10 g; K_2HPO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeCl_3$, 4 drops of a 0.01% solution; agar [Difco], 15 g; distilled water, 1,000 ml; as modified from Jones [5]). Each of the duplicate samples from the vertical casts was mixed with a Vortex mixer, and four plates were made directly from the undiluted sample. After mixing, each sample was subsampled in duplicate by transferring 1.0-ml portions to 9.0-ml dilution blanks. These were mixed with a Vortex mixer, and four plates were prepared from each subsample. Thus, at a given station and depth, eight plates were inoculated directly from the sample and 16 additional plates were inoculated from the diluted sample.

A $\log_{10}$ transformation was used to normalize the variance of the plate counts, and analysis of variance was used to partition the total variance into components due to sampling, subsampling (diluting), and plating (7). If patchiness occurs, then, as the distance between samples or the volume of the subsamples is changed, the relative contribution of that level to the total variance should also change. Knowledge of the components of variance and estimates of the relative costs at each level allow the calculation of confidence limits for the mean plate count at each depth and the design of a sampling scheme that will minimize the total variance at a given cost constraint.

Figure 1 shows the relative abundance and 95% confidence intervals of viable bacteria obtained from the vertical casts, and Table 1 lists values from the analysis of variance performed on each month's data. Note that the three components of variance (samples, subsamples, and plates) are approximately equal. While the lake was mixed, no significant differences between

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depths were detectable. As stratification proceeded, differences between depths became between depths became more significant.

A measure of the efficiency of sampling effort is the product of the variance, $s_{\bar{x}}^2$, and the cost, C, where $s_{\bar{x}}^2 = (s_p^2/[n_p \cdot n_{ss} \cdot n_s]) + (s_{ss}^2/[n_{ss} \cdot n_s]) + (s_s^2/n_s)$ and $C = c_s \cdot n_s + c_{ss} \cdot n_{ss} \cdot n_s + c_p \cdot n_p \cdot n_{ss} \cdot n_p$. The coefficients, c_s, c_{ss}, and c_p, are the costs of obtaining an additional sample, subsample, or plate, respectively. The product, $C \cdot s_{\bar{x}}^2$ is minimized with $n_p = ([c_{ss}/c_p] \cdot [s_p^2/s_{ss}^2])^{1/2}$, $n_{ss} = ([c_s/c_{ss}] \cdot [s_{ss}^2/s_s^2])^{1/2}$, and n_s is chosen to satisfy the total variance or cost constraint (9). Since s_p^2 is not much greater than s_{ss}^2 and c_{ss} is approximately equal to the cost of an additional agar plate, c_p, n_p should be 1. To take into account the probability that a plate will not be readable, we set our lower limit on n_p at 2. Since the cost of taking an additional sample is much greater than that of taking a subsample, the number of subsamples/sample should be large. With a practical restriction to two vertical casts and the above considerations, we could improve the sample design by doing

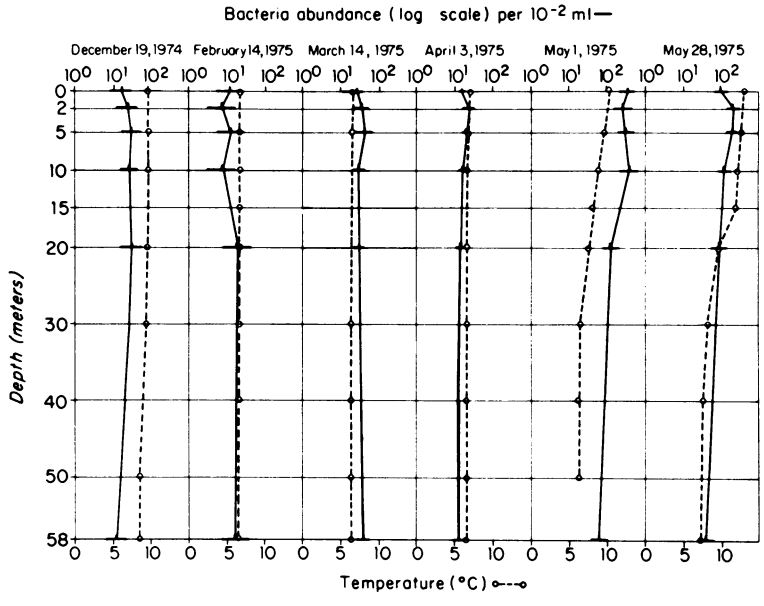

FIG. 1. *Means and 95% confidence intervals of logarithms of counts for each depth of each month and corresponding temperature profiles.*

TABLE 1. *Summary of results from vertical cast experiments*

Determination	Dec.	Feb.	Mar.	Apr.	May	June	Mean
F ratios							
Subsample	3.292[a]	5.275[a]	0.355 (NS)[b]	0.612 (NS)	3.782[a]	3.347[c]	
Sample	6.436[a]	1.448 (NS)	4.134[a]	0.915 (NS)	4.527[c]	0.977 (NS)	
Cast	0.001 (NS)	4.524[c]	1.019 (NS)	0.005 (NS)	0.397 (NS)	4.917[c]	
Depth	1.290 (NS)	1.291 (NS)	1.485 (NS)	3.252[c]	8.785[c]	38.117[a]	
Arithmetic grand mean (colonies per plate)[d]	25.4	15.2	37.8	18.5	274.7	70.9	
Estimates of s_p^2[e]	0.01175	0.04253	0.01419	0.02687	0.00985	0.00793	0.01886
Components of s_{ss}^2[f]	0.00922	0.07658	-0.00105	-0.00157	0.00695	0.00500	0.01586
Variance, s_s^2[g]	0.02773	0.02651	0.00837	-0.00043	0.01449	0.00402	0.01345
Total plates counted	87	88	89	79	95	90	

[a] Significant at the 0.001 level.
[b] NS, Not significant.
[c] Significant at the 0.05 level.
[d] December through May indicates number per 10^{-2} ml; June is number per 0.5×10^{-2} ml.
[e] Plating.
[f] Subsamples.
[g] Sampling.

four subsamples per sample and two plates per subsample without increasing the total number of plates to be counted.

A multiple-syringe sampler was constructed to examine patchiness within 1 m^2 to provide information about aggregation on a scale larger than that of the subsamples and smaller than that sampled in duplicate casts. The sampling device was used to obtain five simultaneous samples from a depth of 2 m in a eutrophic lake, an oligotrophic lake, and Lake Washington. The five sampling points comprised the corners of a 1-m square, positioned horizontally, with an additional point in the middle of the square. There was no replication of subsamples (dilutions) except for one experiment from Lake Washington, in which there were three subsamples from each of the five samples and five plates from each of the 15 subsamples. The samples were otherwise treated in the same manner as the vertical cast samples.

The variance of the data from the horizontal sampling experiment with replicate subsamples was divisible into the following components: $s^2_{\text{plates}} = 0.01046$; $s^2_{\text{subsamples}} = 0.004927$; and $s^2_{\text{samples}} = 0.000970$. The variance between samples was not significant. Fisher's coefficient of dispersion, $d = s_p^2/\bar{x}$, calculated for the untransformed data within 70 horizontal sampling subsamples, with up to 10 plates/subsample, shows significant overdispersion (aggregation) within subsamples.

The picture that emerges is: (i) patchiness is on a scale of 1.0 ml or less, based on the overdispersion of plate counts and significant variance between subsamples; (ii) dispersion on the 1-m scale is relatively random, although the samples may contain numerous aggregates and all of the samples may be within some larger patch; and (iii) samples spaced up to 20 m apart are commonly in different patches.

In other experiments we established that the large variance we have observed between subsamples and plates cannot be explained by imprecision of pipetting or the inability of the Vortex mixer to randomly distribute free bacteria throughout a volume. The use of a blender to mix samples would probably reduce the number of subsamples required and for some purposes would be recommended. However, we did not use such a method, because we wanted to examine bacterial aggregation.

LITERATURE CITED

1. **Bainbridge, R.** 1957. The size, shape, and density of marine phytoplankton concentrations. Biol. Rev. 32:91-115.
2. **Cassie, R. M.** 1959. Micro-distribution of plankton. N. Z. J. Sci. 2:398-409.
3. **Cassie, R. M.** 1962. Micro-distribution and other error components of C^{14} primary production estimates. Limnol. Oceanogr. 7:121-130.
4. **Jones, G. E., and H. W. Jannasch.** 1959. Aggregates of bacteria in sea water was determined by treatment with surface active agents. Limnol. Oceanogr. 4:269-276.
5. **Jones, J. G.** 1970. Studies on freshwater bacteria: effect of medium composition and method on estimates of bacterial population. J. Appl. Bacteriol. 23:679-686.
6. **Paerl, H. W.** 1973. Detritus in Lake Tahoe: structural modification by attached microflora. Science 180:496-498.
7. **Platt, T., L. M. Dickie, and R. W. Trites.** 1970. Spatial heterogeneity of phytoplankton in a nearshore environment. J. Fish. Res. Board Can. 27:1453-1473.
8. **Richerson, P., R. Armstrong, and C. R. Goldman.** 1970. Contemporaneous disequilibrium, a new hypothesis to explain the "paradox of the plankton." Proc. Natl. Acad. Sci. U.S.A. 67:1710-1714.
9. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods, 6th ed. Iowa State University Press, Ames, Iowa.