only safeguard necessary before introducing them to such waters would be to ensure that they did not serve as hosts to pathogenic micro-organisms. They are incapable of surviving an English winter in unheated waters.

A more detailed account of these prawns, with 121 illustrations, is given in a paper published in *Phil. Trans. R. Soc. (B)*, 277, 57–129 (1977). Thanks are given to The Royal Society for permission to use, sometimes in a modified form, illustrations from this paper.

VARIATION IN BACTERIAL POPULATIONS IN TIME AND SPACE

J. G. JONES

Among the many problems which beset the bacterial ecologist, one of the most intractable, and unfortunately the most basic, is that of obtaining a reliable estimate of the bacterial population. At any given time this population will consist of a mixture of autotrophs (organisms deriving carbon from inorganic sources and energy either from inorganic oxidation reactions or sunlight) and heterotrophs (deriving carbon and energy from organic compounds). This nutritional diversity is a major contributing factor to the problem. The bacteriologist may obtain estimates of the 'total' or the 'viable' population in any given sample. The former usually represents the number of particles of bacterial shape and size which have been concentrated on a membrane filter. There are several microscopical techniques for counting these particles but those which have received most attention in recent years are epifluorescence and scanning electron microscopy. The fluorescence technique involves addition to the sample of a dye which reacts with living cell constituents (e.g. acridine-based fluorochromes which are thought to react with DNA and RNA, the nucleic acids which control cell reproduction and growth). The sample is then drawn through a black membrane filter and the particles, including the bacteria, which are trapped on the membrane surface are counted by incident light fluorescence (epifluorescence) microscopy. The light for excitation is directed down through the objective on to the membrane surface. If this light is of a suitable wavelength the particles are excited and emit light of a lower energy (longer wavelength) which passes back up through the objective and via a series of selective filters and mirrors to the eyepiece. There are many variants on this method (Jones 1974; Jones & Simon 1975) but they, like the scanning electron microscope procedure, suffer from the disadvantage that no reliable information is provided on whether the bacteria counted are active or capable of growth and division. To obtain this information we must turn to one of the so-called 'viable counting procedures'. The sample is incubated in the presence of a nutrient medium until visible bacterial growth occurs. This is usually done in this laboratory by spreading known volumes of water on the surface of CPS agar (Taylor 1940; Collins & Willoughby 1962), and counting the colonies which develop after a suitable incubation period. The CPS agar has been thoroughly tested in a number of laboratories and appears to yield higher counts of viable freshwater heterotrophs than other media available at present. We cannot, however, assume that all heterotrophic bacteria produce visible colonies, nor that the medium will support the autotrophic organisms mentioned above. Lake waters in this district yield direct counts which vary between 1×10^6 and 10×10^6 bacteria ml^{-1}, whereas counts of viable organisms are two to three orders of magnitude lower than this at 1×10^3 to 20×10^3 bacteria ml^{-1}. Clearly we are not culturing all the bacteria that are capable of growth, but on the other hand we may be

encouraging growth in species which are not normally active in the field. It is against this background that the bacterial ecologist must attempt to draw meaningful conclusions from the population estimates which have been made. Variations in numbers in time and space must be considered with care, particularly where bacterial indicators of pollution are concerned. The interpretation may affect decisions on water use and management. In more general population studies it is not uncommon for ancillary information to be used to support conclusions drawn from changes in the numbers of bacteria detected. Adenosine triphosphate (ATP), the high energy molecule common to all living cells, may be used as an indicator of biomass, and recent developments in the technique for its determination (Jones & Simon 1977) allow us to detect very low levels without prior concentration of the micro-organisms on membrane filters. Other indicators of microbial biomass and activity, such as electron transport activity and specific substrate uptake kinetics (Hobbie et al. 1972), may also be measured. Electron transport, in one form or other, is common to all living cells whether they are aerobic or anaerobic, autotrophic or heterotrophic. It is the means by which electrons from reduced compounds are linked to the terminal respiratory acceptor (oxygen in the case of aerobes) with simultaneous transfer of energy, as ATP is generated. The rate at which the microbial population responds to different concentrations of radioactively labelled substrates may also be measured, and if the natural substrate concentration is known, then kinetic values such as the maximum theoretical uptake rate (V_{max}) and turnover time (T_t) may be calculated. The results obtained may be of comparative rather than absolute value but could indicate how realistic the trends shown by population estimates have been.

Examples of counts of the total and viable bacteria from firstly a deep and secondly a shallow lake in this district are shown in Fig. 1. Samples were taken in August when both lakes were stratifed and it is evident that the hypolimnion of the deeper lake is cooler and not as depleted in oxygen. The counts of viable bacteria are higher in the epilimnion, where their distribution often corresponds to the stratification of the algal population (Jones 1972). The degree of correlation between the phytoplankton distribution and the bacterial populations depends on the algal species present. We cannot be certain which colonies on a plate count originated as bacteria attached to algae and which as unattached organisms, but we do know that certain algae have more bacteria attached to them than others (Jones 1972). Those which are most heavily colonized by bacteria include the colonial forms, particularly those which produce extracellular mucus or capsules. The algae with hard external coats such as dinoflagellates and diatoms appear to be the least attractive to bacteria whereas the filamentous forms with simple cellulose walls occupy an intermediate position. The degree of attachment of bacteria will therefore depend to some degree on the algal species present, but also on the condition of the particular alga. Exponentially growing cells appear to be relatively free of bacteria whereas

those which have stopped growing, or have become moribund, are rapidly colonized (Jones 1976) and eventually may contribute to the partially decomposed detritus which sediments into the hypolimnion.
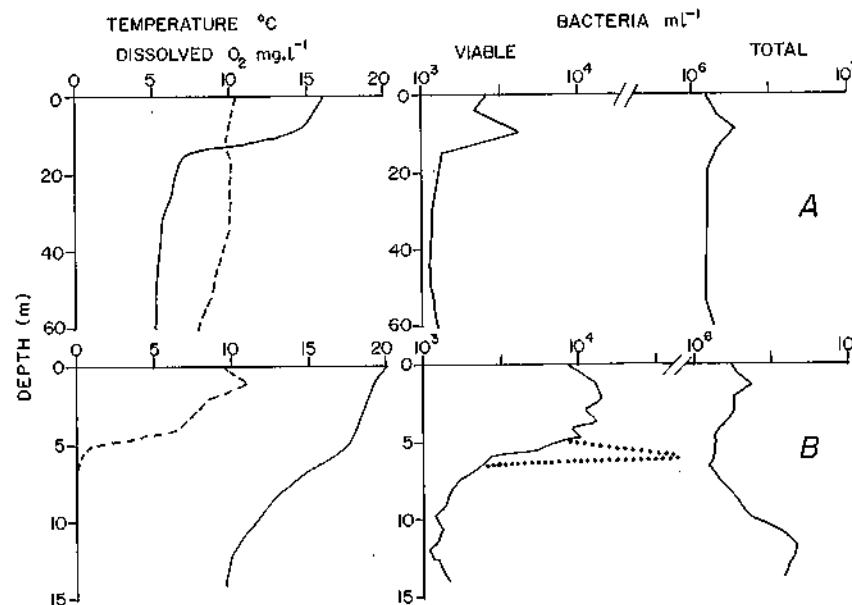

FIG. 1.   The effect of stratification on the vertical distribution of bacteria in a deep and a shallow lake. A, Windermere North Basin, B, Esthwaite Water. —— temperature, – – – dissolved O_2, ······ magnitude of short term population maxima in the metalimnion. (Adapted from Jones, 1977a).

Plate counts of bacteria are usually lower in the hypolimnion than in the epilimnion, particularly in eutrophic lakes where the deeper water becomes anoxic. This might be expected if only aerobic plate counts were made but no significant increase in the count is obtained if plates are incubated in the absence of oxygen. Trials with a number of media have failed to improve the recovery of anaerobes, and emphasize our inability to culture these organisms satisfactorily.

The estimates of the total population are 2 to 3 orders of magnitude higher than those obtained by plate counts, and the degree of difference between the epilimnion and hypolimnion of the deep lake is not as great. In the anoxic hypolimnion of the shallow lake, however, results obtained by the two methods differ markedly. Similar fluctuations with depth are observed in the epilimnion but whereas plate counts decrease with depth into the hypolimnion (except very near the sediment–water interface) an increase in the total count is observed. This increase, often to population numbers which are 5 times greater than those in the epilimnion, occurs in

water which has been enriched by the release of nutrients from the sediment as anoxic conditions develop during the summer. It represents a part of the population not detected by the plate count and probably includes the more demanding heterotrophs as well as photosynthetic and chemosynthetic autotrophs. Fig. 2 also shows that there may be high densities of bacteria in the metalimnion which are of only short duration (Collins & Willoughby 1962; Collins 1970). These may be populations of decomposers which have aggregated in this region along with sedimenting algal remains and other particulate matter, because of temperature and density gradients in the water. On the other hand they may have been carried in by streams which are temporarily in flood and of a temperature similar to that of the metalimnion.
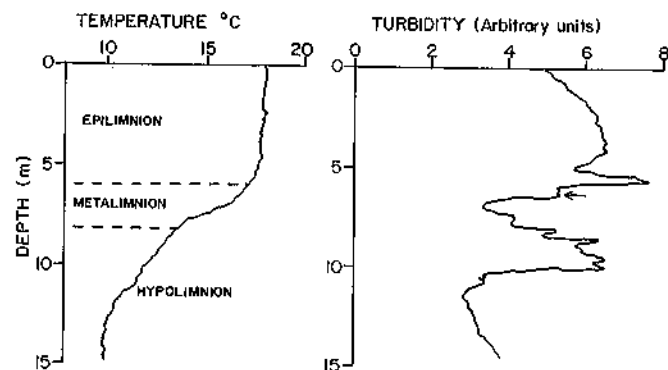

FIG. 2. *In situ* measurements of temperature and turbidity in Esthwaite Water, a eutrophic lake, on 4 Sept. 1975. (Adapted from Jones, 1977b).

We are therefore able to obtain some information on factors controlling bacterial populations from depth profiles such as those illustrated in Fig. 1. It should be remembered, however, that the degree of resolution obtained in the study of distribution patterns will depend on the type of sampling equipment used. The general picture obtained by sampling at 1 m depth intervals, often with large sampling devices up to 1 m in length, may provide an integrated summary but may also obscure a wealth of variation. This will be revealed only by obtaining samples at shorter intervals with a sampler such as that devised by Heaney (1974) or by making continuous measurements in the field. An example of the latter type is illustrated in Fig. 2, where turbidity is measured in situ with a Schenk transparency meter and plotted on an X/Y recorder against the output from a pressure transducer which provides a measure of depth. The major changes in turbidity in the epilimnion and the hypolimnion may be attributed to phytoplankton and water chemistry respectively, but various small secondary peaks are superimposed on the general pattern and require further analysis. Some of the turbidity changes, for example the

shoulder marked with an arrow in Fig. 2, are associated with populations of certain planktonic bacteria, e.g. *Planctomyces* and *Metallogenium* spp., but this has yet to be demonstrated unequivocally. These bacteria may be observed in samples concentrated on membrane filters, but it is difficult to assess their contribution to the overall turbidity of the water and to know for certain that they are active in situ and have not aggregated at a particular depth by purely passive means.

We have, so far, considered bacterial variation in only a single dimension, i.e. depth. Most limnologists, and bacteriologists are no exception, have taken their samples at a single point in a given lake (as in the case of the data in Fig. 2), and variation in the horizontal plane has been largely ignored. Recent results indicate, however, that this variation can be quite considerable. Palmer, Methot & Staley (1976) have demonstrated patchiness on a centimetre and a metre scale. Results from Windermere and Esthwaite Water (Jones 1977a), obtained from sampling cruises organized by Dr Glen George during his stay at this laboratory, show that variation in the horizontal plane may be at least as great as that in the vertical plane. These results were obtained from point samples as well as integrated transects taken continuously across almost the full width of the lakes. One might expect the latter samples to exhibit greater homogeneity, but all showed significant differences with ranges for viable bacteria between 5 and 28×10^3 ml^{-1} and for total bacteria between 1·6 and $4·3 \times 10^6$ ml^{-1}. It might be of interest, at this point, to draw a parallel between the search for finer detail and greater understanding in bacterial spatial distribution and the degree to which we understand the 'fine detail' of the species which make up the population. If, for example, we restrict the species of bacteria studied (e.g. by the use of selective growth media) we will probably obtain a better understanding of changes in their population. In the studies discussed above, the coefficients of variation for horizontal and vertical profiles became greater as the taxonomic groups of bacteria studied became more restricted (i.e. the more the study became an autecological one). In other words, the coefficient increased down the series: total bacteria—viable bacteria—coliforms—*E. coli*. This is, of course, the basis for the general practice of studying variation in water quality through 'indicator species of bacteria'. It is unlikely, therefore, that recent trends towards the use of more general population estimates for assessing water quality will be as useful.

It is hardly surprising, given the variation in space of bacterial populations in the water column, and the fact that water movement continuously changes the picture obtained, that the bacterial ecologist may encounter some difficulty in understanding variation in the final dimension, that of time. Because variables such as temperature, dissolved oxygen concentration, pH, and inorganic nutrient concentration are often inter-correlated, it is difficult to interpret seasonal changes in the water column and to determine which factors might exert a significant effect on the bacterial population. A recent analysis of plate counts and total counts made over

several years (Jones 1977a) has shown that stratification and deoxygenation exert a major effect on the population and that some of the remaining variability can be explained in terms of changes in the algal population and the concentrations of inorganic phosphorus and nitrogen. A sizeable proportion of the variation in bacterial numbers remains unexplained and emphasizes where further work is required. We might further our understanding of population changes at a general level by obtaining more information on changes in dissolved organic carbon compounds, grazing pressure and lake retention time (as a measure of rainfall and input from the drainage basin). At a more specific level we might consider changes over shorter time periods. Much of the work done in the past has been on observations taken at weekly (or longer) intervals, but there is evidence (Saunders, 1976) that the activity of heterotrophs may vary diurnally, in response to that of the primary producers.

I have tried to demonstrate in this brief review that the observed variability in bacterial populations, in any dimension, will depend heavily on the sampling interval used. Perhaps the time has come to search for finer detail, not only in space and time but in the many species of bacteria which are active in the aquatic habitat.

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