

Studies of planktonic bacteria in Lake Valencia, Venezuela

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With 3 figures and 3 tables in the text

Abstract

The planktonic bacteria of Lake Valencia, Venezuela, were studied between 1977 and 1981. Total counts done by epifluorescence microscopy showed that approximately two-thirds of the bacterial cells were solitary coccoid forms spanning a size range of 0.07 to 4 $\mu\text{m}^3/\text{cell}$. Most of the remaining bacterial cells were rod-shaped. The study also documented the steady presence of smaller numbers of distinctive bacterial morphotypes such as *Planctomyces*. More than 90% of the bacteria were unattached to any kind of particulate substrate. In the upper water column, the annual average bacterial abundance was $0.5 \times 10^6 \mu\text{m}^3/\text{cc}$, or about 50 $\mu\text{g}/\text{l}$ bacterial carbon. This corresponded to about 3% of phytoplankton cell carbon over the same interval. Bacterial abundance in the upper and lower water column was essentially uniform as long as the lake was mixing. During the stratification season, however, bacterial abundances in the anoxic deep water declined to very low levels. At irregular intervals, penetration of oxygen into deep water caused by thickening of the mixed layer resulted in increases of bacterial abundance in deep water. In the upper water column, the lowest bacterial abundances occurred after the mixed layer had assumed a stable thickness for an extended interval, and increased whenever the thickness of the mixed layer increased. Tests were made for presence of allelopathic substances during the year 1981. These tests showed that significant suppression of bacterial growth could be accounted for by the presence of allelopathic substances. Allelopathic effects were limited to the stratification season and coincided with periods of decline in bacterial abundance and stable mixed layer thickness. Bacterial abundances are not directly related to phytoplankton abundance, but rather to mixing events, which exercise their effect partially through relationships to allelopathy. Calculations incorporating the rates of phytoplankton metabolism and maximum possible rates of bacterial metabolism demonstrate that the planktonic bacteria of Lake Valencia process a very small fraction of the net primary production. Since primary production is also not efficiently intercepted by zooplankton, sediment bacteria and sediment storage play a very large role in carbon flux of this lake.

Introduction

Since the introduction of direct-count methods based on fluorochromes, it has been possible to estimate the total numbers of bacteria in lakes. Nevertheless, relatively few lakes have been sampled for bacteria over an extended interval. For this reason, many fundamental questions remain unanswered. For example, the number of bacteria in lake water seems to be in the vicinity of 1

million cells/cc (HOBBIE & WRIGHT 1979), but the pattern of variation among lakes of different trophic level or between seasons in a given lake is not yet well understood. The relationship between phytoplankton and bacteria remains controversial, as do the controls on bacterial biomass and the general metabolic activity of bacteria in lakes. In the present paper, we consider these aspects of bacterioplankton ecology for Lake Valencia, Venezuela.

Description of study site

Lake Valencia is situated in the Aragua Valley of north-central Venezuela (10°N, 67°W). The lake has an area of 350 km², a maximum depth of 40 m, and a mean depth of 20 m. The mean temperature of the upper water column is 27 °C; temperature varies only about 2 °C with season and depth in Lake Valencia (LEWIS 1984). The water column is stratified between April and December and mixes completely between December and March (LEWIS 1983), although particular years can differ by a few weeks in the timing of these seasons. During the stratification season, the upper mixed layer has a mean thickness of 12 m but there is considerable variation around the mean in response to irregular changes in wind strength and temperature. During stratification season, the deep part of the water column is anoxic at least part of the time.

Lake Valencia is eutrophic. Although the lake in its completely natural condition was probably productive, algal standing stock has been greatly augmented by nutrients from sewage effluent entering the lake. Phosphorus loading averaged 2.2 g/m²/yr in 1977–1978 and, due to additional sewage diversion, rose to 3.1 g/m²/yr after 1978 (LEWIS & WEIBEZAHN 1983). Phytoplankton standing stock over the 5 years of our study (1977–1981) averaged 19 mm³/l (ca. 40 µg/l chl a), and the number of algal cells per cm³ averaged 445,000 (LEWIS 1985 a). The phytoplankton was heavily dominated by blue-green algae, including such genera as *Microcystis*, *Anabaena*, *Lyngbya*, and *Anabaenopsis* (LEWIS & RIEHL 1982). Diatoms, cryptophytes, and green appeared in quantity at certain times, but the blue-green algae were continually abundant.

Methods

Lake Valencia was sampled at a central station over the deepest part of the lake at weekly intervals during 1977 and 1978 and at bi-weekly intervals during 1979–1981. On each of these routine sampling dates, a complete profile of samples was taken from the top to the bottom of the water column for chemical analysis, bacteria, phytoplankton, and zooplankton. The bacteria samples were taken with an integrating sampler of the type described by LEWIS & SAUNDERS (1979). The sampler takes a 5 m increment of the water column and, since the water depth at the main sampling station was 35 m deep, the entire water column was sampled in 7 contiguous increments of 5 m each.

Each 5 m segment was allowed to pass from the integrating sampler into a mixing chamber where it was homogenized prior to being subsampled. Subsamples from the mixing chamber were drawn into pyrex bottles that had been cleaned with dichromate acid cleaning solution and autoclaved. To each of these bottles, sufficient formaldehyde was added to make a 2% solution, thus preserving the sample. The preservative used for this purpose was first filtered through a Nucleopore filter of 0.2 μm pore size to assure that it did not introduce countable particles into the sample. The samples were kept in the dark while being transported to the laboratory. In the laboratory, the preserved bacteria samples were refrigerated until they were processed. Processing typically occurred within 48 hours of sample collection.

Stained bacteria were counted by epifluorescence microscopy at 1250 \times with a Leitz Dialux and Ploemopak filter combination. Between 1977 and 1980, the method was based on fluorescein isothiocyanate (FITC). Our use of FITC was as described by FLIERMANS & SCHMIDT (1975), but samples were prepared by the methods of HOBBIIE, DALEY & JASPER (1977). Nucleopore filters (0.2 μm pore size) were treated with Irgalen Black BGL dye to darken their color. A small amount of the sample was then passed through the filter following treatment of the filter with a surfactant. A permanent mount was made of each filter according to the method described by RODRIGUEZ & DEINHARDT (1960). Bacteria were then counted in two perpendicular strips across the slide under epifluorescence microscopy by use of a 100 \times oil immersion objective (minimum of 100 cells counted). These counts proved not to be quantitatively sound because of irregular background fluorescence that obscured some bacteria. We therefore use the 1977–1980 data only for qualitative purposes here.

In late 1980, the FITC method was discontinued and replaced with an acridine orange method. The preserved samples were ampulated and stored under refrigeration until they were to be counted. They were then treated with acridine orange according to the procedure described by HOBBIIE et al. (1977) and counted by epifluorescence microscopy immediately after staining. We consider the acridine orange counts quantitatively sound.

Blanks were run with every set of samples. Each blank consisted of water filtered through Nucleopore filters (0.2 μm pore size) to remove bacteria. This water was processed identically to lakewater samples. Except for high blanks during the first few weeks of 1977, when reagents produced contamination, blanks were consistently low enough to account for less than 1% of the bacterial count.

An interlaboratory comparison was done for the acridine orange method. This comparison was done with a sample from the Orinoco River, whose bacteria numbers were being studied simultaneously with those of Lake Valencia. Multiple ampules were prepared from a sample and some of the ampules were sent to J. HOBBIIE & T. CORLISS at the Marine Biological Laboratory, Woods Hole. Our mean number for the split sample was 1.1×10^5 cells per cc. The mean number obtained by HOBBIIE & CORLISS was 1.3×10^5 with acridine orange. HOBBIIE & CORLISS recounted the sample after using DAPI as a stain rather than acridine orange, and obtained 1.4×10^5 . The DAPI probably gave higher counts because it is superior when large amounts of debris are present. However, debris was not characteristic of the Lake Valencia samples, for which the acridine orange counts were used.

Bacterial abundance patterns suggested possible suppression of bacterial growth under some conditions in Lake Valencia. A test was therefore devised for determining whether or not this allelochemical effect was present, and, if so, whether it was present consistently or only at certain times. The allelochemical test was based on the principle that organic allelochemical substances can be destroyed by ultraviolet radiation. Thus

the growth rate of Lake Valencia bacteria supplied with abundant nutrients in lake water that had been treated by ultraviolet radiation, as compared with lake water that had not been so treated, would indicate the presence of organic allelochemicals. Tests of this type were done on most of the routine sampling dates in 1981.

Water to be used in the allelochemistry tests was collected at the surface of Lake Valencia on the morning of routine sampling. The method of collection and handling was identical to that used for routine samples, except that no preservative was added to the samples. The samples were kept in the dark until they reached the laboratory. Two liters of the water was filtered once through Whatman GF/C glass fiber paper to remove phytoplankton, debris, and large bacteria. The water was then refiltered through millipore filters (pore size, 0.22 μm) and again through Nucleopore filters (0.2 μm pore size). The purpose of this filtration was to remove well over 99% of the bacteria in the sample. The water thus filtered was subsampled; subsamples were later counted by epifluorescence. The counts showed that the filtration procedures did consistently remove essentially all of the bacteria from the water (removal of all cells was not necessary for the purposes of the experiment).

The filtered water was divided into two portions. One portion, which we shall refer to here as "UV-treated", was placed in a UV combustion chamber of the type described by MANNY, MILLER, and WETZEL (1971) and irradiated with ultraviolet light for one hour. This resulted in a drastic decline in the concentration of organic matter in the sample from over 5 mg/l C to less than 0.5 mg/l C. Some organic matter remained, but for practical purposes the organic matter in the sample was combusted or drastically altered by partial combustion.

The UV-treated water was used to fill two 300 ml glass-stoppered bottles one-third full (100 ml per bottle). In addition, two bottles of the same type were filled with lake water that had been filtered but not UV treated (control). The bottles, as well as all of the other glassware and materials used to handle the water samples, had been previously autoclaved.

To each of the four sample bottles (two UV-treated, two control) one ml of nutrient solution was added. This nutrient solution consisted of 500 mg of glucose and 250 mg of casamino acids in 100 ml of sterile water. The purpose of these nutrient additions was to establish good nutrient conditions for bacterial growth. After the addition of the nutrient solution, 1 ml of raw lake water from the original sample was added to each of the four bottles. Depending on the date of sampling (see Fig. 2), the number of bacteria added to each bottle would have been between 0.2 and 1.5×10^6 cells. This served as an inoculum from which bacterial growth could occur. If allelochemical substances were present, they would have been transferred along with the inoculum to the treatment bottles, but, because of the small size of the inoculum, would have been very greatly diluted (1:100).

The inoculated bottles with the nutrient solution were placed in the dark (to prevent algal growth) on an orbit shaker, where they were left at ambient temperature ($25^\circ \pm 2^\circ$) for four days. At the end of four days, the samples were processed for acridine orange counting as described for the routine samples.

In evaluating the results of the experiments, we placed each sampling date into one of three categories: (1) no effect, if the UV-treated sample and the control produced approximately the same final abundances of bacteria; (2) UV inhibition, if the bacteria of the UV-treated sample grew more slowly than those of the control; and (3) UV stimulation, if the bacteria of the UV-treated sample grew more rapidly than those of the control. Since the four bottles could be expected to show a certain amount of random variation due to differences in handling and counting variance, experiments were placed in

the "no effect" category if the replicates of the control and the treatment overlapped or if the means were different by less than 20%, which reflects the statistical resolving power of the counting method.

Results

Types of bacteria

Fig. 1 shows the major kinds of bacteria that were observed during the five-year study in Lake Valencia. Simple rods were frequently observed. The median width for such rods was close to $0.5 \mu\text{m}$. Lengths of the rods varied, probably because they included different kinds of bacteria as well as different stages in the division cycle. The small rods were typically in the vicinity of $1\text{--}4 \mu\text{m}$, while the larger ones could be as much as $8 \mu\text{m}$. These simple rods were typically solitary, although occasionally two or more cells were grouped together.

Cocci were very common in the Lake Valencia samples. These also appeared in a number of sizes, and were typically single cells. The smallest cocci had diameters in the vicinity of $0.5 \mu\text{m}$. A slightly larger type of cell, with a diameter in the vicinity of $1.5 \mu\text{m}$, was also relatively common. Even larger cells, with diameters in excess of $2 \mu\text{m}$, were seen but were not common. Colonies of cocci were also occasionally observed (Fig. 1). Typically these colonies contained in the vicinity of 100 cells, and the cells were of the small type (ca. $0.5 \mu\text{m}$). Neither the colonies nor the individual coccoid cells were commonly associated with algae.

More than 90% of the bacteria that were observed by epifluorescence were unattached to any kind of substrate. While bits of debris and dead algae did

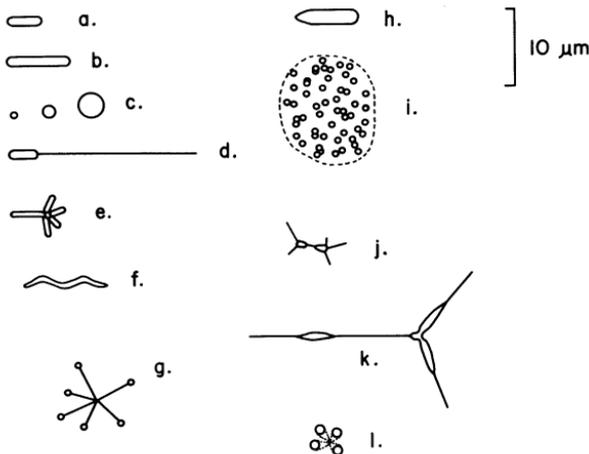


Fig. 1. Scale drawings of bacteria from Lake Valencia. See text for details.

Table 1. Major categories of microbes in Lake Valencia and their relative contributions to numbers and biomass in 1978.

Category	Numerical Abundance, %	Size of Individual, μm^3	Biomass %
Very Small Rods	7.6	0.20	2.3
Small Rods	24.5	0.77	28.3
Large Rods	4.6	1.50	10.4
Very Small Cocci	26.6	0.07	2.7
Small Cocci	31.7	0.52	24.9
Large Cocci	5.0	4.17	31.4

support bacterial growth, the attached bacteria were far outnumbered by the unattached ones.

Table 1 provides a percentage breakdown of the most important categories of microbes observed during the epifluorescence counting. The bacteria were not counted by categories over the entire study period; the data in Table 1 are for the year 1978. Numerically, the cocci account for about two-thirds of the cells and the rods account for the remaining third. The size of individual cells varied greatly. Because of the great size range among cells, the percentage contribution of the various categories to biomass is different from that of the numerical contributions.

Certain types of bacteria were detectable even in relatively small numbers because of their distinct morphologies. Even though these bacteria did not make a major numerical or biomass contribution to the total standing stock of bacteria, their consistent appearance in the plankton makes them worth mentioning. Fig. 1 shows the distinctive bacterial types that appeared repeatedly. A special kind of rod that was observed repeatedly but never in great abundance we have dubbed the "pointed rod", as shown in Fig. 1. This type of rod was consistently attached to algae, especially the mucoid coat of *Microcystis*. It is distinguished from other rods by its slightly pointed end (the narrow end is proximal to the alga) and its greater diameter (ca. $1.5 \mu\text{m}$) and length (ca. $8 \mu\text{m}$) than most rods. Although these bacteria were associated directly with algae, the vast majority of algal cells and colonies were bacteria-free.

Another type, shown as item D in Fig. 1, was a small rod with a long filament attached. The morphology of this organism, including both the length of the filament and the size of the cell to which it was attached, was strikingly consistent. Another distinctive morphotype, which was somewhat less abundant, consisted of a stellate arrangement of ordinary looking rods (item E, Fig. 1). *Vibrio* forms also appeared consistently, although they were never abundant (item F, Fig. 1). Items J and K in Fig. 1 are organisms whose cells are vaguely spindle shaped, and bear projections or spines. These appear similar, at least superficially, to the genus *Rhabdochromatium* (HUBER-PESTALOZZI 1975), a

Table 2. Abundance of distinctive bacterial types counted routinely with phytoplankton, expressed as averages over the interval 1977–1981.

Category	Abundance cells/cc
Large spindle form (k)*	840
Small spindle form (j)	140
Vibrio (f)	11700
Stellate (e)	200
Rod with Filament (d)	224
Small <i>Planctomyces</i> (l)	49
Large <i>Planctomyces</i> (g)	1320

* letter refers to drawings in Fig. 1.

genus associated with sulfur metabolism. Also observed were at least two types of the genus *Planctomyces* (items G and L, Fig. 1). This peculiar stellate form, which is attributed to the Caulobacteriales, has been found in several European lakes (HAJDU 1974), in Lake Mendota (PEDROS-ALIO & BROCK 1983), in the English Lake District (JONES 1977), and in Lake Lanao, Philippines (LEWIS, unpublished).

Because the distinctive morphotypes mentioned above could be easily recognized during phytoplankton counting, they were tabulated over the entire 5 years along with the phytoplankton. The average abundances of the distinctive morphotypes are given in Table 2.

Some filamentous bacteria were also observed during the course of the study, but these were not tabulated. They were typically present in relatively small numbers and were difficult to separate from the much more numerous filaments of *Lyngbya limnetica*.

Average abundances and seasonal changes

Table 3 shows the average numerical abundance for bacteria in Lake Valencia over the year 1981 at all depths in the water column. The averages are es-

Table 3. Average abundance of bacteria of all depths in Lake Valencia for the year 1981.

Depth m	Thousands of cells/cc	Cell volume $\mu\text{m}^3/\text{cc}$	Cell carbon $\mu\text{g}/\text{l}$
0–5	765	508000	51
5–10	766	509000	51
10–15	878	583000	58
15–20	777	505000	51
20–25	605	402000	40
25–30	557	370000	37
30–35	525	342000	34

essentially identical for 0–5 m and 5–10 m, as would be expected in view of the fact that the upper mixed layer in Lake Valencia has a mean thickness of 12 m. The abundances at 10–15 m, where the uppermost thermocline is often found during the stratification season, had slightly higher average abundances of bacteria. Below 15 m there was a steady decline in average abundances toward the bottom of the lake. This segment of the water column is subject to anoxia and relatively high concentrations of hydrogen sulfide at certain times of the year.

The numerical abundances in Table 3 have been converted to biomass on the basis of a weighted average cell volume for bacteria in Lake Valencia. The source of this average cell volume is Table 2, which gives the relative abundance of various morphotypes and the cell volume of each. The cell volume of each morphotype in Table 2 weighted by its proportional contribution to total bacteria numbers gave an average of $0.66 \mu\text{m}^3$ per cell.

The total bacterial cell volume in Lake Valencia in 1981 averaged between $0.3 \times 10^6 \mu\text{m}^3/\text{cc}$ in the lower water column and $0.5 \times 10^6 \mu\text{m}^3/\text{cc}$ in the top 20 m. In the top 20 m, phytoplankton cell volume averaged $15.0 \times 10^6 \mu\text{m}^3/\text{cc}$ over the same interval. Thus bacterial cell volume in the growth zone averaged 3.3% of phytoplankton cell volume. Table 3 also provides estimates of bacterial carbon; these estimates are based on the assumption that cell carbon is 10% of wet weight for bacterial cells (SOROKIN & KADOTA 1972).

Fig. 2 shows the pattern of bacterial abundances at the surface and in deep water in 1981. Oxygen concentration, a good indicator of mixing, is also shown for the surface and deep water. Total phytoplankton cell volume is shown in the bottom panel of Fig. 2.

At the surface, bacterial abundances were relatively low at the first of the year, during the mixing season, after which they climbed to the vicinity of the annual average, 0.5×10^6 cells/cc. Abundances varied as much as 50% around this average during the entire stratification. There was an especially marked decline in late June. This decline may have been caused by a preceding extended interval of stable layering, as indicated by steady oxygen concentrations near the surface and complete anoxia in deep water. Such periods of stable, high-lying thermoclines result in nutrient stress that can be severe enough to reduce phytoplankton populations (LEWIS 1985a and panel 3 of Fig. 2). The sharp minimum in bacterial abundance gave way to more average abundances of bacteria and then to a peak after weather changes brought up water from deeper layers, as indicated by the oxygen decline at the surface in July.

Deeper in the water column, the early bacteria concentrations were very similar to those at the surface of the lake. Later in the year, however, the bacteria abundances in deeper water were consistently lower than those near the surface. The lower abundances of bacteria in deep water were a by-product of stratification, since they coincided with the occurrence of severe anoxia in deep water during the stratification season. Bacterial abundances in deep water fell

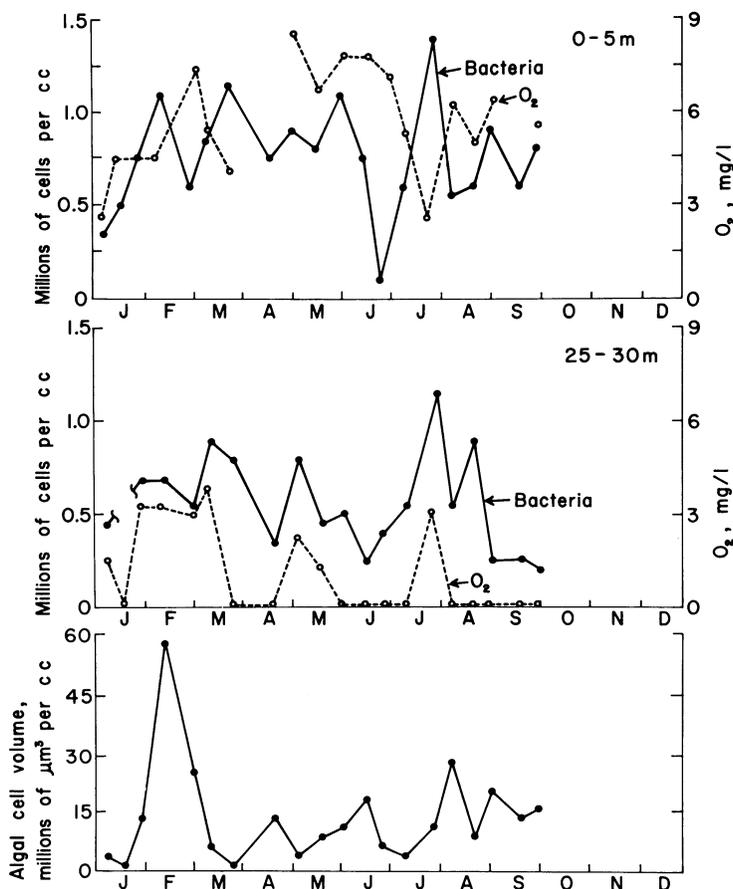


Fig. 2. Abundances of bacteria and amounts of oxygen at the top of the water column (top panel) and bottom of the water column (middle panel) and algal abundances in Lake Valencia, 1981.

to a low level after an extended period of very stable stratification in June and declined again in September for the same reason. Oxygen pulses near the bottom, representing thickening of the mixed layer (atelomixis: LEWIS 1983), were accompanied by rises in bacterial abundance.

There is no statistical relationship between phytoplankton cell volume and bacterial abundance at the surface or in deep water (Spearman Rank Correlation Coefficient, $p > 0.05$). In effect, bacterial abundance is uncoupled from phytoplankton abundance in Lake Valencia.

Tests for bacterial growth suppression by algae

One possible explanation of the low bacterial standing stock in general and the tendency for bacterial standing stock to decline after long periods of stabil-

stratification season on which bacterial abundances in the lake were lowest. These were dates on which the upper mixed layer had been stable and relatively thin. Thus the timing of the stimulation responses suggests that an allelopathic effect coincides with, and thus may help explain, periods of declining bacterial abundance. Furthermore, the results suggest that the allelopathic effect is coincident with conditions under which nutrient stress for the phytoplankton community is most pronounced due to extended growth in a relatively thin mixed layer.

The periods of stimulation did not correspond to the abundance pattern of any particular genus or class of the phytoplankton. However, since the production of dissolved organic carbon by phytoplankton is known to vary in quantity and in quality according to the conditions for phytoplankton growth (FOGG 1975), the lack of any simple correlation between the allelopathic effects and the presence of certain taxa is not surprising.

Discussion

The sizes of bacterial cells observed in Lake Valencia overlap with those found in other lakes. On a numerical basis, most of the cells in the plankton of lakes have cell volumes in the vicinity of $0.1 \mu\text{m}^3$ (HOBBIE & WRIGHT 1979; PEDROS-ALIO & BROCK 1983; STRASKRABOVA & KOMARKOVA 1979). However, a contingent of larger cells, whose median cell volume is in the vicinity of $1 \mu\text{m}^3$, is consistently reported from lakes, including Lake Valencia. It is generally thought that the larger cells are simply in better nutritional condition (PEDROS-ALIO & BROCK 1983), although it is also known that the smaller cells are capable of taking up dissolved organics from the water column (HOBBIE & WRIGHT 1979). The weighted average cell volume in a lake is very strongly dependent on the exact percentage of larger cells. The weighted average for Lake Valencia ($0.66 \mu\text{m}^3$) is higher than for some other lakes where averages have been calculated (PEDROS-ALIO & BROCK 1983; STRASKRABOVA & KOMARKOVA 1979). This may reflect differences in the nutritional condition of bacteria among lakes.

The small percentage of attached bacteria in Lake Valencia is by no means unusual. HOBBIE & WRIGHT (1979), generalizing from the literature, indicate that most planktonic bacteria will be unattached. In a careful year-long study, PEDROS-ALIO & BROCK (1983) found between 1 and 30% of bacteria in the upper layers of Lake Mendota attached to particles. SIMON & TILZER (1982) report only 5% average attached bacteria in Lake Constance. Thus while the percentages may be higher in some lakes (see the literature survey by PEDROS-ALIO & BROCK 1983), the rule seems to be that far fewer than 50% of the cells will be attached. Lake Valencia, which conforms to this trend, may be even more extreme than most of the lakes that have been studied. A key observation

here is the extreme rarity of detrital organic matter in Lake Valencia, as evident during plankton counting: live phytoplankton are 10 to 100 times as abundant as nonliving particles at all times of the year. This appears to be a common characteristic of tropical lakes (see LEWIS 1974), possibly because the high water temperatures cause rapid breakdown of particulate organics.

Abundances of bacteria in Lake Valencia fall well within the range of values reported for lakes generally, but could be considered low among values reported specifically for eutrophic lakes. In their survey of the literature, HOBBIÉ & WRIGHT (1979) indicate that the expected value for abundances in the water column of eutrophic lakes is between 1 and 2×10^6 cells/cc. OLAH (1973) reported approximately 1.5×10^6 cells/cc for Lake Balaton, and COVENEY et al. (1977) found abundances between 3 and 13×10^6 cells/cc in Lake Bysjon, Sweden. However, lakes Balaton and Bysjon are both very shallow, and are thus subject to resuspension of bacteria from sediments and possibly have a richer supply of organic materials from sediments. Eutrophic Lake Mendota has abundances as low as 0.1×10^6 cells/cc in the winter and as high as 4×10^6 cells/cc in the summer (PEDROS-ALIO & BROCK 1983). Among lakes of more moderate productivity, the values reported by JONES (1977) for Lake Windermere in the English Lake District fall generally 0.6 and 2.4×10^6 cells/cc, as would be expected from the predictions of HOBBIÉ & WRIGHT (1979).

Values for tropical lakes are generally not available for comparison with those of Lake Valencia. KILHAM (1981) found very high abundances of bacteria in four saline lakes in Africa, but these may well have been caused by resuspension, since these lakes were very shallow.

Depth distributions of bacteria are highly variable among lakes. According to HOBBIÉ & WRIGHT (1979), abundances will typically be higher in the epilimnion than in the hypolimnion. Numerous lakes, including Lake Valencia, show this pattern. On the other hand, JONES (1978) working in the English Lake District, found the highest abundances of bacteria in the hypolimnion, despite hypolimnetic anoxia, and STRASKRABOVA & KOMARKOVA (1979) found virtually no variation with depth in a Czech reservoir. For Lake Valencia, the strong suppression of bacterial abundance by anoxia in the deep water is very significant in that it indicates slow, and possibly negligible, decomposition of organic matter over much of the stratification season. However, deep penetration of oxygen caused by movement of the thermocline relieves anoxia in an irregular manner, allowing sporadic bacterial regrowth.

From the studies that have been done thus far on temperate lakes, very few generalizations can be made about causes of temporal and spatial variation in bacterioplankton abundance. Abundances during the growing season can be quite variable, but causes of variation are far from clear. Some authors attempt to interpret variations in abundance by reference to temporal changes in the availability of labile organic matter originating from phytoplankton. For ex-

ample, COVENEY et al. (1977) argue that higher abundances of bacteria following algal blooms in Lake Bysjon, Sweden, are explained by the release of labile organic matter from the senescent algae. Without direct evidence, however, one could just as logically appeal to allelopathic suppression of bacterial growth during the period of algal bloom or even to factors only indirectly related to phytoplankton. The Lake Valencia data do not suggest any simple proportional coupling between phytoplankton and bacterial abundances. For Lake Valencia, the experimental evidence based on the growth of natural bacterial assemblages in water freshly drawn from the lake shows that bacterial populations are at times severely suppressed by inhibitory substances. This effect is not steady, but it is predictable to the extent that bacterial growth suppression occurs during extended periods of algal growth in a thin mixed layer likely to lead to algal nutrient stress. Mixing appears to be more directly related to bacterial abundance than any other factor, but this is at least partly because mixing affects the presence, effectiveness, or dilution of allelochemicals. Extended isolation of an upper mixed layer seems to lead to decline in bacterial abundances, whereas enrichment of the mixed layer with water from deeper in the water column is accompanied by increases in abundance. In this connection it is interesting that JONES (1977) concluded from statistical studies of bacterial abundance in the English Lake District that the events surrounding stratification were more important than other factors in accounting for variance in bacterial abundance.

Dating back at least to the studies of PRATT (1942), there is evidence for the production by algae of organic substances that suppress bacterial growth. More recent studies include those by CHROST (1975) and JØRGENSEN (1962). Although the possibility of algal-bacterial interaction has been appreciated in principle for quite some time, its operation has seldom been studied in nature. Most of the work on allelochemicals is limited to the demonstration that an arbitrarily selected laboratory alga can produce substances that affect a cultivated bacterial taxon. Such studies fail to take into account the mutual adaptations of coexisting natural populations of bacteria and algae. Thus very little is known of the general operation of this principle in nature. There is little room for doubt about the importance of allelopathy for bacterial growth in Lake Valencia, although the exact sources and triggers for allelochemical release remain unclear.

It is instructive to make some calculations relevant to the influence of bacteria on carbon flux given the standing stock of bacteria, the rate of photosynthesis, and the probable ranges of metabolic activities for bacteria in Lake Valencia. Considering the range of mean abundances with depth as shown in Table 3 and the proportion of water in various layers of the lake, the annual average bacterial standing stock is very close to 1.0 g C/m^2 . The net primary production by algae, as shown by oxygen difference measurements, is close to

2 g C/m²/day (LEWIS, unpublished). From grazing studies and ratios of zooplankton to phytoplankton, it is possible to show that zooplankton cannot consume more than 5–10% of phytoplankton production per day (SAUNDERS 1980). Also, the lake lacks an outlet through which carbon could be lost. Thus carbon amounting to almost 2 g/m²/day is stored in the sediments or passed through the detrital chain. Although the metabolic activity of the planktonic bacteria is not known, a number of indicators point to quite low bacterial biomass turnover rates. Most of the bacteria are in the small size categories that are generally considered to include inactive cells (PEDROS-ALIO & BROCK 1983). Furthermore, despite the presence of considerable amounts of organic matter in the water column (>5 mg C/l), bacterial populations remain low to moderate throughout the year. There are no known sources of mortality focused on bacterial populations that would account for the continual disappearance of biomass produced by rapid bacterial growth. Protozoans have a standing stock in Lake Valencia of close to 5 µg C/l (LEWIS 1985 b). This is insufficient to account for major losses of bacteria, especially since the protozoans are known to feed partly on algae. Although larger zooplankton (rotifers, crustaceans) can consume bacteria, it is impossible to invoke this as a significant cause of bacterial biomass loss in Lake Valencia without supposing that all of the zooplankton in Lake Valencia concentrate specifically on bacteria while passing over the much more abundant algae. Gut content analysis of the zooplankton in Lake Valencia has shown that the zooplankton feed heavily on algae (INFANTE 1981). Thus it seems highly likely that bacterial loss rates are low, and low loss rates for bacteria imply slow growth rates.

From the bacterial abundances it is possible to make rough approximations of the maximum observed growth rates *in situ* over extended intervals in Lake Valencia. None of the growth rates represented in Fig. 2 exceed 20% per day. Although this may seem surprising in view of the high growth potential of such small organisms under ideal conditions, long doubling times have been demonstrated in a number of different ways for field populations of bacteria in other lakes (JONES 1977; HOBIE & RUBLEE 1975; OVERBECK 1974). If the bacteria were growing steadily at the highest observed rates of 10% per day, they would have had to take in roughly 0.02 g C/m²/day of organic nutrient. This is only 1% of the net primary production. Since the estimate is generous in that it is based on the highest observed net growth rates, in all likelihood the processing of carbon by bacteria in the open water is considerably below this. It therefore seems likely that the processing of organic carbon in Lake Valencia occurs at the sediment-water interface or is short circuited by carbon storage in the sediments.

It is not difficult to see how processing of organic carbon at the sediment-water interface could exceed that of the overlying water column. It is known that sediments can contain as much as 100 times as many bacteria per unit

volume as the overlying water (HOBBIE & WRIGHT 1979). Since the sediment-water interface is more abundantly supplied with concentrated organic matter than the plankton zone, it is likely that a much larger proportion of these cells is metabolically active. Thus the sediment-water interface over a water column averaging 20 m thick could easily have a metabolic capacity ten or more times greater than the overlying water. Carbon in water of the upper mixed layer sweeping over sediments could be processed by these bacteria, thus accounting for processing that cannot be accounted for by planktonic bacteria. When carbon storage and hypolimnetic sediment decomposition are added to this, the net carbon fixation by phytoplankton can be accounted for. Such an accounting shows, however, that carbon processing in Lake Valencia, and possibly in numerous other moderately deep eutrophic lakes, occurs principally in the sediments, while the production of carbon occurs in the open water. Many authors, dating back to RUTTNER (1932), have stressed the importance of planktonic bacteria in lakes. Since numerous studies indicate a predominance of small, inactive bacterial cells in open water, long generation times for planktonic bacteria, and high inventories of organic matter coexisting with only moderate bacterial populations, it seems appropriate to question the general importance of planktonic bacteria in explaining the metabolism of lakes. Perhaps many lakes, including Lake Valencia, should be viewed as divided systems that produce carbon in open water but pass this carbon to the detrital chain almost entirely through sediment surfaces.

Zusammenfassung

Die planktischen Bakterien des Valenciasees, Venezuela, wurden zwischen 1977 und 1981 studiert. Gesamtzahlen mittels Epifluoreszenzmikroskopie zeigten, daß ungefähr zwei Drittel der Bakterienzellen einzelne Kokkenformen waren, die eine Größenskala von 0,07 bis zu $4 \mu\text{m}^3/\text{Zelle}$ umspannten. Die Mehrzahl der übrigen Bakterienzellen waren stabförmig. Die Untersuchung erwies auch die stetige Anwesenheit kleinerer Zahlen charakteristischer Bakterienmorphotypen, wie zum Beispiel *Planctomyces*. Mehr als 90 Prozent der Bakterien waren keiner Partikelchenunterlage angehaftet. In der oberen Wassersäule betrug die jährliche Durchschnittsbakterienmenge $0,5 \times 10^6 \mu\text{m}^3/\text{cc}$, oder ungefähr 50 $\mu\text{g}/\text{l}$ Bakterienkohlenstoff, was in derselben Zeitspanne ungefähr drei Prozent Planktonkohlenstoff entsprach.

Die Bakterienmenge in der oberen und unteren Wassersäule war etwa gleich, so lange der See zirkulierte. Während der Schichtungsperiode fiel aber die im anoxischen Tiefenwasser befindliche Bakterienmenge auf sehr niedrige Zahlen. In unregelmäßigen Zeitabständen aber veranlaßte der ins Tiefenwasser durch Dichteänderungen des Mixolimnion verursachte Sauerstoffimport einen Zuwachs an Bakterienmenge. In der oberen Wassersäule kamen die geringsten Bakterienmengen vor, nachdem das Epilimnion für eine längere Zeit stabil geworden war. Die Bakterienmengen wurden aber größer, wenn die durchmischte Schicht dicker wurde.

1981 wurde versucht, die Anwesenheit allelopathischer Substanzen festzustellen. Die Versuche ergaben eine bedeutende Hemmung des Bakterienwachstums, die sich durch Einwirkungen allelopathischer Substanzen erklären läßt. Diese in den Proben

festgestellten allelopathischen Einwirkungen beschränkten sich auf die Stagnationszeit. Sie fielen mit Perioden zusammen, in denen die Bakterienmenge abnahm und auch die Dicke der durchmischten Schicht stabil war. Im allgemeinen korrelierte die Bakterienmenge nicht direkt mit der Phytoplanktonmenge, sondern mit den Mischungsvorgängen, deren Einwirkung teilweise in Beziehung zu Allelopathie gedeutet wird.

Vergleichende Berechnungen über die Phytoplanktonmetabolismusraten und maximal mögliche Bakterienstoffwechselraten zeigen, daß die planktischen Bakterien im Valenciasee einen sehr kleinen Bruchteil der Nettoprimärproduktion verarbeiten. Da die Primärproduktion auch in keiner Weise durch Zooplankton wirksam beeinflusst wird, spielen die Sedimentbakterien und das Sedimentlager eine sehr große Rolle im Kohlenstofffluß dieses Sees.

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