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Bacterial Population Dynamics in a Meromictic Lake

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Polyclonal antibodies against nine different bacteria isolated from Lake Sælenvannet in western Norway were produced, and the population dynamics of these strains in the lake were monitored through two spring seasons by immunofluorescence staining. The total counts of bacteria varied over time and space from 1.5×10^6 to 1.5×10^7 cells ml^{-1}. The counts of specific bacteria were in the range of 10^3 to 10^4 cells ml^{-1} or less; in sum, they generally made up less than 1% of the bacterial community. Some populations showed significant changes in abundance, with blooms lasting 1 to 3 weeks. The rate of change (increase and decrease) in abundance during blooms was estimated to be 0.2 to 0.6 day^{-1}. The average virus-to-bacteria ratio was 50, and there was a significant correlation between the abundances of virus and bacteria. Both protozoan grazing and lytic virus infection were assessed as possible mechanisms driving the variations in bacterial population density.

In most ecological studies, bacteria are considered simply as bacteria, i.e., as one population where all individuals have the same or similar properties. The obvious reasons for this are that bacteria in general lack morphological characteristics which may be used to recognize them as members of species and that the fraction which may be cultured on agar plates (CFU), isolated, and characterized often makes up less than 1% of the total community (for a review, see reference 1). Most other organisms exhibit characteristic morphological traits and lend themselves more easily to taxonomic classification. As a consequence, population dynamics, species succession, and diversity have been studied and are well-known for many organisms, but for bacteria almost nothing is known. From pure-culture studies, it is known that bacteria have properties which are as diverse as those of any other group of organisms. There is thus no a priori reason to believe that bacteria in natural aquatic ecosystems should be less diverse and show fewer temporal and spatial variations than do other populations, such as phytoplankton or microzooplankton, living in the same habitat.

With the development of molecular techniques, it has become possible to detect bacterial species by their specific DNA or RNA base sequences without cultivation. These techniques have been used to characterize the bacterial community in natural seawater and to study variations over time and space. Using total genomic DNA cross-hybridization to study spatial and temporal variations in three ocean basins and coastal waters, Lee and Fuhrman (21) concluded that natural bacterial assemblages differ as much as do ambient environmental factors and that particular populations are dominant under certain conditions. By comparing 16S rRNA genes from Atlantic and Pacific bacteria, it has been concluded that the bacterial communities in these waters are diverse but that they also contain closely related groups (13, 23). Höfle and Brettar (18) estimated the diversity of the bacterial community in the central Baltic Sea by an analysis of low-molecular-weight RNA. They concluded that throughout the water column, the community was dominated by three to seven abundant bacterial species and that the diversity increased in layers with increased microbial activity. Rehnstam et al. (27) used hybridization of 16S rRNA gene probes to community DNA to assess temporal variations in the bacterial community off Scripps Pier. With four different probes, Rehnstam-Holm found a clear successional pattern and blooms of bacterial genotypes lasting several days to weeks (28). In some cases, the bacterial community was found to be dominated by one or a few bacterial species.

Another approach that has been applied to detect specific bacteria in natural waters is immunostaining with antibodies conjugated to fluorescent dyes (3). This technique is simple and sensitive but requires that the organism has previously been isolated for antibody production. Immunofluorescence has been used to study the distribution of specific groups or strains of bacteria, such as ammonia and nitrite oxidizers (37), denitrifiers (38), methylotrophs (26), and cyanobacteria (8). This method has also been used to study the population dynamics of marine organotrophic bacteria (10, 20). (For reviews of this method and its applications, see references 3, 7, and 36).

The aim of this investigation was to study the population dynamics in a bacterial community in natural waters. The strategy we used was to isolate several bacterial strains from a given ecosystem, to produce antibodies against these strains, and then to use these antibodies to assess the in situ abundances and dynamics of the source populations in the ecosystem. The habitat we used for this study was chosen because of its limited horizontal water flow and vertical mixing so that large-scale patches and drifting water masses with different community compositions would not influence the results.

MATERIALS AND METHODS

Description of Lake Sælenvannet. Lake Sælenvannet is a meromictic lake situated some 11 km south of Bergen, Norway. The surface area is 0.6 km^2, and the maximum depth is 26 m (19). A narrow channel (roughly 0.5 m deep by 2 m wide) connects the lake to the sea. The direction and magnitude of the water flow through the channel depend on tidal height and precipitation. The normal tidal difference is 20 to 30 cm. Lake Sælenvannet is characterized by an oxic surface layer (0 to 2.5 m), with salinity increasing from 0 to 20‰ with depth, and an anoxic bottom layer (2.5 to 26 m), with a salinity of about 20‰ (see Fig. 1). The narrow inlet and sharp pycnocline restrict water transport and deep mixing and make Lake Sælenvannet well suited for studying defined water masses.

Sampling. For the collection of water samples, we submersed a 1-liter bottle loosely capped with a silicone rubber stopper. The rubber stopper was pulled out at the sampling depth by the aid of a string. This sampling procedure allowed sampling at defined depths (± 10 cm). The first sampling period for this inves-

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tigation started on 9 March 1995, just as the ice cover broke up, and ended on 23 May 1995 (Julian days 68 to 143). Water samples were collected at a depth of 1.5 m and at intervals of 1 to 14 days. The sample used for the isolation of bacterial strains was collected on 15 March 1995 (Julian day 74).

The second sampling period started on 5 March 1996 and ended on 6 May 1996 (Julian days 65 to 127). The samples for that year were collected at depths of 1 and 2 m, just above the chemocline, where the water was colored green by a dense population of *Euglena* spp. (see Results). The samples were collected at intervals of 1 to 6 days, but due to unsafe ice, sampling could not take place between 8 March and 12 April 1996, when the ice cover finally broke up (Julian days 68 to 103).

On 6 March 1996 (Julian day 66), while the lake was frozen, water samples were collected at several depths to investigate the vertical distribution of the microbial community in the water column. The temperature and salinity were measured with a salinoterm (model 33 S-C-T meter; Yellow Springs Instruments, Yellow Springs, Ohio), oxygen was measured with an oxygen electrode (model 57 oxygen meter; Yellow Springs Instruments), and sulfide was determined colorimetrically by the method of Grasshoff et al. (14). In all cases, samples for counting bacteria (100 ml) were preserved with 2% formaldehyde (final concentration) within 1 h of sampling.

Total counts of bacteria and viruses. During the first sampling period (i.e., in 1995), samples for total counts of bacteria were prepared within 2 days of sampling. During the second sampling period (i.e., in 1996), all samples were prepared on the day of sampling. Sample water was filtered onto black Nuclepore filters (pore size, 0.2 μm) and stained with DAPI (4′,6-diamidino-2-phenylindole) (10 μg ml^{-1}) for 1 to 2 min (25). The filters were dried and mounted on glass slides in liquid paraffin. At least 200 cells from at least 15 fields were counted at a magnification of ×1,000 with a Nikon Microphot epifluorescence microscope with a UV-1A filter block and a PlanApo 100/1.4 fluorescence objective. Samples for total counts of viruses were centrifuged onto electron microscopy (EM) grids by the method of Bratbak and Heldal (4). To avoid problems with samples where the particle density was high, we diluted the samples 1:1 in filtered distilled water or we used cutoff centrifuge tubes accommodating 3 ml of water. Between 50 and 350 virus-like particles (VLP) were counted in 35 to 70 fields of view at a magnification of ×100,000 with a JEOL 100CX transmission electron microscope.

Isolation and culturing of bacterial strains for vaccines. Agar plates were made from liquid M65 medium with the addition of 15 g of agar $liter^{-1}$. Liquid M65 medium was prepared by dissolving 0.5 g each of yeast extract, tryptone, and peptone (Difco) $liter^{-1}$ in filtered (GF/C; pore size, 0.2 μm) water from the sampling location and by autoclaving. The agar (Difco) was purified prior to use by washing in double-distilled water, ethanol, and acetone (39). The water samples for counts of CFU and isolation of bacterial strains were collected in Lake Sælenvannet on 15 and 16 March 1995 (Julian days 74 and 75). The water samples were diluted 10^{-2} to 10^{-4} in filtered and autoclaved water from Lake Sælenvannet, and aliquots of 100 μl were plated on M65 agar plates within 1 h of sampling. The plates were incubated at 15°C, and after 5 to 7 days, colonies that looked different were picked and subcultured on a new set of plates to ensure purity. Then clones were picked, transferred to liquid M65 medium, incubated with shaking at 15°C, and after 2 or 3 days harvested for the preparation of vaccines.

At the end of the first sampling period (23 May 1995 [Julian day 143]), we attempted to enrich the strains that had been isolated in March by nutrient addition. A water sample of 10 ml was added to 90 ml of M65 medium and incubated at 15°C in the dark on a rotary shaker. Subsamples were collected from the culture after 24 and 65 h of incubation, preserved, and prepared for total counts and immunostaining as described above.

Production of PAb against isolates. Polyclonal antibodies (PAb) were prepared by the method of Christensen et al. (9). Vaccines were prepared from 100 ml of liquid cultures. Cells were collected by centrifugation (6,000 × *g* for 15 min) and suspended in 10 ml of phosphate-buffered saline (PBS; pH 7.4) with 2% formaldehyde. Cells were fixed for 2 to 12 h and then washed twice with PBS. Cell density was determined by counting DAPI-stained cells. Cell suspensions were prepared with 10^9 cells ml^{-1} in PBS. PAb were produced by injecting rabbits intravenously in the ear with increasing amounts (from 0.1 to 1.0 ml) of vaccine during a period of 19 days. Booster injections with or without incomplete Freund adjuvant were given 3 days before blood samples were harvested.

Immunoassays and staining. An enzyme-linked immunosorbent assay (ELISA) was used to determine the titers of antisera and to analyze the cross-reactivities of antisera and various bacterial strains. This ELISA was carried out by using the horseradish peroxidase color development system (6) and a Titertek Multiskan Plus MKII microplate reader.

Fluorescent antibody (FA) staining of bacteria was carried out by the method of Hoff (17). The samples were filtered onto black Nuclepore filters (pore size, 0.2 μm), labeled with antibody, stained with fluorescein isothiocyanate-conjugate, and mounted on glass slides in liquid paraffin. FA-stained cells were counted at a magnification of ×1,000 with a Nikon Microphot epifluorescence microscope with a B-2A filter block and a PlanApo 100/1.4 fluorescence objective. We inspected 60 to 200 (usually around 100) view fields for each specimen, and the limit of detection was better than 1,000 cells ml^{-1}. Some samples were prepared in duplicate or triplicate to check the accuracy of the procedure.

In 1995, many samples had to be stored for up to 70 days before the antibodies were ready for use. These samples were therefore also prepared for total counting of cells as described above to check for any loss of cells during storage (15, 33, 34). In 1996, FA staining of cells was carried out on the day of sampling.

Phenotypic characterization. For phenotypic characterization of strains, we used BIOLOG GN MicroPlate test panels containing 96 different carbon sources. The strains were grown on M65 agar plates for 2 or 3 days and then transferred to 30 ml of sterile saline (11.5‰ NaCl). The cell density was adjusted to give an optical density of 0.5 to 0.8 at 590 nm, and 150 μl was then added to each well on the BIOLOG plates. The plates were incubated at 15°C, and growth was recorded with a Titertek Multiskan Plus MKII microplate reader after 7 (strains SV5 and SV7) or 12 (strains SV1 to SV4, SV6, and SV8 to SV10) days of incubation.

TABLE 1. Properties of the 10 isolated strains

Strain	Morphology	Motility[b]	Growth on liquid M65	Antibody cross-reaction[c]
SV1	Rod	−	Good	—
SV2	Curved rod	+	Moderate	—
SV3	Rod	+	Moderate	SV4
SV4	Rod	−	Good	—
SV5	Short rod	−	Slow	—
SV6	Cocci in chains[a]	−	Slow	—
SV7	Cocci	−	Slow	—
SV8	Rod	−	Moderate	—
SV9	Rod	−	Moderate	—
SV10	Rod	−	Moderate	—

[a] Cells were not always in chains (hardly ever in natural water samples).
[b] +, flagellated; −, no flagella.
[c] —, no cross-reaction observed.

RESULTS

Isolation and characterization of strains. The numbers of CFU in the water samples collected on 15 and 16 March 1995 were 0.61×10^4 and 2.29×10^4 CFU ml^{-1}, respectively. A number of different looking colonies on the plates from 15 March were selected, and finally 10 strains (SV1 to SV10) were used to produce antisera in rabbits. Transmission EM, epifluorescence microscopy, phenotypic characterization with BIOLOG plates, and immunoassays showed that the 10 isolates were different from each other (Table 1). Cluster analysis of the data from BIOLOG plates by using simple matching distance and complete link showed that SV2 and SV10 had a similarity of 90%, SV5 and SV7 had a similarity of 85%, and all other combinations had a similarity of <80%. If 80% similarity is used as the criterion for grouping (31), cluster analysis of the BIOLOG data indicates that we have isolated eight different biotypes of bacteria. Amplification of the V3 region of 16S rRNA with subsequent denaturant gradient gel analysis revealed that the percent GC compositions of the 10 isolates varied nearly as much as did the percent GC compositions in the entire community and that certain pairs of strains, SV1 and SV7, SV4 and SV9, and SV6 and SV10, had approximately the same percent GC compositions (23a).

All vaccinations, except those with SV5, gave antisera with adequate titers. SV5 was thus excluded from further study. Several antisera gave cross-reactions at dilutions of 1:10 to 1:1,280 when antisera were tested on ELISA plates, and some weak cross-reactivities were also seen when antisera were tested on glass slides. Uncertain cases were tested on filters, and on these filters, the only cross-reaction seen was strain SV3 reacting to antisera for SV4. Strain SV4, however, did not react to antisera for SV3. A 1:200 dilution of antiserum gave good staining results for all strains both on glass slides and on filters, and this dilution was used in the subsequent immunostaining of samples.

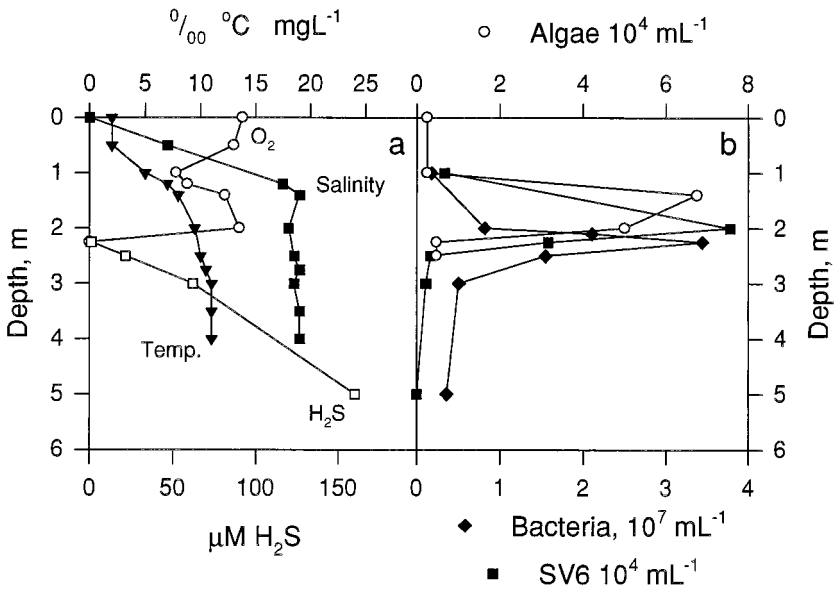


FIG. 1. Depth profile of Lake Sælenvannet sampled on 6 March 1996 (Julian day 66). (a) Vertical distributions of oxygen, sulfide, temperature (Temp.), and salinity; (b) vertical distributions of phytoplankton (dominated by *Euglena* spp.), the bacterial community (total counts), and bacterial strain SV6, as enumerated by immunofluorescence staining.

Vertical distribution of the microbial community in Lake Sælenvannet. Figure 1 shows a characteristic depth profile of Lake Sælenvannet. The abundance of bacteria peaked at a depth of ca. 2.25 m, at the oxic-anoxic interface. The color of the water samples from this depth was red due to a dense population of phototrophic bacteria. An in vivo spectrum of intact cells showed main absorption maxima at 460 and 715 nm, characteristic of green sulfur bacteria. Just above this red layer, the color of the water was green due to a dense population of *Euglena* spp. (Fig. 1b). The abundance of strain SV6 showed a major peak in the green layer at a depth of about 2 m.

Development of the bacterial community and population dynamics. The total counts of bacteria in the surface layer were in the range of about 1.5×10^6 to 5×10^6 cells ml^{-1} (Fig. 2 and 3a). Each year there was a marked increase in bacterial abundance for a period of ca. 10 days after the melting of the ice cover (Julian days 70 to 80 in 1995 [Fig. 2] and Julian days 103 to 113 in 1996 [Fig. 3a]). The total counts of viruses varied each year from about 2×10^7 to 30×10^7 ml^{-1} (Fig. 2 and 3a).

At a depth of 2 m, just above the chemocline, the abundances of both bacteria and VLP were two to three times higher than those at 1 m (Fig. 3). The mean virus-to-bacteria ratios (VBR) ± standard deviations in the surface layer and at 2 m were 40 ± 15 and 50 ± 30, respectively.

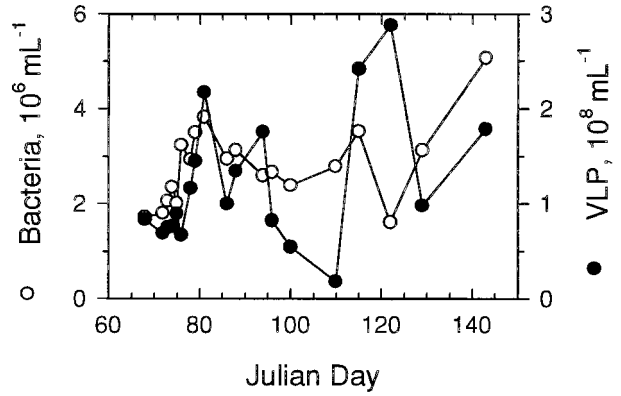


FIG. 2. Total counts of bacteria and VLP in Lake Sælenvannet from 9 March to 23 May 1995 (Julian days 68 to 143). All samples were collected at a depth of 1.5 m.

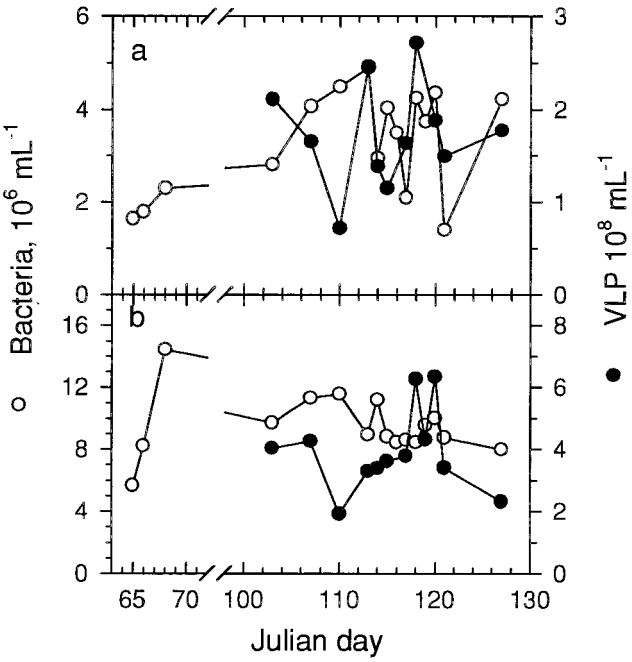
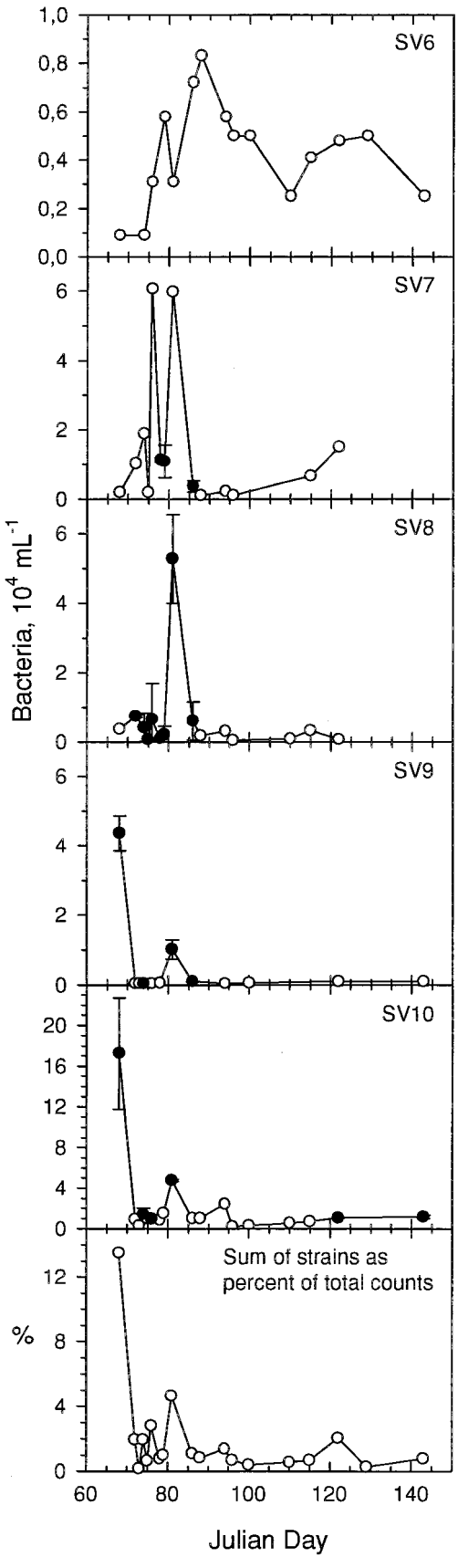


FIG. 3. Total counts of bacteria and VLP in Lake Sælenvannet from 5 March to 6 May 1996 (Julian days 65 to 127) at depths of 1 m (a) and 2 m, just above the chemocline (Fig. 1) (b).



Five of the nine strains isolated were found in 1995 in abundances of $>10^3$ cells ml^{-1} (Fig. 4). Strain SV10 was the most abundant strain at the beginning of the sampling period (16×10^4 cells ml^{-1}). Strains SV7, SV8, SV9, and SV10 had short periods of higher cell abundance, but most of the time they were found in low numbers ($<10^4$ cells ml^{-1}). Strain SV6 was found at a steady but low concentration of between 2×10^3 to 8×10^3 cells ml^{-1}. The abundance of bacteria detected by immunostaining that year (1995) generally made up <2% of the total counts (median, 0.8%). Exceptions were seen at the beginning of the experiment, when strain SV10 made up about 13% of the total cell count, and on Julian day 81 (22 March 1995), when strains SV7, SV8, and SV10 together made up about 5% of the total cell count (Fig. 4).

During the 1996 sampling period, the abundances of strain SV3 and SV7 were always low (generally $<2 \times 10^3$ cells ml^{-1}) and strains SV2, SV4, and SV6 were found in significant abundances only on the first sampling dates (Julian days 65 to 68) (Fig. 5). Strains SV1, SV9, SV10, and to some extent SV8 showed significant population changes at both sampling depths for most of the 3-week sampling period after the breaking of the ice cover (Fig. 5). At a depth of 3 m, blooms of SV1, SV9, and SV10 lasted for about 6 to 12 days (Fig. 5). The abundance of these bacteria increased at a rate of 0.2 to 0.5 day^{-1}, and they disappeared at a comparable rate, 0.2 to 0.6 day^{-1}. At a depth of 1 m, where the total counts of bacteria were lower, blooms of these strains lasted for about 15 to 20 days and the rates of increase and decrease in abundance were 0.2 to 0.3 and 0.2 to 0.4 day^{-1}, respectively. These rates were calculated only for trends lasting 4 to 12 days and included three to six data points. The abundance of bacteria detected by immunostaining that year (1996) made up on average 0.35% of the total counts.

Enrichment of strains in 1995. The total count of bacteria in the enrichment culture increased from 0.5×10^6 to 3×10^8 cells ml^{-1} in 65 h. Strains SV9 and SV10 increased from initial densities of 0.1×10^3 cells ml^{-1} (0.02% of total counts) to 1.5×10^7 cells ml^{-1} (5% of total counts) and 1.3×10^5 cells ml^{-1} (0.04% of total counts), respectively. The antisera for SV3, SV4, SV6, SV7, and SV8 stained between 1×10^5 and 3×10^7 cells ml^{-1}, but the fluorescence was weak and not characteristic. In addition, SV6 and SV7 cells were cocci or very short rods, but all the cells stained in the enrichment culture were rods.

DISCUSSION

Isolation of bacterial strains for studying population dynamics. Based on phenotypical characterization, serotyping, and denaturant gradient gel analysis, we concluded that all 10 bacterial strains we isolated from Lake Sælenvannet were different. All strains, except one (SV3), used in this investigation were detected in significant and variable abundances, but in sum they made up only a small fraction of the bacterial community.

In the two samples we collected for the isolation of bacterial strains, the numbers of CFU made up 2.6 and 11.4% of the total bacterial counts. These values are high compared to those usually reported for CFU in aquatic environments (1). The use

FIG. 4. Immunofluorescence counts of five different bacterial strains (SV6 to SV10) in Lake Sælenvannet from 9 March to 23 May 1995 (Julian days 68 to 143). The results for samples counted in duplicate or triplicate are indicated with filled symbols. Standard deviations are indicated with error bars when they are larger than the symbols. All samples were collected at a depth of 1.5 m.

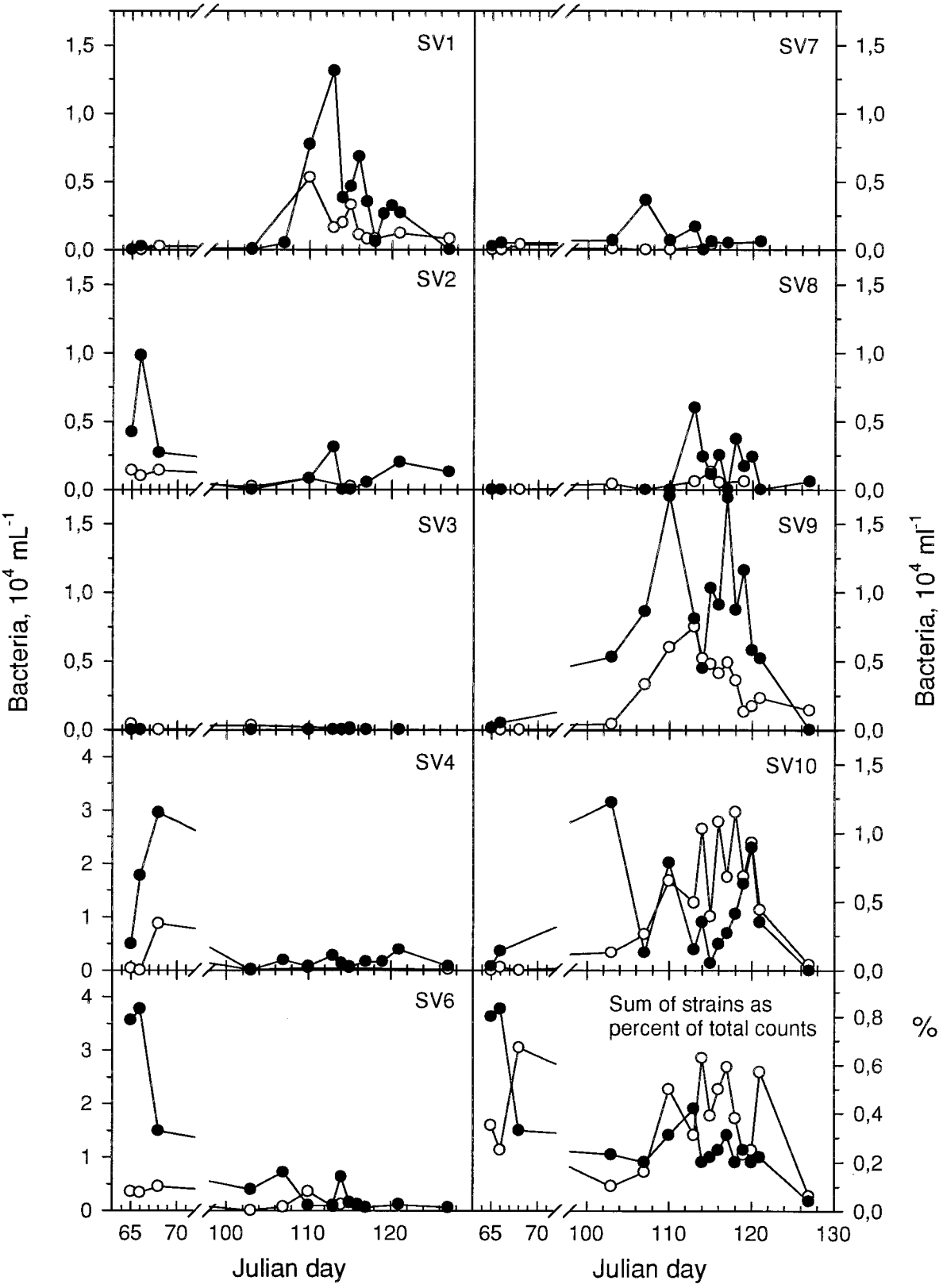


FIG. 5. Immunofluorescence counts of nine different bacterial strains (SV1 to SV4 and SV6 to SV10) in Lake Sælenvannet from 5 March to 6 May 1996 (Julian days 65 to 127) at depths of 1 m (open circles) and 2 m, just above the chemocline (Fig. 1) (filled circles).

TABLE 2. Data in the literature for the abundances of specific bacterial strains, as counted by immunofluorescence, expressed as percentages of total counts

Habitat	Strain(s) (*n*)	% of total count (range)[a]	Reference
Norwegian fjord (3- and 10-m depths)	Marine isolates (5)	0.16–1.3	10
Microfouling film	Marine isolates (5)	4–60 (25)	40
Southern California bight	Nitrifying strains (4)	0.1–0.8	37
Monterey Bay (0- to 90-m depths)	*Pseudomonas stutzeri*	0.02–0.08	38
Pluss See (0- to 10-m depths)	*Nitrosomonas marina* serotypes	0.005–0.05	36

[a] The mean from microfouling film is given parenthetically.

of purified agar may have contributed to this result. By immunostaining, we found that nine of the strains we isolated in sum made up 1.9 and 0.6% of the total counts in these samples, respectively. When we tried to enrich these strains 2 months later (in May 1995), we found that only one of the nine strains (SV9) showed a significant increase in abundance, measured as a fraction of the total count. Thus, most of our isolates appeared not to be copiotrophs that were present in low numbers most of the time but had the ability to grow fast and become dominant when nutrient input was high.

Detecting specific bacterial strains in natural water samples. PAb appeared to be quite specific for each strain as only one antiserum had a cross-reaction with one other strain. Nonspecific labeling, however, was sometimes observed as weakly stained cells in some of the water samples. These cells were not included in the cell counts. The number of cells counted per sample was between 0 and 260, and the counting error is therefore considerable (about 25% for the mean number of cells counted). As care thus should be exercised in interpreting point-to-point variations, we focused on trends including several successive samplings.

The nine strains we counted generally made up a minor fraction of the bacterial community (typically 0.2 to 2%). Other studies using immunofluorescence for the enumeration of specific strains in natural waters have reported similar figures (Table 2). These results may be interpreted as indicating that the bacterial community is very diverse and that most species make up in the order of 0.1 to 1% of the community or less. It is, however, also possible that the bacterial populations isolated on agar plates make up a small fraction of planktonic communities so that immunostaining of specific strains, which have been isolated due to their ability to grow on agar plates, in most cases comes up with low numbers. This view is not supported by Rehnstam et al. (27), who concluded that cultured bacteria in some cases may dominate the bacterial community in Californian coastal waters. Fuhrman et al. (12), however, have challenged the conclusion of Rehnstam et al. by arguing that it is difficult to ascertain the specificity of their approach, which was based on total microbial community DNA hybridization with oligonucleotide probes targeted at bacteria they had isolated by agar plating. Studying the community composition of marine fouling film, Zambon et al. (40) found that five strains in sum could make up as much as 60% of the community (Table 2). One major difference between planktonic and fouling film bacterial communities may be the ability to grow on solid surfaces such as agar plates. Strains isolated in the latter case are thus more likely to represent numerically dominating forms than is the case for planktonic systems. Thus, to isolate a species that makes up a numerically significant fraction of the community, if one does, remains a major obstacle for any approach that depends on the isolation of specific strains.

Decay and disappearance of bacteria in samples preserved with glutaraldehyde and other fixatives are problems that have been brought to attention during the last few years (15, 33, 34). The preparation of antibodies took about 2 months, in which time in the order of 50% of cells (total counts) in the earliest samples had disappeared. It is not known if all bacteria decay at the same rate, if there are differences between species, or if the decay rate depends on the physiological conditions of cells at the time of fixation. To investigate these matters, a few fresh samples were immunostained on the day of sampling and again after some days of storage. Strain SV10 was detected in the same abundance after 1 month of storage, and the abundance of strain SV1 decreased to 50% in 10 days and that of strain SV7 decreased to <10% in 1 month. A variable decay rate obviously had a significant impact on the results and probably explains why the abundances of some strains, such as SV1, were always very low. The apparently low decay rate of SV10 suggests that the variations observed for this strain are not encumbered with larger errors. In contrast, the initial abundance of SV7 in the samples collected during the first weeks of the experiment and stored for about 2 months may have been underestimated by a factor of between 10 and 100. Thus, this strain alone may, at its maximum, have made up at least 8% of the bacterial community.

The data from the 1996 sampling series were not affected by the uncertainties discussed above for the 1995 sampling series because in this case all samples were prepared and counted within 1 day of sampling. The abundances of the individual strains we observed in 1995 and 1996 were comparable. However, considering the decay of cells during the storage of samples in 1995, the abundances of most strains may have been considerably higher in 1995, the year strains were enriched and isolated, than in 1996. This is the case both for average abundances and for the peak concentrations of individual strains. One possibility is that the differences between the successive years reflect a shift in the composition of the bacterial community. It is, however, also possible that by coincidence, we sampled during a period when these strains bloomed in 1995 and that they did not bloom to the same extent during the 1996 sampling period.

Dynamics of the bacterial community and its relation to viral lysis and protozoan grazing. The bacterial populations investigated in this study made up only a small fraction of the bacterial community. The peaks in abundance of specific populations did in some cases coincide with peaks in total counts, but they could not alone account for all the increases in total counts (e.g., SV7 to SV10 on Julian day 81 [22 March 1995] [Fig. 2 and 4]). In most cases, changes in specific counts were too small to show up in the total counts.

The population control factors that may explain the observed variations in population density are protozoan grazing and viral lysis. Protozoan grazers may discriminate between particles on the basis of size and quality, i.e., food versus nonfood particles (11, 24, 29, 35). However, it is difficult to

envision how positive food selectivity could work on a species level with prey populations of 10^3 to 10^4 cells ml^{-1}, making up <1% of the community. However, if a bacterial population stops growing while grazing continues, the population density will, neglecting other causes of mortality, decrease in abundance with a rate equal to the grazing clearance rate. The clearance rate of bacteria in natural waters is in the order of 0.2 to >1 day^{-1} (11, 30), and the observed decreases in population abundance, which were estimated to be <0.6 day^{-1}, may thus be explained by nonspecific grazing on the bacterial community. The factors regulating growth and cessation of growth of individual populations in a mixed community remain to be investigated.

There was a significant correlation between bacterial and viral total counts ($r^2 = 0.587$, $P < 0.001$, $n = 42$), and the VBR was thus relatively constant throughout the entire study with a mean ± standard deviation of 50 ± 30. It is, however, difficult to draw a connection between virus abundance (total counts) and any of the bacterial populations studied. Assuming that the observed decreases in abundance of the different populations are due to viral lysis of populations that are not growing and that each cell upon lysis releases 50 viral particles (16), we estimate that the concurrent increases in viral abundance at most would be in the order of 10^6 to 10^7 viruses ml^{-1}. This is <10% of total virus counts and would not represent a significant increase in this parameter.

With a population of 10^4 cells ml^{-1} and a VBR of 50, we estimate that 7.5×10^2 viruses per ml per h will encounter a host cell (2, 32). Assuming that each encounter results in infection and cell lysis, the bacterial population will have a half-life of ca. 9 h. Thus, infection by lytic phages does seem to be a possible explanation for the bacterial population dynamics as the observed decreases in population density (Fig. 5) are equivalent to a half-life of 20 to 80 h. Temperate viruses do not affect the population density in lysogenic host-virus systems, but spontaneous formation of lytic mutants remains a possibility (5, 22). Mass induction of lysogenic populations is possible, but whether such induction may act as a population-specific mechanism in natural ecosystems is unknown.

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