Temporal distribution of viruses, bacteria and phytoplankton throughout the water column in a freshwater hypereutrophic lake

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ABSTRACT: Analytical flow cytometry (AFC) and denaturing gradient gel electrophoresis (DGGE) were used to investigate the diversity and dynamics of viruses, bacteria and phytoplankton communities in a hypereutrophic freshwater lake. Samples were taken from different depths throughout the water column over an annual cycle. Priest Pot is a small lake in the Lake District in NW England (UK), and has been well characterised in previous studies; however, little is known of the diversity and dynamics of the virus community. Virus abundance was shown to change over both spatial and temporal scales, and appeared to be closely linked to other biotic and abiotic parameters. The highest virus concentrations occurred in the deepest part of the lake at a depth of 3.2 m, in the anoxic layer, at the same time as a peak of abundance of green sulphur bacteria. Sequence analysis of a DGGE band that occurred at the same time suggested a bacterium similar to *Chlorobium* spp., a green sulphur bacterium, comprised part of this bacterial bloom.

KEY WORDS: Viruses · Microbial community dynamics · Freshwater · Flow cytometry

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INTRODUCTION

Viruses are the most numerically abundant entities in aquatic habitats. Virus concentrations have been shown to range between 4.8×10^6 and 1.4×10^7 ml⁻¹ in marine systems (Bergh et al. 1989, Wommack & Colwell 2000, Weinbauer 2004), and numbers are often an order of magnitude higher in freshwater (Maranger & Bird 1995). Virus abundance is also generally higher in habitats with higher nutrient status in both freshwater (Bettarel et al. 2003b) and marine systems (Bratbak et al. 1994), possibly as a result of higher bacterial numbers in these environments. The impact of viruses on their host communities is significant, with as much as 34 % of bacterial production in freshwater estimated to be lost through viral lysis (Hennes & Simon 1995, Mathias et al. 1995, Bettarel et al. 2003a).

Although many studies have investigated virus–host interactions in freshwater habitats (Mathias et al. 1995, Weinbauer & Höfle 1998a, Fischer & Velimirov 2002, Bettarel et al. 2004), none have been carried out over both spatial and temporal scales in any detail. This is the first study of a complete freshwater microbial community, including viruses, that extends over an entire annual cycle. We aimed to gain a clearer picture of virus community dynamics throughout the water column of Priest Pot, a small hypereutrophic lake in the English Lake District (Cumbria, UK), over a whole annual cycle. Priest Pot has been extensively studied, and the seasonal changes that occur in the physical and chemical characteristics of the water column, and the phyto- and zooplankton communities have been well documented (Finlay & Maberly 2000). The water column is mixed and oxygenated in the winter and

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early spring. During early summer, the lake stratifies and high nutrient concentrations support a large microbial biomass, which leads to oxygen depletion at depth. During the autumn, the water cools and the stratification eventually breaks down as oxygen becomes available at depth. The stratified water column provides a large number of diverse niches in which different organisms can proliferate, generating a dynamic and complex microbial community. Although Priest Pot has been well studied, the role of viruses within the microbial community has received little attention.

The different microbial communities were enumerated by analytical flow cytometry (AFC), an accurate way of screening large numbers of samples; this technique has been shown to be useful in studies of microbial ecology, and has been used to enumerate viruses (Marie et al. 1999, Larsen et al. 2001), bacteria (Gasol et al. 1999) and phytoplankton (Olson et al. 1985, Binder et al. 1996, Crosbie et al. 2003) from a range of aquatic systems. The diversity of potential bacterial and phytoplankton host populations was determined by denaturing gradient gel electrophoresis (DGGE) separation of PCR amplified 16S and 18S rDNA, respectively. DGGE has been widely used to monitor diversity and dynamics within aquatic microbial populations, and remains a powerful tool in this field (Casamayor et al. 2002, Ovreas et al. 2003). A previous study suggested that the virus community in Priest Pot was closely coupled to both the bacteria and phytoplankton communities in the surface layer (Goddard et al. unpubl.). In the present study we investigated the effect of depth, and the steep physical and chemical gradients that develop during the year in Priest Pot, on the dynamics of microbial and virus communities.

MATERIALS AND METHODS

Sampling. Samples were taken from Priest Pot every 2 wk from March to May 2002, then monthly until April 2003. Samples for flow cytometry (2 to 5 ml) were taken using a pneumatically operated sampler modified from the design of Heaney (1974), so that samples of up to 180 ml could be collected simultaneously from each of 16 depths. All samples were taken from the centre of the lake. Samples for bacteria and phytoplankton diversity were taken using an acid and sample rinsed 250 ml glass bottle sealed with a rubber bung attached to a line, and weighted with a brass weight. The bottle was lowered to the appropriate depth, then the bung was jerked off with a sharp tug; the bottle was returned to the surface when full. All samples were processed or fixed immediately on arrival at the laboratory.

Physical and chemical measurements. Nitrate, phosphate, silicate and oxygen analyses were carried out using the methods detailed in Mackereth et al. (1978). Algal chlorophyll *a* and bacterial chlorophylls *c* and *d* were analysed using the method of Caraco & Puccoon (1986).

Phytoplankton, bacteria and virus enumeration. All counts were carried out on a FACScan flow cytometer (Becton Dickinson), equipped with an air cooled laser providing 15 mW at 488 nm, with the standard filter set-up. For phytoplankton enumeration, fresh samples (collected from the 26 March 2002 to the 17 December 2002) were analysed for 3 min at a flow rate of 90 $\mu\text{l min}^{-1}$, using red fluorescence as the trigger. When viewing the scatter plots, the majority of the different groups were easier to distinguish when plotted on the basis of orange fluorescence versus side scatter. Samples for virus and bacteria enumeration (collected from the 26 March 2002 to the 8 April 2003) were fixed in a final concentration of 0.5 % glutaraldehyde (Sigma, EM grade) overnight at 4°C, and stored at –80°C for analysis at a later date. (Note that recent research by Brussaard [2004] has shown that this method may have underestimated virus numbers.) For virus enumeration, 10 μl of fixed sample was diluted in 980 μl TE (Tris-ethylenediaminetetraacetic acid; 10 mM Tris, 1 mM EDTA) and incubated at 80°C for 10 min with 10 μl 1/1000 concentration SYBR Green 1 (Molecular Probes) in the dark. Samples were analysed for 2 min at a flow rate of 30 $\mu\text{l min}^{-1}$ with the discriminator set on green fluorescence. For bacteria enumeration, 100 μl of fixed sample was diluted 10-fold in TE, and incubated with 10 μl of 1/1000 concentration SYBR Green 1 for 15 min at room temperature in the dark, and samples analysed as above. In each case, distinct virus, bacteria or phytoplankton groups were identified from scatter plots of side scatter versus fluorescence using Cellquest software (Becton Dickinson) (Fig. 1).

Bacteria and phytoplankton diversity. The diversity of bacteria and phytoplankton communities was measured by DGGE of PCR amplified 16S and 18S rDNA fragments, respectively. Prokaryotes and eukaryotes were harvested from approximately 100 to 200 ml water onto 47 mm diameter 0.2 μm Durapore filters (Millipore) by vacuum filtration. The filters were cut into strips and stored at –80°C until processing. DNA extraction was carried out using an adaptation of the CTAB (1 % N-cetyl N,N,N-trimethylammonium bromide) method (Goddard et al. 2001). Half filters were incubated in lysis buffer (0.5 % sodium dodecyl sulphate [SDS], 20 $\mu\text{g ml}^{-1}$ Proteinase K) for 1 h at 55°C. Eighty μl of 5 M NaCl and 150 μl of CTAB buffer (2 % [w/v] hexadecyltrimethylammonium bromide [Sigma], 1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl [pH 8.0]) were added and incubated for a further 15 min at 65°C,

followed by chloroform isoamyl alcohol (24:1) extraction. Extracted DNA was then precipitated in 0.6 volumes of isopropanol at room temperature and pelleted by centrifugation at $14\,000 \times g$ for 30 min. Recovered DNA was resuspended in 100 μ l of TE (pH 8.0) and stored at -20°C .

PCR reactions were carried out using primers 8f (Weisburg et al. 1991) and 534r (GC) (Muyzer et al. 1993) for amplification of a section of the bacterial 16S rRNA gene, including the variable V3 region, and 1427f (GC) and 1616r (van Hannen et al. 1998) for amplification of a fragment of the eukaryotic 18S rRNA gene. All PCR reactions were carried out using 1 μ l of a 1/100 dilution of extracted DNA as template. This was added to a reaction mix containing 1 U of *Taq* DNA polymerase (Promega), 1 $\times$ PCR buffer (Promega), 0.25 mM deoxynucleoside triphosphates (dNTPs), 2.5 mM MgCl_2 and 20 pmol of each primer (8f/534r[GC] for 16S rDNA amplification and 1427f[GC]/1616r for 18S rDNA amplification). The PCR was conducted on a PTC100 thermocycler (MJ Research) with 30 cycles of 1 min denaturing at 94°C , 1 min annealing at 52°C and 1 min extension at 72°C . An initial denaturing step of 3 min at 94°C and a final extension of 5 min at 72°C were also included. PCR products were verified by agarose gel electrophoresis.

DGGE separation of PCR products was carried out on a parallel gradient of 30 to 50% denaturant in an 8% acrylamide gel (where 100% denaturant is 7 M urea and 40% deionized formamide). Electrophoresis was carried out on a DCode universal mutation detection system (Biorad) for 3 h at a constant temperature of 60°C . Gels were stained in a 1 $\times$ solution of SYBR gold (Molecular Probes) for 30 min at room temperature, and band patterns visualised on a UV transilluminator on a low intensity setting, and photographed with the Gel Doc 2000 system (Bio-Rad).

Selected bands for sequence analysis were picked from the gels by removing plugs with wide bore 200 μ l tips. Polyacrylamide plugs were placed in 200 μ l of TE

Fig. 1. Flow cytometry scatter plots showing characteristic groups obtained from Priest Pot water samples. (A) Virus Groups V1 to V4 obtained from samples stained with SYBR Green I (Molecular Probes), run using flow cytometry settings specific for the detection of viruses. (B) Bacteria Groups B1 and B2 obtained from samples stained with SYBR Green I, run using settings specific for the detection of bacteria. Scatter plots A and B were both obtained from the same sample taken on 26 March 2002. (C) Phytoplankton Groups P1 to P6 obtained from unstained samples, using settings specific for the detection of autofluorescent phytoplankton and picoplankton. Sample from 8 May 2002. (D) Groups P7 and P8 detected in unstained samples, using the same settings as for C. Sample from 8 October 2002

and heated to 94°C for 10 min, then cooled to 4°C. Eluted bands were then stored at –20°C prior to reamplification. For the 16S rDNA bands, 1 µl of eluate was used as the template for reamplification using the PCR conditions detailed above, with modifications. Reamplification was carried out in half volume reactions (25 µl), the annealing temperature was increased to 60°C and 25 cycles were carried out instead of 30. For the 18S rDNA bands, 1 µl of 1/100 diluted eluate was used as the template for reamplification using the modified conditions detailed above. Reamplified bands were verified on agarose and DGGE gels as described above.

Reamplified 16S rDNA and 18S rDNA DGGE bands for sequencing were treated with Exo SAP-IT (USB) to remove unused primers and nucleotides, according to the manufacturer's instructions. Sequencing reactions were carried out on a PTC100 thermocycler (MJ Research), using a BigDye Terminator v3.1 cycle sequencing kit (ABI), with 8f and 1616r as the sequencing primers for 16S rDNA and 18S rDNA bands, respectively. Cycle sequencing was carried out following the manufacturer's instructions, the only modification being an increased annealing temperature of 60°C during the cycle sequencing reactions. Sequences were determined on an ABI 3100 capillary sequencer, and similarity to known sequences determined using the BLAST database (www.ncbi.nlm.nih.gov). The sequences obtained in this study are available in GenBank (see Table 1 for accession numbers).

RESULTS

Spatial and temporal variations in environmental conditions

Changes in water temperature, dissolved oxygen, nitrate, phosphate, algal chlorophyll *a* and bacterial chlorophyll *c* and *d* concentrations were measured at different depths throughout the annual cycle from March 2002 to March 2003 (Fig. 2). Water temperature ranged between 3.7 and 18°C over the annual cycle, with the highest temperatures recorded in the summer in the surface waters. During this time, the difference between the surface temperature and that in proximity to the sediment was between 2 and 4°C. During the winter, the temperature was consistent throughout the water column (Fig. 2A). Dissolved oxygen (dO₂) ranged from 0 to 490 µmol l⁻¹, with the development of an anoxic layer below 150 cm occurring between 8 May and 8 October 2002 (Fig. 2B). Nitrate was undetectable in Priest Pot throughout the water column between 23 April and 8 October, and increased markedly between 8 October and 19 November (Fig. 2C). Nitrate concentrations then increased to a peak of 865 µg l⁻¹ on 12 February 2003,

before dropping again, and did not vary greatly with depth. The majority of phosphate was detected in the deeper part of the water column (below 200 cm) between 21 May and 8 October (Fig. 2D). Concentrations ranged from 1.3 to 439 µg l⁻¹. Algal chlorophyll *a* concentrations ranged from 5.2 to 387.5 µg l⁻¹, with a peak at 100 cm on 8 May, and were generally higher in the upper half of the water column (Fig. 2E). Algal chlorophyll *a* was not detected in the anoxic layer, but was present at very low levels at all depths from October 2002 to February 2003. Bacterial chlorophylls *c* and *d* were detected at high levels (up to 650 µg l⁻¹) during the late summer and autumn months (between 18 June and 8 October) in the anoxic layer below 150 cm, and only low concentrations (<7.5 µg l⁻¹) were detected in other parts of the water column (Fig. 2F).

Abundance and succession of bacteria, viruses and phytoplankton

Several different groups of bacteria, viruses and phytoplankton were identified in scatter plots of green fluorescence versus side scatter for viruses and bacteria, and red or orange fluorescence versus side scatter for phytoplankton (see Fig. 1). Concentrations of bacteria ranged from 9.46×10^5 to 2.17×10^7 ml⁻¹ (Fig. 3A). Two distinct bacterial groups were observed in the AFC scatter plots (Fig. 1B), thought to represent high and low DNA fluorescence bacteria. Similar bacteria groups have previously been observed by flow cytometry (Gasol et al. 1999). The seasonal distribution of the 2 groups was found to be very similar (data not shown), and it was decided to take the 2 bacterial groups as one community. The bacterial abundance reported here therefore reflects the sum of both bacterial regions. Although patchy, bacterial abundance increased during the spring months (26 March to 21 May) and remained relatively constant during the summer (18 June to 10 October), although concentrations were lower in the surface waters during September and October. Abundance remained consistently low (2×10^6) during the winter months (between 19 November and 17 March). The following spring (17 March to 8 April 2003) bacteria concentrations started to increase again, with little variation over depth.

Total virus concentrations (the sum of all 4 virus groups determined from AFC scatter plots shown in Fig. 1A) ranged from 7.06×10^6 to 2.03×10^8 ml⁻¹ (Fig. 3B). During the spring months (26 March to 21 May), temporal variations in virus abundance were observed, but concentrations remained relatively consistent with depth. In contrast, during the summer months (18 June to 8 October), higher concentrations of viruses were observed in the deeper anoxic layers,

with the highest virus concentrations ($2 \times 10^8 \text{ ml}^{-1}$) observed at a depth of 330 cm (near the bottom) on 8 October. At the beginning of the winter months (between 8 October and 19 November) virus concentrations decreased markedly throughout the water column, and remained low until the following spring (17 March

2003), when concentrations began to increase once more. The seasonal distributions of Groups V1, V2 and V3 were similar to that of the total virus community (data not shown). However, Group V4 had a distinct seasonal pattern, with a peak in abundance on 16 July at 100 cm depth (Fig. 3C).

The phytoplankton community was differentiated into 8 distinct groups. Six groups were distinguished from scatter plots on the basis of side scatter and orange fluorescence signals by AFC (P1 to P6, Fig. 1C). A further 2 groups were distinguished from scatter plots of red fluorescence and side scatter (P7 and P8, Fig. 1D), which were distinct from those previously identified on the basis of orange fluorescence. Total phytoplankton concentrations ranged from 6.39×10^2 to $5.94 \times 10^5 \text{ ml}^{-1}$. Most of the phytoplankton groups had peaks of abundance that were both spatially and temporally distinct (Fig. 4). For example, Group P2 had a peak of $5 \times 10^3 \text{ ml}^{-1}$ on 8 May at a depth of 150 cm (Fig. 4B), and Groups P3 and P4 were most abundant on 10 April ($3.2 \times 10^2 \text{ ml}^{-1}$) and 16 July ($1 \times 10^3 \text{ ml}^{-1}$), respectively (Fig. 4C,D). Two phytoplankton groups, P1 (Fig. 4A) and P6 (Fig. 4F), had peaks in abundance of 3.6×10^4 and $2.7 \times 10^2 \text{ ml}^{-1}$, respectively, on 8 May at 100 cm depth, at the same time and depth at which the highest algal chlorophyll *a* levels were recorded. Group P8 was the numerically dominant group detected using these settings, with concentrations reaching 4.5×10^5 on 16 July below 200 cm depth (Fig. 5B). Group P7 was also present in relatively high numbers, with concentrations reaching a peak of $7.5 \times 10^4 \text{ ml}^{-1}$ on 10 September below 300 cm depth, in the anoxic layer (Fig. 5A). Neither Group P7 nor P8 were detected above 200 cm, nor in the spring or winter months. The peaks in abundance of these 2 groups also occurred at the same time as the peaks in bacterial chlorophylls *c* and *d* (Fig. 2F), suggesting that they did not comprise phytoplankton, but rather autofluorescent phototrophic bacteria growing anaerobically in the anoxic layer. Phytoplankton counts were measured throughout the winter, but all groups were undetectable during this time.

Bacteria and eukaryote diversity

Spatial and temporal changes in the diversity of prokaryotic and eukaryotic members of the microbial community were monitored by DGGE separation of PCR amplified 16S and 18S rDNA, respectively. Samples were taken from 8 depths on 10 occasions between 26 March 2002 and 8

Fig. 2. Contour plots showing spatial and temporal variations in Priest Pot of (A) water temperature ($^{\circ}\text{C}$), (B) dissolved oxygen (dO_2) concentration ($\mu\text{mol l}^{-1}$), (C) nitrate concentration ($\mu\text{g l}^{-1}$), (D) phosphate concentration ($\mu\text{g l}^{-1}$), (E) algal chlorophyll *a* concentration ($\mu\text{g l}^{-1}$), and (F) bacterial chlorophyll *c* and *d* concentration ($\mu\text{g l}^{-1}$). x: times and depths of samples taken

Fig. 3. Spatial and temporal dynamics of (A) bacteria and (B) virus total communities (ml^{-1}) in Priest Pot over an annual cycle, from 26 March 2002 to 8 April 2003. (C) Spatial and temporal distributions of virus Group V4 (ml^{-1}) during the annual cycle

October 2003, and the DGGE profiles observed. Comparison of DGGE profiles showed that the structure of the bacterial community, reflected by the distribution of the most abundant strains, changed over both spatial and temporal scales. Fig. 6 shows 3 gels from 10 April, 8 May and 10 September 2002 that are representative of these changes. The number of obvious bands ranged from 3 to 9, with the highest number of bands observed in May. In the early part of the year (in March and April), the profiles consisted of 4 bands of similar intensity, suggesting that they were all of similar abundance. These profiles were consistent throughout the water column. In the sample from 8 May, DGGE patterns from different depths reflected marked differences between the bacterial community in the top 200 cm of the water column and the bottom 150 cm, as the anoxic layer became established. The highest number of bands observed during the annual cycle was seen in the oxic water at this time, and both band number and intensity decreased with depth.

There was also a marked shift in bacterial community structure at 3.2 m depth (at the bottom of the lake) be-

tween 26 March and 8 October. Fig. 7 shows the temporal variations in 16S rDNA DGGE profiles at 3.2 m, before, during and after the establishment of the anoxic layer. The profiles obtained from 3.2 m in the samples from 26 March and 10 April generated 9 bands each and were very similar to each other; however, the profiles from subsequent samples differed markedly from both these and each other. Between 23 March and 8 October, 3 to 5 bands were observed in each sample, and few bands were present more than once, suggesting a dynamic bacterial community. The lower diversity observed by DGGE in the latter part of the annual cycle coincided with the increase in autofluorescent prokaryotes seen by AFC (Fig. 5). A small subsample of 4 bands was excised from the gel for reamplification and sequencing, to confirm that they were of bacterial origin (Fig. 7, Bands 1 to 4). The sequences obtained were compared to the BLAST database to determine the closest matches (Table 1). Band 1, detected on 10 April was most similar to an uncultured *Actinobacterium*. The sequence data obtained for Bands 2 and 3 contained a high number of degeneracies, and were therefore not submitted to GenBank; however, BLAST searches were carried out, and both bands, isolated from 16 July (that co-occurred with the high numbers of photo-synthetic bacteria), were both most similar to bacteria in the *Chlorobium*–*Pelodictyon* group, a group of anaerobic green sulphur bacteria. Band 4 isolated on 10 September was also most similar to a *Chlorobium* species. Although the overall

number of bands was low, there was a relatively high background smearing, suggesting high diversity.

Changes in the composition of the eukaryotic community were observed by DGGE separation of PCR amplified 18S rDNA. The eukaryotic community showed different DGGE profiles based on both the number of bands observed and the distance of migration, throughout the annual cycle. Fig. 8 shows 3 gels that are representative of the spatial and temporal variations observed by DGGE during the annual cycle. There was little variation in either the number of bands or the band profiles with depth on 26 March, 10 April and 23 April (represented by the gel in Fig. 8A), but the intensity of the most dominant bands did decrease with increasing depth (Fig. 8A). From 8 May until 8 October (represented by the gels in Fig. 8B,C), however, the DGGE profiles from different depths reflected substantial differences between aerobic and anaerobic assemblages. Profiles from the oxic part of the water column varied little with depth, but very few 18S rDNA bands were detected in the anoxic layer (Fig. 8B,C). Two bands were excised from the gel from the 10 April sample and se-

quenced to confirm they were of 18S rDNA origin. The sequences were compared to the BLAST database and the closest matches determined (Table 1). Band 1 was most similar to a *Pterocystis* species (a heliozoan) and Band 2 to *Cryptomonas ovata*, a microalga.

DISCUSSION

The aim of this study was to improve our current understanding of the spatial and temporal dynamics of bacteria, viruses and phytoplankton over an annual cycle in Priest Pot, a small hypereutrophic lake in the English Lake District. Priest Pot has been extensively characterised in previous studies, and the seasonal changes in physical and chemical parameters have been well documented (Finlay & Maberly 2000). Although Priest Pot is a small lake of only 1 ha in area, and 350 cm depth, steep physical and chemical gradients develop during the spring and summer, giving rise to a wide range of habitats which support a diverse and dynamic microbial community.

Analytical flow cytometry (AFC) was used as the tool of choice to determine concentrations of different groups within the whole microbial community, from viruses and bacteria, through to phytoplankton. The unique sensitivity of AFC allows it to easily discriminate between different groups within these microbial components, using scatter plots of either natural fluorescence (in the case of phytoplankton), or an added chemical dye such as SYBR Green I that binds to nucleic acid (in the case of bacteria and viruses) versus side scatter. Particles with similar fluorescence and side scatter properties form an individual group on a scatter plot, allowing different groups of microorganisms to be distinguished (Chisholm et al. 1986, Marie et al. 1999). This technique allowed us to study the community dynamics of phytoplankton, bacteria and viruses in the natural environment, simultaneously, something not previously achieved in any similar freshwater study.

In this study, 4 distinct groups of viruses were observed by AFC (Fig. 1A). Similar AFC groups were also observed in samples from the English Channel (Marie et al. 1999) and in a marine mesocosm study, during a study of a bloom of the microalga *Emiliania huxleyi*, and its termination by viruses (Jacquet et al. 2002). Group V2 was the most abundant, and was thought to comprise bacteriophages. Marie et al. (1999) found that the abundance of a similar group followed that of heterotrophic bacteria in a study of virus abundance in the Pacific Ocean, and both Lambda and T7 phages were shown to form distinct groups within this region when cultures were enumerated by AFC (Brussaard et al. 2000). Group V1 had a larger green fluorescence intensity, suggesting the presence of larger particles, with larger genomes, perhaps suggesting this group could comprise viruses that infect microalgae. Group V3 was unlike any that

Fig. 4. Spatial and temporal dynamics of phytoplankton Groups P1 to P6 (ml^{-1}) in Priest Pot over an annual cycle, from 26 March 2002 to 8 April 2003

Fig. 5. Spatial and temporal dynamics of Groups P7 and P8 (ml^{-1}) in Priest Pot over an annual cycle, from 26 March 2002 to 8 April 2003

has been reported previously. The larger side scatter and green fluorescence readings suggest this group could comprise large viruses, or small bacteria, although the group was not removed by filtration through a $0.2 \mu\text{m}$ filter (data not shown). The distribution of abundance of Groups V1, V2 and V3 over the 2002/2003 annual cycle did not differ greatly from each other, and were very similar to the total virus distribution. No correlation was observed between these groups, or any of the bacteria or phytoplankton groups determined by AFC (data not shown). In contrast, a study by Larsen et al. (2004) reported that virus numbers in an AFC group similar to V2 increased during a bloom of picoeukaryotes in coastal waters off Norway, suggesting that this group was dominated by viruses infecting a major component of the bloom. Although overall abundance of microalgae was high, their diversity was also high, as illustrated by both AFC (Fig. 4) and DGGE analysis of 18S rDNA (Fig. 8). This suggests that the high microalgal diversity could be maintained by continuous infection by viruses, hence virus concentrations remained relatively consistent and high from April to October in the top 250 cm (Fig. 3). Essentially, it may be interpreted that

Fig. 6. PCR-DGGE analysis of the bacterial community (16S rDNA) in Priest Pot at 8 depths (cm) throughout the water column, on 3 sampling occasions: (A) 10 April 2002, (B) 8 May 2002, and (C) 10 September 2002, using the 16S specific primers 8f and 534r (GC)

Fig. 7. PCR-DGGE analysis of the bacteria community (16S rDNA) in Priest Pot, at 330 cm depth, over an annual cycle, from the 26 March to the 8 October 2002, using bacterial 16S specific primers 8f and 534r (GC). Bands excised for sequence analysis are indicated (1 to 4) on the gel (details in Table 1)

virus activity could be responsible for preventing the main phytoplankton groups from reaching intense monospecific blooms. This is consistent with the 'kill the winner' hypothesis (Thingstad & Lignell 1997). Similar findings were also observed in a marine mesocosm study (Larsen et al. 2001). The resolution afforded by AFC is, at present, insufficient to determine differences between individual groups of bacteriophage in environmental samples, and although individual cultures of Lambda and T7 phages can be distinguished (Brussaard et al. 2000), the diversity observed in Priest Pot was too high to distinguish all the different groups within the major regions used in this and other studies. Group V4 had a scatter profile typical of those obtained for large algal viruses such as members of the *Phycodnaviridae* including *Chlorella* viruses (authors' unpubl. data). A similar AFC group was observed by Jacquet et al. (2002) in a study of the role of viruses in the termination of a bloom of the coccolithophorid *Emiliania huxleyi*. In Priest Pot, this group had a peak of abundance on 16 July at a depth of 100 cm, but did not coincide with high numbers of any of the phytoplankton groups determined by AFC.

Phytoplankton enumeration was also undertaken by AFC. Groups P7 and P8 were the most abundant determined using these settings (Fig. 5). These groups had relatively high side scatter and red fluorescence readings, and were originally thought to represent the

Fig. 8. PCR-DGGE analysis of the eukaryotic phytoplankton community (18S rDNA) in Priest Pot at 8 depths (cm) throughout the water column, on 3 sampling occasions: (A) 10 April 2002, (B) 21 May 2002, and (C) 8 October 2002, using the 18S specific primers 1427f (GC) and 1616r. Bands excised for sequence analysis are indicated (1 and 2) on the gel (details in Table 1)

Table 1. 16S and 18S DGGE band sequence accession numbers and closest BLAST matches. Bands 1–4 of 16S: see Fig. 7; bands 1–2 of 18S: see Fig. 8

Band	Accession no.	Closest BLAST match	e value	% similarity	Source
16S Band 1	AY81286	<i>Actinobacterium</i> sp.	e^{-170}	99	Urbach et al. (2001)
16S Band 2	Not submitted	<i>Chlorobium ferrooxidans</i>	$4e^{-71}$	96	Heising et al. (1999)
16S Band 3	Not submitted	<i>Chlorobium ferrooxidans</i>	$1e^{-34}$	96	Heising et al. (1999)
16S Band 4	AY81287	<i>Chlorobium ferrooxidans</i>	e^{-153}	96	Heising et al. (1999)
18S Band 1	AY81288	<i>Pterocystis</i> sp.	$6e^{-71}$	95	Nikolaev et al. (2004)
18S Band 2	AY81289	<i>Cryptomonas ovata</i>	$4e^{-44}$	94	Deane et al. (2002)

picoplanktonic cyanobacteria. However, both groups were found to flourish under anaerobic conditions in the stratified water column during the late summer and autumn (Fig. 5), and were therefore thought to comprise anaerobic green sulphur bacteria. Despite the dominance of cyanobacteria in many marine (Liu et al. 2002) and freshwater (Callieri & Stockner 2002) habitats, they were not detected in Priest Pot by AFC at any point during this study. Sequence analysis of a DGGE band whose appearance also coincided with the high Group P7 concentration suggested that a component of the bloom was closely related to *Chlorobium ferrooxidans*, an obligate anaerobic green sulphur bacterium that grows photoautotrophically using sulphide as the electron donor (Holt et al. 1994). *Chlorobium* species have previously been found in the anoxic layer of stratified lakes (Casamayor et al. 2000) and another member of the green sulphur bacteria has been observed in Priest Pot, and was tentatively identified as *Clathrochloris hypolimnica*, on the basis of TEM analysis (Davison & Finlay 1986). The large cell size of Group P7 suggested by AFC scatter plots is in accordance with other studies that have found bacteria in the anoxic hypolimnion to be up to twice the size of those in oxic waters (Cole et al. 1993). Strains within both the *Chlorobium* and *Clathrochloris* genera are known to form filaments, which could also explain the relatively high side scatter reading. High bacterial chlorophylls *c* and *d* levels recorded at the same time as the peaks in abundance of both Groups P7 and P8 (577 and 643 $\mu\text{g l}^{-1}$, respectively) indicate that this is an actively growing population (Fig. 2F).

The highest virus concentrations detected during this study were also observed in the anoxic layer, at the same time and depth as the P7 peak of abundance, suggesting possible interactions between these 2 groups. Larger bacteria have been shown to be more susceptible to viral infection than smaller ones in a eutrophic lake (Weinbauer & Höfle 1998b), and high virus concentrations, enumerated as the frequency of visibly infected bacterial cells, have previously been reported in the anoxic hypolimnion of the eutrophic Lake Plußsee, Germany, where viruses were shown to

account for up to 94 % of bacterial mortality (Weinbauer & Höfle 1998a).

In Priest Pot, there was a great deal of variation in bacterial community composition, at 320 cm depth, during and after the establishment of the anoxic layer. Although variation in bacterial community composition in the anoxic hypolimnion of freshwater lakes has been shown to be less marked than in communities from oxygenated parts of the water column (Casamayor et al. 2002), bacterial community composition is known to vary in response to changing environmental parameters. The level of photosynthetically available radiation (PAR) is an important factor in structuring anoxic bacterial communities, with organisms with different light harvesting adaptations proliferating under a range of light conditions (Vila & Abella 2001). Temporal and spatial fluctuations in microbial community composition in response to changes in limnological parameters have also been well documented (Casamayor et al. 2000). Once stratification is established in Priest Pot, data from this and previous studies have shown that physical and chemical parameters remain relatively stable in the anoxic layer (Finlay & Maberly 2000), so it is probable there are other forces driving the dynamic change observed within the bacterial community. The data reported in this study support those by others in suggesting that viruses could play an important role in structuring anoxic bacterial communities.

In addition to Groups P7 and P8, 6 distinct phytoplankton groups were determined by AFC analysis during this study (Fig. 1C), and were distinguishable on scatter plots of orange fluorescence versus side scatter. We compared the groups observed in this study with the scatter plots generated by individual cultured strains from the Culture Collection of Algae and Protzoa (CCAP) at the Centre for Ecology and Hydrology, Windermere, UK, in an attempt to determine possible identities for each (data not shown). Group P1 had a similar AFC signature to the diatom *Stephanodiscus hantzschii*. Groups P2 and P3 were most similar to the chrysophytes *Synura petersenii* and *Cryptomonas ovata*, respectively. *C. ovata* was also

identified from 18S rDNA DGGE profiles (Table 1), suggesting that it is an important component of the phytoplankton community in Priest Pot. Group P5 was most similar to another diatom, *Asterionella formosa*, and Group P6 was similar to a large cryptomonad *Cryptomonas*, isolated from Priest Pot. None of the cultured strains tested showed similar scatter plot signatures to Group P4, but the higher side scatter reading suggests it is larger than the chrysophyte *C. ovata*. AFC has been used previously to monitor the community dynamics of picophytoplankton in estuaries (Li & Dickie 2001, Liu et al. 2004), and many studies of phytoplankton seasonal distribution using AFC have been carried out in oceanic environments, where the eukaryotic phytoplankton have been considered as a single group, and compared with the numerically dominant picoplanktonic cyanobacteria (Campbell et al. 1997, Liu et al. 2002). However, studies of the spatial and temporal distributions of freshwater eukaryotic phytoplankton, determined by AFC, are rare.

The phytoplankton community in Priest Pot was shown by AFC to consist of a succession of small-scale blooms, or peaks of abundance of numerically dominant groups throughout the annual cycle. Of the 6 distinct groups observed, only 2 (P1 and P6) occurred at the same time and depth. The peaks of abundance of the remaining 4 groups were either spatially or temporally distinct, although they were all present at lower concentrations throughout the annual cycle. Similar observations have been reported in the estuary of the Swan River in Australia, where the major phytoplankton groups (enumerated by microscopy) were shown to be separated in both space and time (Chan & Hamilton 2001). Four out of the 6 groups observed in Priest Pot had peaks of abundance on 8 May 2002, as the water column made the transition from mixed to stratified. This is in agreement with the findings of Crosbie et al. (2003), who monitored the seasonal and vertical distributions of phytoplankton in an oligomesotrophic alpine lake. These authors identified 2 distinct eukaryotic phytoplankton groups that were responsible for a spring bloom at the end of isothermal mixing phase and the beginning of stratification in the lake. This period is one of dynamic change in the physical and chemical characteristics of the water column, which, combined with increasing solar radiation and temperature, lead to an increase in phytoplankton growth. The resulting succession dynamics of the different phytoplankton groups are clear. Similar findings were reported in a mesocosm study investigating the effect of nutrient addition on phytoplankton succession (Estrada et al. 2003). These authors reported that while the qualitative composition of phytoplankton assemblages remained consistent between replicates, significant variability in the abundance of dominant taxa

was observed, suggesting that minute differences in conditions within the different replicates put different selective pressures on the phytoplankton assemblage, causing different patterns of succession within the community.

Phytoplankton succession observed by AFC was supported by the DGGE data for which, again, clear succession dynamics were observed in the band patterns (Fig. 8). DGGE also showed far greater diversity within the eukaryotic community than was observed by AFC. On 8 October 2002, high numbers of phytoplankton Groups P1, P2 and P3 were observed by AFC between the surface and 2 m depth (Fig. 4). The DGGE profiles show a similar pattern, with 5 bands present in samples from the surface down to 2 m, below which all but 1 of the bands disappeared, suggesting that these bands may represent the dominant components of AFC Groups P1, P2 and P3.

Two distinct groups of bacteria were observed in this study (Fig. 1B), distinguishable by their high and low DNA fluorescence upon staining with SYBR Green I. Similar groups have been observed previously in bacterial populations, and are thought to represent bacteria containing high and low DNA. The high DNA bacteria are considered to represent the active fraction (Vaque et al. 2001), with the dynamics of bacterial production closely related to changes in the abundance of this group. The distribution of the 2 groups in this study was very similar, and is in accordance with data shown by Li & Dickie (2001), who enumerated bacteria from the surface of coastal waters over successive annual cycles and found that the patterns of seasonal abundance were very similar for the 2 groups. Bacterial abundance in Priest Pot was high, with concentrations reaching $2.6 \times 10^7 \text{ ml}^{-1}$. This is higher than epifluorescence microscopic counts of acridine orange stained bacteria from the eutrophic lake Plußsee, where 2.6×10^6 and $2.7 \times 10^6 \text{ ml}^{-1}$ were reported in the upper and lower water column, respectively (Weinbauer & Höfle 1998b). High bacterial biomass is a well-known characteristic of eutrophic systems. Bacterial production and chlorophyll *a* measurements were shown to be significantly higher in the eutrophic basin of Lake Erie, Ohio, USA, than in the less productive basins (DeBruyn et al. 2004).

This is the first study to investigate the diversity and dynamics of microbial communities from phytoplankton to bacteria and viruses, throughout the water column of a freshwater lake, over an entire annual cycle. The combined approach, using AFC and DGGE gives much more information about the ecology of these groups than either method on its own. We only undertook a limited amount of sequence analysis from the DGGE gels, as the value of these data are in the succession observed in the band profiles over spatial and

temporal scales, rather than in the identification of all bands. The data presented here suggest close coupling between the virus, bacteria and phytoplankton communities in Priest Pot. The high turnover of phytoplankton could be driving bacterial succession and abundance, which in turn maintains a diverse and abundant bacteriophage community. Priest Pot is a dynamic ecosystem in which a complex array of environmental factors combine to shape a rich and diverse microbial community.

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