Microbial Ecology

Abundance, Distribution, and Diversity of Viruses in Alkaline, Hypersaline Mono Lake, California

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Abstract

Mono Lake is a large (180 km^2), alkaline (pH ~ 10), moderately hypersaline (70–85 g kg^{-1}) lake lying at the western edge of the Great Basin. An episode of persistent chemical stratification (meromixis) was initiated in 1995 and has resulted in depletion of oxygen and accumulation of ammonia and sulfide beneath the chemocline. Although previous studies have documented high bacterial abundances and marked seasonal changes in phytoplankton abundance and community composition, there have been no previous reports on the occurrence of viruses in this unique lake. Based on the high concentrations and diversity of microbial life in this lake, we hypothesized that planktonic viruses are also abundant and diverse. To examine the abundance and distribution of viruses and bacteria, water samples were collected from four stations along 5 to 15 vertical depths at each station. Viral abundance ranged from 1×10^8 to 1×10^9 mL^{-1}, among the highest observed in any natural aquatic system examined so far. Increases ($p < 0.1$) in viral densities were observed in the anoxic bottom water at multiple stations. However, regression analysis indicated that viral abundance could not be predicted by any single environmental parameter. Pulsed field gel electrophoresis revealed a diverse viral community in Mono Lake with genome sizes ranging from ~14 to >400 kb with most of the DNA in the 30 to 60 kb size range. Cluster analysis grouped the anoxic bottom-water viral community into a unique cluster differentiating it from surface and mid-water viral communities. A hybridization study using an indigenous viral isolate as a probe revealed an episodic pattern of temporal phage distribution with strong niche stratification between oxic and anoxic waters.

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Introduction

Viruses are an integral part of aquatic microbial communities and can be a significant source of bacterial mortality [27]. They are also capable of mediating processes such as transduction, lysogenic conversion, and species successions and help to maintain microbial diversity [6]. Over the past decade, viral ecology has been studied in a wide range of aquatic habitats including rivers, lakes, oceans and seas, sea ice [16], and solar salterns [7]. The results indicate that viruses are truly ubiquitous, though their impact on microbial communities can be variable. To our knowledge, the ecology of viruses has not yet been investigated in alkaline, hypersaline lakes (aka soda lakes), an unusual habitat type represented by Mono Lake.

Mono Lake is a large, alkaline (pH ~ 10), hypersaline (70–85 g kg^{-1}) lake lying at the western edge of the Great Basin. Mono Lake is a chloride–carbonate–sulfate "triple water" lake of nonmarine origin. Following exceptionally high runoff in 1995, an episode of persistent chemical stratification (meromixis) was initiated and remained through the period of this study. This has resulted in the depletion of oxygen and accumulation of ammonia and sulfide in the monimolimnion or region beneath the chemocline [18].

The lake has a simple food web, with the brine shrimp *Artemia monica* as the sole macrozooplankter [4, 13]. Phytoplankton productivity is relatively high (269–1060 gC m^{-2} yr^{-1}) [10], consistent with reports from other soda lakes [2, 5]. Primary production decreases immediately following the onset of episodes of meromixis because of lower vertical nutrient fluxes and depletion of nutrients stored in the mixolimnion [10]. The phytoplankton community contains relatively few species dominated by a recently described unusual phytoplankter, *Picocystis salinarum* [14], several bacillarophytes,

mainly *Nitzschia* spp. (20–30 μm) [17], and unidentified small flagellates and coccoid cyanobacteria (Jellison, unpubl. data).

Bacterial populations in Mono Lake reach their highest density in winter ($2–8 \times 10^7$ mL^{-1}), decrease during the spring and summer months, and increase again in conjunction with the autumn phytoplankton bloom [17, 21]. There is evidence of metabolically diverse populations of prokaryotes in the lake including methanogens, methylotrophs, sulfate reducers, nitrate reducers, ammonia oxidizers, anoxygenic and oxygenic photoautotrophs, and selenate respirers [3, 22]. Overall productivity is limited by nitrogen and, because external inputs of nitrogen are low, bacterial consortia provide a key ecosystem service by regenerating ammonia [12].

This study provides the first documentation of the abundance and diversity of viruses in an alkaline, hypersaline environment. Our results revealed that viral abundance in Mono Lake was higher than has been observed in any other marine or freshwater system, but comparable to concentrations observed in a hypersaline evaporator ponds used to produce salt from seawater [7]. DNA–DNA hybridization analysis and viral community fingerprints also revealed horizontal and vertical spatial variability of viral community composition.

Materials and Methods

Sampling Sites and Procedures. Both pelagic and nearshore stations (Fig. 1) were sampled during different seasons. Stations 3, 6, and 12 were sampled during May 2000 (spring) and February (winter) and August (summer) 2001. The nearshore Station 1 was sampled during spring 2000 and summer 2001. Additional samples for viral diversity and isolation were collected from station 3 in August 2000 and station 8 in August 2001. Water samples were collected from 5 to 15 depths at each station using 5-L Niskin bottles. Temperature, conductivity, and dissolved oxygen were measured using a conductivity-temperature-depth recorder (Seacat 19, Sea-Bird Electronics, Bellevue, Washington).

Bacterial and Viral Direct Count. Water samples were fixed with 0.02-μm-filtered formaldehyde (1% final conc.) within 4 h of collection. Bacteria and viruses were counted by epifluorescence microscopy using a SYBR Green I (Molecular Probes, Eugene, OR) staining method [20]. In brief, preserved samples were filtered onto 0.02 μm pore size, 25 mm diam. aluminum oxide filters (Anodisc, Whatman Inc., Clifton, NJ), stained with SYBR Green I (1:2000 dilution in PBS) for 15 min, mounted in anti-fade solution (0.1% ethylenediamine in glycerol:PBS 1:1), and counted under blue excitation on an Olympus BX60 microscope. Most slides for bacterial and viral direct counts were prepared within 24 to 48 h of sample collection. Slides were either counted immediately after preparation or stored in freezer for ≤ 2 weeks before being counted. Both bacteria and viruses were counted from the same slide, and at least 200 particles of each kind per filter were counted from 10 randomly selected fields per slide. Counts in this study were based on duplicate slides.

Analysis of Viral Genome Sizes by Pulsed Field Gel Electrophoresis (PFGE). The genome size distributions in natural viral assemblages were analyzed by PFGE [24, 29]. Samples of 10 ml were prefiltered through 0.22-μm

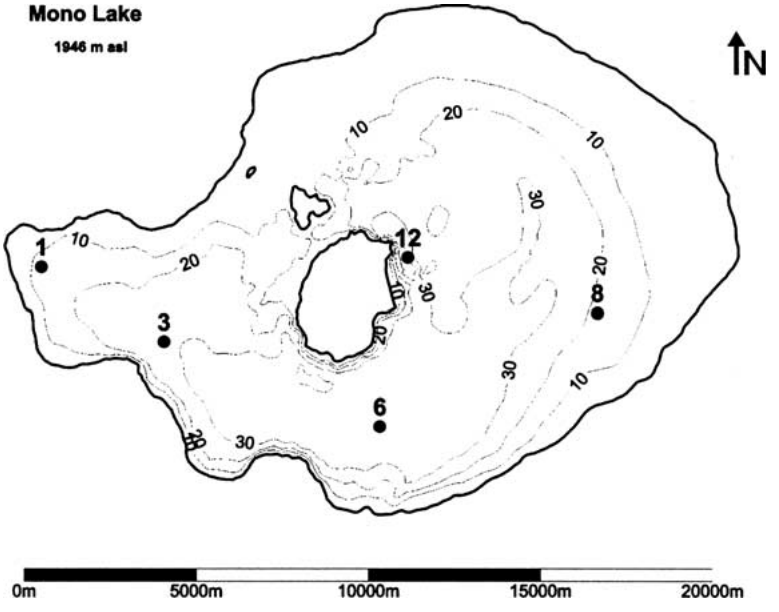

Figure 1. Map of Mono Lake, California, indicating the stations sampled. The depths for stations are station 1, 15 m; station 3, 30.3 m; station 6, 42.5 m; station 8, 19.3 m; station 12, 35 m.

pore size Sterivex GS filters (Millipore Corp., Billerica, MA) to remove bacteria and phytoplankton. This prefiltration step had little impact on viral recovery according to an early study [27]. The filtrates were concentrated to ~1 mL using Centriprep centrifugal ultrafiltration units (Millipore Corp.) with 100-kDa nominal molecular weight cutoff (NMWCO) membranes. This first-step concentrate was then transferred to a Centricon-100 (Millipore Corp.) and further concentrated to ~100 μL. Alternatively, viral particles in 10-mL samples were pelleted by ultracentrifugation (Beckman, rotor SW41/Ti) at 28,800 *g*, 10°C for 1.5 h. The final viral concentrate or viral pellet was resuspended and washed twice with 5× TE buffer in a Microcon-100 to a final volume of 30–50 μL. Concentrated products were heated to 60°C for 10 min to release viral nucleic acid. DNA was quantified using the PicoGreen dsDNA quantification kit (Molecular Probes). For samples used in cluster analysis, 150 ng of DNA was mixed with loading dye and separated in a 1% PFGE-grade agarose gel on a CHEF DR II electrophoresis apparatus (Bio-Rad; Hercules; CA). Electrophoresis was at 6 V/cm for 18 h at 14°C in 0.5× TBE buffer with a linear ramp in switch time from 1 to 10 s. Gels were stained with SYBR Gold (Molecular Probes) and images recorded with a digital gel documentation system (UVP). Mid Range 1 PFGE Marker (New England Biolabs Inc., Beverly, MA) and the high DNA Mass Ladder (Invitrogen Corp., Carlsbad, CA) were run to determine size and mass respectively, of DNA in sample bands.

Genome size distributions among samples were compared by performing cluster analysis using Gel Compar II (Applied Maths, Sint-Martens-Latem, Belgium). Each gel was normalized using molecular weight ladders run on both sides of the gel. Curve-based similarity analysis was used to take into account both position and density of each DNA band. Pearson correlation was used to generate the dendrogram and cophenetic correlation was used to reveal the stability of clusters.

Isolation of Bacteriophage. A bacterium was isolated on marine agar (Zobell 2216) amended with 1% additional NaCl from a sample collected in February 2001 at Station 12 at the depth of the chlorophyll *a* maximum (18 m). This isolate was used as a host for phage isolation from the same water sample using the top agar overlay method [1]. In brief, 1 mL of log-phase bacterial culture was mixed with 2 mL of molten agar and 1 mL of water sample in a culture tube. The entire mixture was poured over a marine agar plate, incubated for 4 days at room temperature, and monitored for plaque development. An individual plaque was picked and repropagated using the same method three times before the phage was considered a pure isolate. The phage appears to be lytic and forms clear plaques on its host. This phage isolate was designated Φ*Mono1*. Sequence analysis of the 16S rRNA gene of the host bacterium indicates that the closest known relatives are members of the gamma subdivision of the Proteobacteria. The sequence is 94% similar to a Mono lake isolate reported earlier (NCBI GenBank accession no. AF14006) and 91% similar to *Idiomarina baltica* isolated from surface water of the central Baltic Sea (GenBank accession no. AJ440215).

Hybridization Analysis of Viral Community. Φ*Mono1* genomic DNA was extracted from 0.2-μm-filtered, DNase- and RNase-digested viral lysate using the Wizard Lambda Preps DNA Purification System (Promega, Madison, WI) following the manufacturer's instruction. The total viral genome was labeled with digoxigenin using the Dig-High Prime DNA Labeling system (Roche Diagnostics Corp., Indianapolis, IN) following the manufacturer's instructions. The hybridization conditions were optimized to detect 0.1 ng of homologous DNA without cross hybridization with Lambda phage DNA and host genomic DNA (negative controls) at equal or greater concentrations.

The Φ*Mono1* probe was hybridized to viral community DNA in a dot-blot format. The viral fraction of samples collected in February 2001 were concentrated from ~30 mL to ~300 μL using Centripreps with 100 kDa NMWCO. Two hundred μL was spotted onto nylon membranes (MagnaGraph, MSI) using a dot-blot apparatus (Minifold II, Schleicher and Schuell Bioscience Inc., Keene, NH). Three viral concentrates collected in May 2000, from surface, oxycline, and anoxic layers, respectively, and preserved with 1% sodium azide were also spotted onto membranes. Lambda and Φ*Mono1* DNA were used as negative and positive control, respectively, for probe hybridization. Membranes were treated with 1.5 M NaCl, 0.5 M NaOH for 15 min and neutralized by 1.5 M NaCl and 0.5 M Tris (pH 8.0) for 3 min. DNA was fixed onto the membrane by UV cross-linking (UV Cross-Linker, Fisher Scientific, Pittsburgh, PA). Hybridizations were performed at 37°C overnight. Stringency washes were performed twice in 0.5× SSC, 0.1% SDS at 65°C, for 30 min each with constant agitation. Immunological detection of positive hybridization signals was performed following the manufacturer's instructions (Roche Diagnostics).

Statistical Analysis. Analysis of variance was performed to test the statistical significance of bacterial and viral distribution along vertical profiles and between sampling stations using direct counts from 20 fields for each data point. Regression analysis was used to test the relationship between viruses and bacterial abundance, temperature, conductivity, and dissolved oxygen concentration. Pearson correlation was performed using all data points of viral and bacterial abundance.

Results

Hydrographic Description. The stations selected for this study included a shallow (18 m) nearshore station which seasonally mixed to the bottom, and three deep (28 to 38 m) pelagic stations with anoxic monimolimnia. While the temperature in the anoxic monimolimnion was fairly constant between 4 and 5°C, surface water temperature varied seasonally from <2°C in February to >20°C in August 2001 (Fig. 2). A strong halocline was present at ~25 m depth at all sampling times. Deep mixing in the winter resulted in correspondence of the thermo- and oxyclines with the halocline at 25 m (February). Progressive thermal stratification in spring and summer resulted in shallower thermo- and oxyclines ranging from 14 to 20 m (May) and 10 to 12 m (August).

Viral and Bacterial Abundance and Distribution. Bacterial abundance ranged from 0.3 to 4.4×10^7 mL^{-1} for all stations throughout the seasons (Fig. 3). Viral abundances were generally more than one order of magnitude greater than those of bacteria, ranging from 0.1 to 1×10^9 mL^{-1}. Significant changes ($P < 0.01$) in bacterial and viral abundance along the vertical profiles were detected at nearly all stations except station 1 during summer 2001. Viral and bacterial abundances in the anoxic layer were significantly higher ($P < 0.01$) than in overlying waters at all mid-lake stations during August 2001 and at Station 12 during May 2000 (Fig. 3). However, a significant difference between the anoxic layer overlying waters was not detected at station 3 and station 12 in February 2001. There were no significant differences in viral and bacterial abundance between seasons and stations when all data for each station were used in the statistical analysis. Regression analysis using viral direct counts as a dependent variable and bacterial abundance, temperature, salinity, conductivity, and dissolved oxygen as independent variables did not reveal any significant correlation between viral abundance and any single independent factor tested ($P > 0.05$) at any individual station. Pearson correlation analysis using the combined viral and bacterial abundances from all seasons and all stations yielded an r-value of −0.28.

Viral Genome Size Distributions. PFGE was used to examine the distribution of viral genome sizes in samples collected from various depths within the oxic layer at station 3 in August 2000 and from the oxic, oxycline, and anoxic waters at station 12 in May 2000 (Fig. 4). For all samples, the sizes of virus-like genomes ranged from ~14 to >400 kb with most of the DNA in the 30- to 60-kb size range. Banding patterns were generally quite similar among the 0 to 20 m samples at station 3, but slight changes are visually detectable. When pooled samples from station 12 were examined, the banding patterns for oxic and oxycline waters were similar to one another, but distinctly different from the pattern observed in the anoxic waters.

Spatial variations in viral genome size distributions were examined in more detail using PFGE banding patterns for 25 samples of viral DNA collected from various depths at four stations in August 2001. Cluster analysis of the banding patterns grouped the samples into four major clusters (Fig. 5). The samples tended to group according to depth in the water column with distinct surface, mid-water, and deep clusters. One exception was the surface water samples from station 3, the majority of which formed a distinct cluster along with a mid-water sample from station 6.

Dynamics of Viral Isolate ΦMono1. To look at the dynamics of an individual virus, DNA from an indigenous viral isolate Φ*Mono1* was used to probe viral community DNA collected at different locations and sampling times. There was strong hybridization of Φ*Mono1* DNA to community viral DNA collected on the same day, from the same station and at similar depths to when and where Φ*Mono1* was isolated (Fig. 6). There was little hybridization to DNA from samples collected from a different station on the same date, and there was no detectable hybridization to DNA collected at the same station 9 months earlier.

Discussion

Abundance. Our estimates of virus concentrations in Mono Lake are among the highest reported for any natural aquatic environment to date. Although high, the virus concentrations in Mono Lake are consistent with the observed bacterial concentrations in the lake, given that bacterial and viral abundance display a general positive correlation over a wide range of environments [15]. The lack of a significant correlation between bacterial and viral abundance within the Mono Lake data set may simply reflect a more limited spatial and temporal scale over which this data was collected. Positive correlations seen for data sets spanning wide ranges of bacterial and viral abundance probably reflect gross, averaged differences in overall ecosystem productivity. Changes on shorter time and spatial scales, on the other hand, will be heavily influenced by the simultaneous predator–prey dynamics for many virus-host systems and may thus appear more chaotic.

Concentrations of viruses comparable to those in Mono Lake have been reported for hypersaline solar salterns used to crystallize salt from seawater [7]. In that study, the concentrations of prokaryotes and viruses increased along a salinity gradient, reaching ~10^8 and 10^9 mL^{-1}, respectively, in ponds with salinities in excess of 200 parts per thousand and in which protozoa and

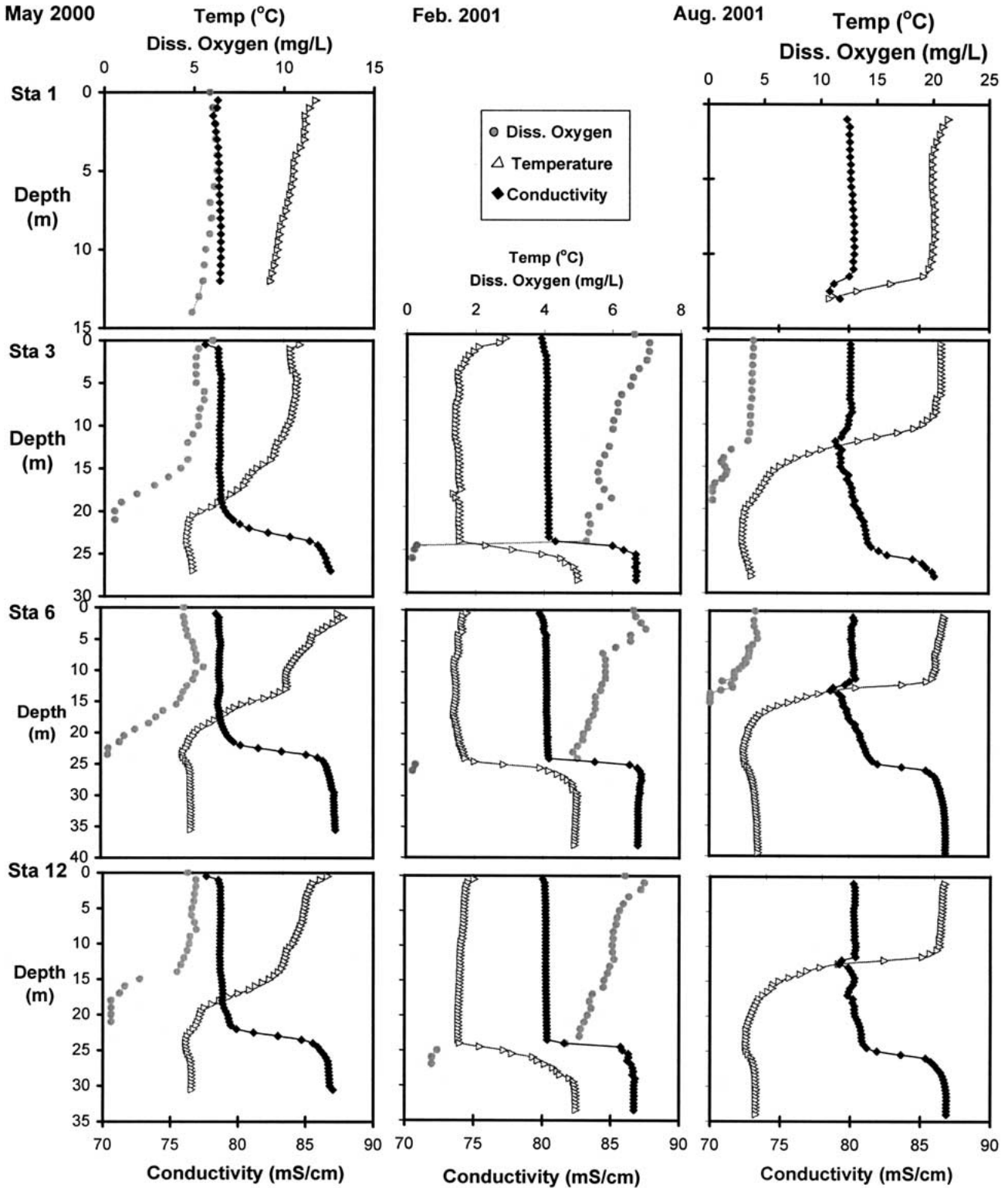

Figure 2. Depth profiles of temperature, conductivity, and dissolved oxygen concentration at Mono Lake stations sampled in May 2000 and February and August 2001.

bacterivory were not detectable or not expected to occur. The dramatic increase in viral and bacterial abundance in these ponds might be related to a release from top-down control on the bacterial abundance. We have no data on the abundance or grazing rate of protozoa in Mono Lake during the period of this study, but protozoa have been

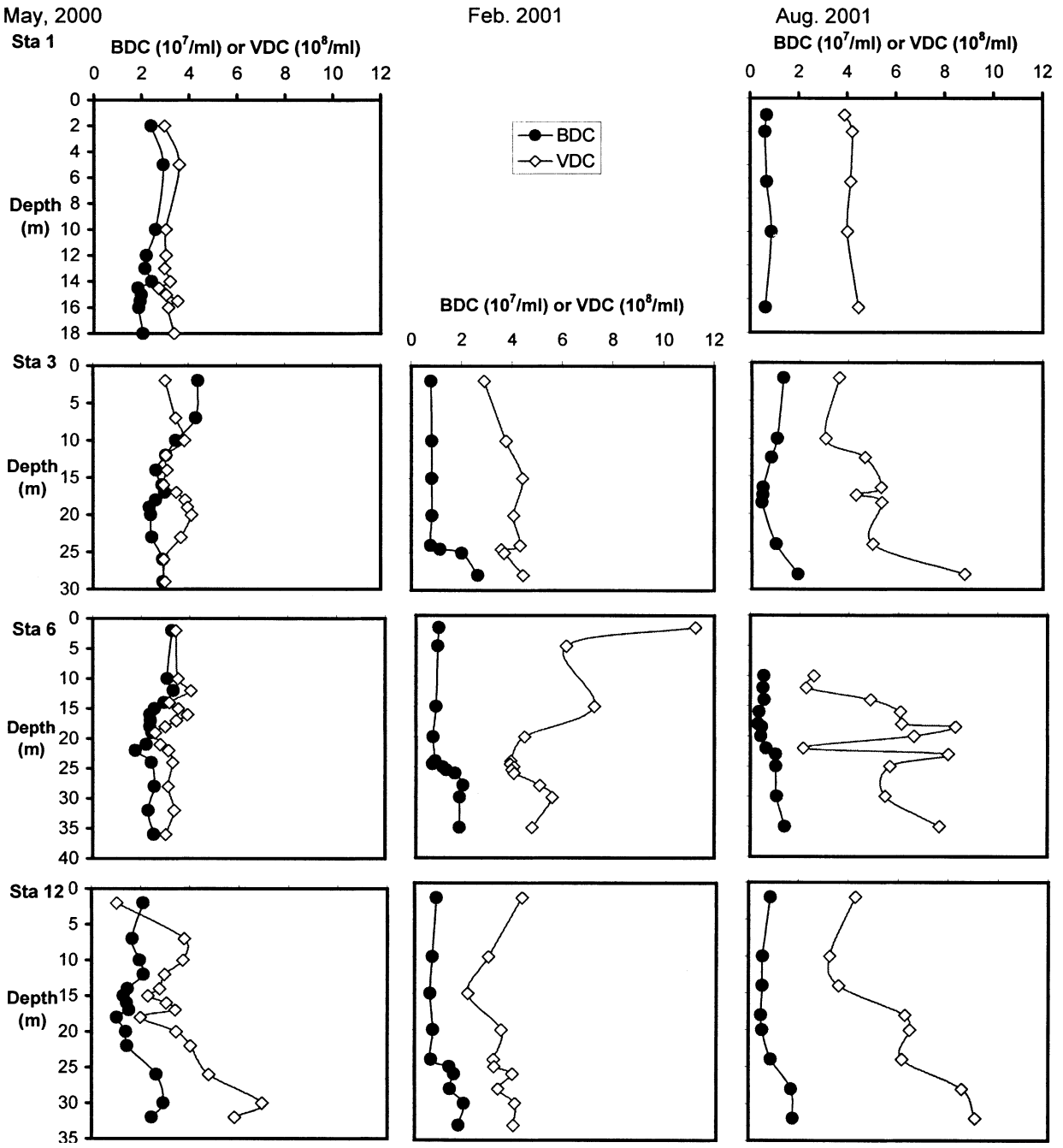

Figure 3. Depth profiles of bacteria and virus-like particles at Mono Lake stations sampled in May 2000 and February and August 2001. Each data point represented counts from 20 selected fields from two slides. BDC, Bacterial direct counts; VDC, viral direct counts.

observed in Mono Lake, which is only moderately hypersaline [17]. Thus the mechanisms leading to the high abundance of viruses in this lake may well differ from those operating in solar salterns.

The peaks in viral abundance we found in the anoxic water could be attributed to viruses infecting anaerobic bacteria, though it is possible that viruses are transported to the bottom waters by adsorption to sinking particles. Results from the dimictic Lake Plußsee [26] provide support for the former interpretation. Measurements of viral infection and grazing rates in that study suggested that viruses were a more important source of mortality in anoxic waters while protozoan grazing was more important in oxygenated surface waters. Their data imply that a high viral production in anoxic waters was at least partly responsible for the elevated concentrations of viruses. Investigations of viral infection rates in Mono Lake are currently underway to determine whether the results found for the freshwater lake Plußsee hold true in this moderately hypersaline lake.

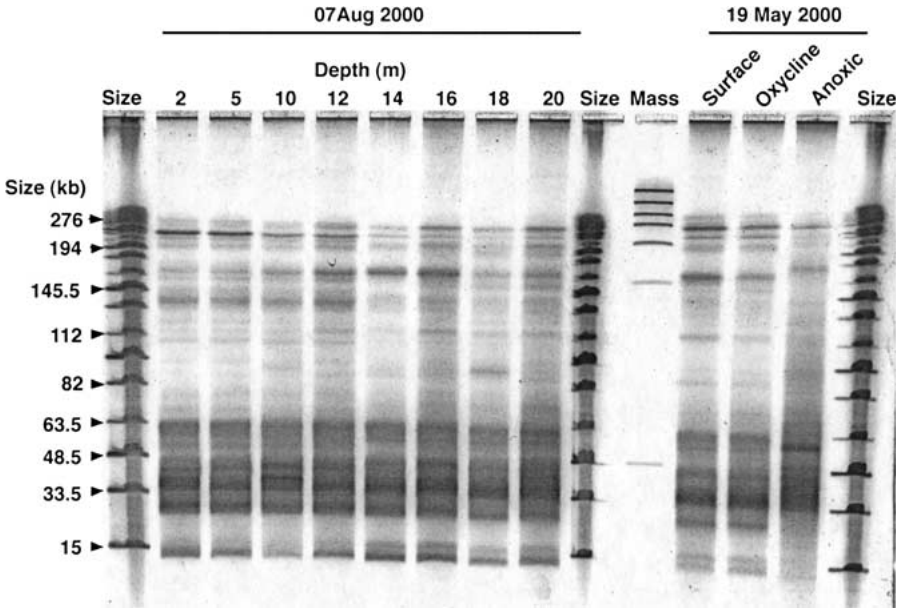

Figure 4. Image of fluorescently stained virus-like DNA after size separation in an agarose gel by pulsed field gel electrophoresis. Samples were collected from Mono Lake at station 12 and station 3 on May 19 and August 7, 2000, at various depths as indicated.

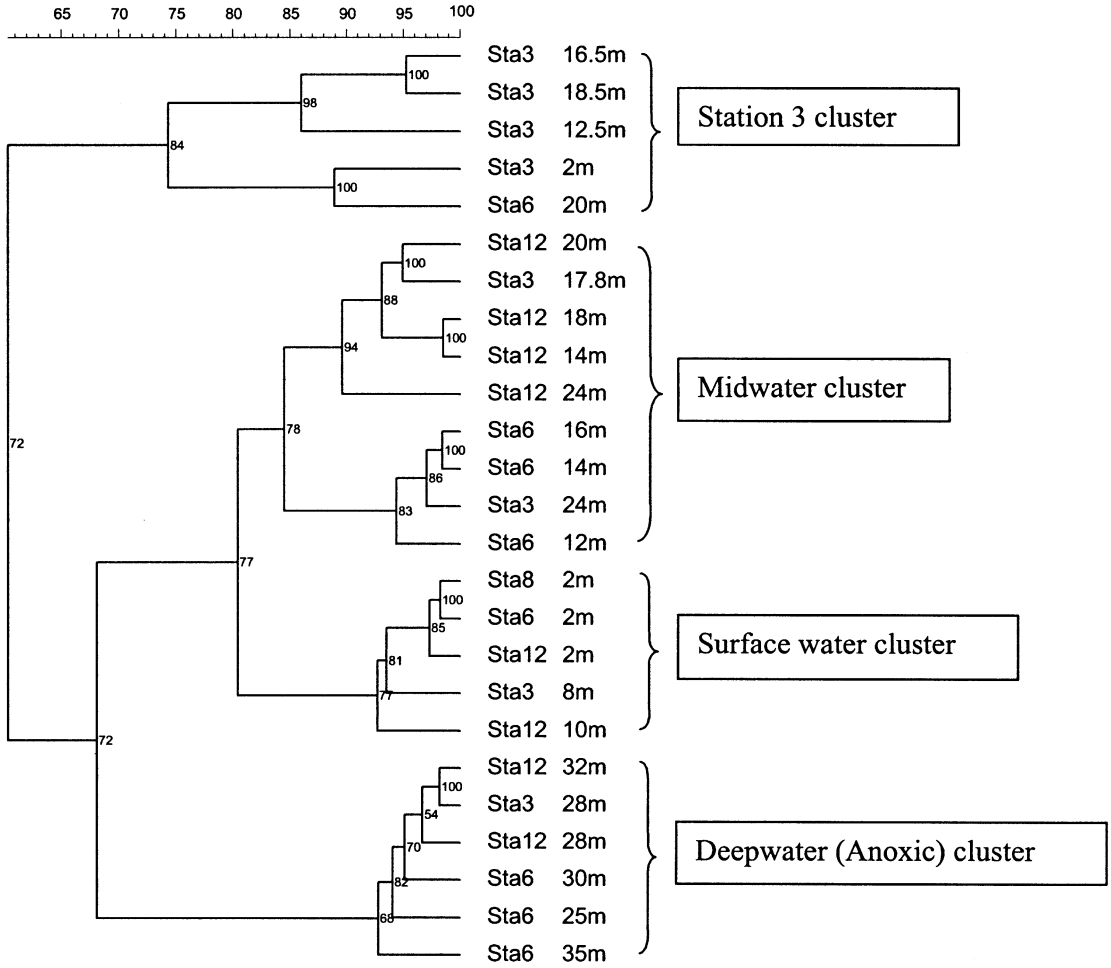

Figure 5. Diagram illustrating relative similarities in the viral genome size distributions among samples collected at stations 1, 3, 4, and 6 in August 2001 as determined by cluster analysis of DNA banding patterns by pulsed field gel electrophoresis.

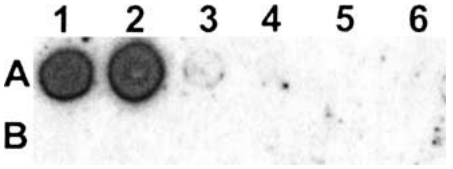

Figure 6. Dot-blot hybridization of a Φ*Mono1* whole-genome probe to total viral community DNA from various depths and stations collected in February 2001 (row A) and May 2000 (row B). Samples were collected from the following locations. A1: station 12, chlorophyll *a* max (18 m); A2: station 12, 2 m; A3; station 12, 15 m; A4: station 6, 5 m; A5: station 6, 20 m; A6: station 6, 14.5 m; B1: station 12, pooled surface water; B2: station 12, pooled oxycline water; B3: station 12, pooled anoxic water.

Diversity and Distribution. The distribution of viral genome sizes in Mono Lake as revealed by PFGE, is similar to that observed in marine [25] and estuarine [29] environments with the most common size of the virus-like genomes being ~35 kb. Although identity cannot be determined from genome size alone, the bulk of the DNA falls in the range typical for viruses infecting prokaryotes [25]. The presence of high-molecular-weight bands (>200 kb) suggests the presence of algal viruses, which typically have larger genomes [23]. It was interesting to note that the larger genomes were present in both oxic and anoxic waters.

Up to 27 bands could be resolved providing a minimum estimate of diversity. This is higher than some previous reports, but the difference is likely due to differences in methods used for preparing the samples for electrophoresis and the equipment used to document the gel images. An analysis of a marine viral community from Monterey Bay, using the same methods as employed in this study, yielded up to 35 resolvable bands [23]. Because much of the diversity in the viral fingerprints can be hidden within regions of unresolved bands, the conclusions one can draw about relative diversity among samples based solely on these fingerprints are limited. The fingerprints can, however, reveal some of the specific differences in composition among samples and the scale on which viral community composition varies. The variability of banding pattern between successive samples on one of the more detailed profiles, for example, indicates that at least some members of the community varied in relative abundance on a vertical scale of 2 m or less. The depth-dependent variability in the viral community is consistent with reports of vertical stratification of the bacterial community during periods of physical stratification of the water column [8, 9].

A more comprehensive comparison of PFGE banding patterns by cluster analysis revealed that viral community composition varied significantly on both the vertical and the horizontal scales. The probing results with the isolate Φ*Mono1* further indicate that the distribution of at least some bacterial viruses in the lake can be highly variable temporally as well as spatially. Similar results were found for the Chesapeake Bay [30]. In that study, probes prepared from a specific viral population only hybridized to total community DNA collected at the same time and place as the DNA used to prepare the probe. These results are in contrast to those from a viral diversity study conducted at a subtropical station in the Pacific Ocean, where hybridization data suggested that genetically similar phage could be detected in the deep water one year after isolation of the first phage [11].

Our data provide a first glimpse at the abundance and distribution of viruses in an unusual environment, but their significance as a source of bacterial mortality is not yet known. Mono Lake is unusual in having a relatively simple food web with very high phytoplankton production and brine shrimp as the sole macrozooplankton grazer. The brine shrimp, along with alkali flies inhabiting the littoral region, are in turn a staple food source for millions of migratory birds. Because of the high host densities and the short food chain, viral infections may have a more direct influence on biomass production at the highest trophic levels compared to the impact predicted for other environments [19]. Future studies will focus on temporal and spatial variations in viral abundance and determining the rate of viral infection to more fully investigate the impact of viruses on the microbial dynamics of this unique ecosystem.

Acknowledgments

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