Microbial Ecology

The Occurrence of Lysogenic Bacteria and Microbial Aggregates in the Lakes of the McMurdo Dry Valleys, Antarctica

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Abstract

The McMurdo Dry Valleys of Antarctica form the coldest and driest ecosystem on Earth. Within this region there are a number of perennially ice-covered (3–6 m thick) lakes that support active microbial assemblages and have a paucity of metazoans. These lakes receive limited allochthonous input of carbon and nutrients, and primary productivity is limited to only 6 months per year owing to an absence of sunlight during the austral winters. In an effort to establish the role that bacteria and their associated viruses play in carbon and nutrient cycling in these lakes, indigenous bacteria, free bacteriophage, and lysogen abundances were determined. Total bacterial abundances (TDC) ranged from 3.80×10^4 to 2.58×10^7 cells mL^{-1} and virus-like particle (VLP) abundances ranged from 2.26×10^5 to 5.56×10^7 VLP mL^{-1}. VLP abundances were significantly correlated ($P < 0.05$) with TDC, bacterial productivity (TdR), chlorophyll *a* (Chl *a*), and soluble reactive phosphorus (SRP). Lysogenic bacteria, determined by induction with mitomycin C, made up between 2.0% and 62.5% of the total population of bacteria when using significant decreases and increases in TDC and VLP abundances, respectively, and 89.5% when using increases in VLP abundances as the sole criterion for a successful induction event. The contribution of viruses released from induced lysogens contributed <0.015% to the total viral production rate. Carbohydrate and protein based organic aggregates were abundant within the water column of the lakes and were heavily colonized by bacteria and VLPs. Alkaline phosphatase activity was detected within the matrix of the aggregates, implying phosphorus deficiency and consortial nutrient exchanges among microorganisms.

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Introduction

The McMurdo Dry Valleys of Antarctica (160°–164°E, 76°20′–78°20′S) form one of the most extreme habitats for life on Earth. A unique feature in the dry valleys is the presence of perennially ice-covered lakes [32, 45]. The water columns of these lakes are hydraulically stable due to the 3- to 6-m thick permanent ice cover that impedes wind-driven mixing, low advective stream inflow, and steep salinity gradients over relatively short vertical distances [50]. These lakes are also highly stratified with respect to dissolved gases (e.g., oxygen, nitrous oxide, hydrogen sulfide), temperature, organic and inorganic constituents, and microbial distribution [27, 43, 44, 53, 54]. Molecular diffusion dominates vertical mixing of constituents within the water column [50]. Adequate sunlight to drive primary productivity exists for only 6 months of each year, producing systems where the ratio of contemporary net annual production to respiration is <1 [47]. Even during the 6-month period of sunlight, the ice cover reduces light penetration by >95%. Despite these conditions, microbial activity persists in the lakes throughout the year [23, 47, 60].

A previous study of these lakes showed that bacterial and viral abundances and their respective productivities were similar to those in temperate waters [20]. We present here mechanistic studies on a number of dry valley lakes (Bonney, Fryxell, Hoare, and Vanda) in the Taylor and Wright Valleys of the McMurdo Dry Valley system. Specifically, we present the first study of bacteriophage and lysogen interactions in these microbially dominated systems. We also characterized water column aggregates to show they harbor bacterial and viral particles as well as photoautotrophic organisms in what appear to be consortial "hot pots" [2] for biogeochemical transformations of inorganic phosphorus, a nutrient shown to be limiting to microbial growth in these lakes [11].

Methods

Sample Sites and Collection. Samples were collected once during the study from four depths in the deepest portion of the east and west lobes of Lake Bonney (11 and 12 December 1999, respectively), Lake Fryxell (1 December 1999), Lake Hoare (25 November 1999), and Lake Vanda (6 December 1999) using a 5-L Niskin sampler through holes melted in the permanent ice covers (see [43] for location and morphometric data on the lakes). The approximate maximum depths of these lakes, measured from the hydrostatic water level in the sampling hole, were 39 m for both lobes of Lake Bonney, 19 m for Lake Fryxell, and 21 m for Lake Hoare. The sampling depths were chosen to include the phytoplankton deep-chlorophyll-*a* maximum that exists in all of the lakes [25], as well as a sample from immediately beneath the ice cover and one in the aphotic waters below the chemocline. Samples were immediately transferred to sterile bottles and stored in a dark container near 0°C for transport to a lakeside laboratory. Upon return to the laboratory (<1 h), each sample was prefiltered through a Whatman GF/C filter (2.7 μm effective retention size) to remove potential protozoan grazers and immediately processed through the bacteriophage induction assay described below. Samples screened for the presence of lake aggregates were not prefiltered before processing.

Induction Assay. The induction assay used was a modification of that previously described by Jiang and Paul [18]. Briefly, each primary sample was gently mixed and 125-mL subsamples were transferred to two sterile bottles. Aliquots (25 mL) from each subsample were immediately transferred to sterile tubes and preserved with glutaraldehyde (2% v/v final concentration) and stored at 4°C for background (i.e., time zero) bacterial and viral abundance estimates. Mitomycin C (1 μg mL^{-1}) (Sigma) was added to the first bottle, while the second bottle served as an unamended control (no inductant was added). Both bottles were gently mixed and incubated at 4°C in the dark. Samples (25 mL) were collected from each bottle at 4, 8, and 24 h following the start of the incubation and preserved and stored for enumeration as described for the background sample. For each depth in the respective lakes, the time point sample that demonstrated the greatest decrease in bacterial abundance and increase in VLP abundance, relative to the control sample for that same time point, was used as the representative sample for that lake's depth.

Total Direct Bacterial and Virus-like Particle Counts. Each preserved sample was first filtered through a 0.2-μm pore-size filter (GTPB, Millipore Corp.) to capture the bacteria. The filtrate was collected and subsequently filtered through a 0.02-μm pore-size filter (Anodisc, Whatman, Inc.) to retain virus-like particles (VLP). Both filters were stained using SYBR Gold (supplied at 10,000×, final concentration 25×) (Molecular Probes, Inc.) as described by Chen et al. [7] and Noble and Fuhrman [38]. Replicate filters were prepared for both bacterial and VLP abundances from the time-zero samples collected during the induction experiment described previously. Fluorescent bacteria and VLP on the filters were counted using a Nikon Eclipse 600 epifluorescent microscope, equipped with B-2E (FITC), Y-2E (Texas Red), and EF4UV (DAPI) filter cubes, at a final magnification of 1250×. Minimums of 400 bacterial cells or VLP were respectively counted per filter [12].

Lake Aggregate Staining. Lake aggregates were stained with SYPRO Orange protein stain (Molecular Probes), ELF 97 phosphatase substrate (Molecular Probes), as recommended by the manufacturer, and 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma). DAPI staining was at a final concentration of 10 μg mL^{-1} during a 15-minute dark incubation [41]. Lake aggregates were also stained with alcian blue and Coomassie Brilliant Blue for the detection of polysaccharide and proteins, respectively [26]. Stained aggregates were photographed with a Kodak MDS 290 system (Eastman Kodak Co.) fitted to the Nikon Eclipse 600 microscope using either direct illumination or epifluorescence.

Background Chemical, Physical, and Biological Data. The parameters listed in Table 1 were measured using standard techniques employed by the National Science Foundation's McMurdo Long Term Ecological Research (LTER) Project [42, 43, 54]. Conductivity and temperature data were collected using a SeaBird SBE 25 [50].

Statistical Analyses. Comparison of means for selected treatments were performed using a two-tailed *t*-test on $\log_{10}$-transformed [i.e., $\log_{10}(x + 1)$] bacterial and viral count data. Log transforms of these data provided more even weighting to the widely dispersed data. The other variables used in the statistical analysis demonstrated a significantly reduced range of data dispersion and did not require transformation. Pearson product moment correlation coefficients (r) were calculated on the pairwise comparisons between bacterial and viral count data and selected chemical and nutrient parameters, using pooled data from all the lakes. Before calculating the correlation coefficients, each pair of variables was tested to ensure that there was a linear relationship between the variables and the residuals were normally distributed around the regression line with constant variance [13]. Significant correlations were determined at the $\alpha = 0.05$ level. Statistical analyses were performed with Minitab, version 13 (Minitab, Inc., State College, PA).

Table 1. Physical, chemical and biological characteristics for dry valley lakes

Lake	Sample date	Depth (m)	Temp. (°C)	Cond. (mS cm^{-1})	DO (mg L^{-1})	DIN (μmol L^{-1})	SRP (μmol L^{-1})	Chl *a* (μg L^{-1})	TdR (nmol L^{-1} d^{-1})
Lake Bonney (east lobe)	6 Nov 99	5	1.6	1.46	32.85	7.00	0.09	1.15	0.0000
		13	6.2	23.41	41.87	30.54	0.03	0.64	0.0030
		18	6.2	75.02	28.13	90.59	BDL	0.10	0.0019
		20	6.3	113.52	10.30	144.12	BDL	0.29	0.0013
Lake Bonney (west lobe)	11 Nov 99	10	3.1	5.88	34.45	8.01	BDL	0.79	0.0017
		13	2.5	22.53	41.50	14.04	BDL	3.60	0.0063
		15	1.1	89.64	4.70	103.80	0.03	1.10	0.0039
		20	−1.4	103.59	1.95	146.27	0.04	0.24	0.0006
Lake Hoare	17 Nov 99	4.5	0.1	0.34	45.20	5.37	BDL	5.08	0.0263
		10	0.3	0.53	45.05	1.00	0.06	2.47	0.0092
		14	0.1	0.72	33.45	3.69	0.04	0.60	0.0023
		19.5	0.1	0.72	21.25	8.18	0.07	0.40	0.0000
Lake Fryxell	27 Nov 99	6	0.4	2.15	23.90	0.12	5.65	5.44	0.2503
		9	1.8	3.74	25.80	0.86	20.32	12.03	0.1993
		11	2.1	5.49	0.00	17.04	BDL	7.92	0.0944
		12	2.2	6.26	0.00	43.28	0.08	3.60	0.0861
Lake Vanda	6 Dec 01	50	8.1	2.18	20.85	6.28	0.20	0.01	0.0075
		55	12.2	8.98	20.50	119.11	0.27	BDL	0.0088
		60	18.7	51.01	15.85	231.95	0.33	BDL	0.0101

[a]Abbreviations, Temp: temperature; Cond: conductivity; DO: dissolved oxygen; SRP: soluble reactive phosphate; Chl *a*: chlorophyll *a*; TdR: bacterial productivity. Method Detection limits are SRP = 0.03 μmol L^{-1}, Chl *a* = 0.01 μg L^{-1}. Below Detection Limits (BDL), No Data (ND).

Results

Physical and Chemical Data. The physical and chemical profiles in the lakes reveal the highly stratified vertical structure of the water column within each lake and the variability among lakes (Table 1). The east and west lobes of Lake Bonney both have steep conductivity gradients over the portion of the water column considered in our study with values ranging from 1.46 to 113.52 mS cm^{-1} and 5.88 to 103.59 mS cm^{-1}, respectively. The water temperature in Lake Bonney reaches a maximum in the middle of the water column and never exceeds 6.3°C in the east lobe and 3.1°C in the west lobe. Lakes Fryxell and Hoare also exhibit increasing conductivity with depth, but the gradients are not as steep and are up to two orders of magnitude less than those values found in Lake Bonney. Temperatures in Lake Fryxell peak at 2.2°C in the region of the chemocline (12 m). The water columns of these lakes do not mix on an annual scale (mixing time is about 50,000 years [50]) and probably have not done so for more than 1000 years [27]. Hence, the same gradients persist from year to year and have done so for at least 40 years (Priscu, unpublished data).

The dissolved oxygen in the euphotic zone of all lakes is highly supersaturated with respect to the mixing ratio in air above the lake (Table 1). Dissolved oxygen decreases precipitously beneath the chemoclines in all lakes, reaching anoxic levels in Lakes Fryxell and near-anoxic levels in the west lobe of Bonney (see [43] for detailed profiles of dissolved oxygen and redox potentials). Dissolved inorganic nitrogen increases below the chemoclines of all lakes, whereas SRP is low and relatively consistent with depth except for a peak at 9 m in Lake Fryxell [11].

Bacterial and Virus-like Particle (VLP) Abundances. The average bacterial total direct counts (TDC) ranged from 3.80×10^4 (Lake Vanda) to 2.58×10^6 (Lake Fryxell) cells mL^{-1} (Table 2). Average VLP abundances ranged from 2.26×10^5 (Lake Hoare) to 5.56×10^7 VLP mL^{-1} (Lake Fryxell) and were similar to TDC in the east lobe of Lake Bonney and Lake Hoare, about an order of magnitude greater than TDC in the west lobe of Lake Bonney and 1.0 to 1.5 orders of magnitude greater than those in Lakes Fryxell and Vanda.

Samples were shipped (dark at 4°C) to our United States laboratory for TDC and VLP abundance determination (within 5 months of collection) because logistical constraints did not allow us to process samples in the field. Storage of water samples has been shown to reduce TDC [15, 51, 53, 58] and VLP [10, 72] abundance values. Takacs and Priscu [53] and Spinard et al. [51] both developed equations to describe the kinetics of bacterial loss during storage. Based on these published equations and the length of time our samples were stored, the abundance values in Table 2 may be between 41.1% and 45.7% [53] or 60.2% and 65.4% [51] too low, depending upon which equation is used. We know of no systematic studies that provide a kinetic function to estimate VLP decay rates during storage. However, Danovaro et al. [10] observed that viruses recovered from marine sediments

Table 2. Bacterial abundance (TDC ± standard deviation, $\times 10^6$), virus-like particle abundance (VLP ± standard deviation, $\times 10^6$) and virus-bacteria ratios (VBR) for various depths in the study lakes[a]

Lake	*Depth (m)*	*TDC (mL^{-1})*	*VLP (mL^{-1})*	*VBR*
Lake Bonney (east lobe)	5	0.141 ± 0.031	0.327 ± 0.171	2.3
	13	0.102 ± 0.057	0.336 ± 0.117	3.3
	17	0.569 ± 0.271	0.510 ± 0.626	0.9
	20	0.549 ± 0.043	0.701 ± 0.121	1.3
Lake Bonney (west lobe)	10	0.120 ± 0.031	3.83 ± 0.86	31.9
	13	0.516 ± 0.102	2.06 ± 0.55	4.0
	15	0.791 ± 0.152	1.67 ± 0.59	2.1
	20	0.455 ± 0.109	1.15 ± 0.34	2.5
Lake Hoare	4.5	0.754 ± 0.209	0.457 ± 0.432	0.6
	10	0.675 ± 0.190	0.520 ± 0.433	0.8
	14	0.240 ± 0.070	0.226 ± 0.249	0.9
	19.25	0.151 ± 0.047	0.340 ± 0.383	2.3
Lake Fryxell	6	1.16 ± 0.29	53.8 ± 26.0	46.3
	9	1.05 ± 0.33	55.6 ± 33.3	53.0
	11	1.74 ± 0.79	38.1 ± 20.0	21.9
	12	2.58 ± 1.10	34.4 ± 20.5	13.3
Lake Vanda	50	0.038 ± 0.019	0.318 ± 0.143	7.5
	55	0.050 ± 0.027	1.09 ± 0.24	9.0
	60	0.161 ± 0.045	1.94 ± 0.28	12.1

[a]The depth is relative to the hydrostatic level in the sampling hole.

and preserved with glutaraldehyde decreased by 30% to 40% after 7 days of storage, with no significant increase in VLP loss after 90 days. If we apply the decay rate estimate of Danovaro et al. to our VLP abundances in Table 2, our reported VLP values could be 30% to 40% too low, relative to what they were at the time of collection. Given the high uncertainty surrounding the use of loss kinetics during sample storage, we use our measured values, but the reader should beware that the absolute values we present may be low; all relative comparisons are accurate.

Lake Hoare had the lowest average and most consistent virus-to-bacterium ratios (VBR), while Lake Fryxell's average VBR were considerably higher than the other lakes over the range of depths sampled (Table 2). The highest VBR values occurred at 10 m in the west lobe of Lake Bonney (31.9) and 6 and 9 m in Lake Fryxell (46.3 and 53.0, respectively). Correlation coefficients between bacterial and VLP abundances and Chl *a*, bacterial productivity (TdR), and soluble reactive phosphate (SRP) are listed in Table 3. These variables were selected for inclusion in our statistical analysis because previous studies have shown the existence of positive but inconsistent correlations with bacterial and VLP abundance data [71] and biological productivity in the dry valley lakes has been shown to be phosphorus limited [11]. The bacterial abundance data were significantly and positively correlated with all parameters tested, with the exception of SRP. The VLP abundance data, like the bacterial abundance data, were significantly and positively correlated with all tested parameters, including SRP.

Table 3. Pearson Product Moment Correlation coefficients (r) and probability levels (P value) for comparisons between bacterial abundance (TDC, cell mL^{-1}) and virus-like particles (VLP, particles mL^{-1}) and chlorophryll *a* (Chl *a*), bacterial productivity (TdR), and soluble reactive phosphorus (SRP)

	TDC		*VLP*	
Parameter	*r*	*P value*	*r*	*P value*
VLP	0.586	0.0080		
Chl *a*	0.628	0.0040	0.739	0.0001
TdR	0.490	0.0330	0.804	0.0001
SRP	0.214	0.3800	0.572	0.0110

Aggregates of bacteria and VLP's often occurred within a transparent to-opaque matrix in many of the SYBR-Gold stained samples observed by epifluorescence microscopy (Fig. 1a), leading us to further investigate the role of aggregates in the water columns of the study lakes. Aggregates were present in a majority (35–100%) of the microscope fields of preserved samples we examined and were similar in size to unpreserved samples from all lakes. The chemical and biological composition of aggregates from the east and west lobes of Lake Bonney was revealed by differential staining obtained with SYPRO Orange and ELF 97 for the detection of total protein and alkaline and acid phosphatase activity, respectively. The red Chl-*a* autofluorescence in Fig. 1b reveals numerous phototrophic phytoplankton associated with nonautofluorescing proteinaceous particles denoted by the yellow-green fluorescence of SYPRO Orange. The unstained sample shown in Fig. 1c shows further detail of autofluorescence associated with the many spherical chlorophyll-*a*–containing phytoplankton. Localized areas of phosphatase activity were detected in or on lake aggregates following exposure to the ELF 97 substrate and were associated with bacteria in the aggregates (Fig. 1d). The aggregates also contained relatively large amounts of polysaccharide and proteins following staining with alcian blue and Coomassie blue, respectively (Figs. 1e and 1f).

Induction of Lysogenic Bacteria. Table 4 summarizes data from induction experiments for selected depths in each lake. An induction experiment was considered significant when one of the three time-point samples had a statistically significant increase in VLP counts with a concomitant statistically significant reduction in TDC, relative to the control sample at that same time point [18]. The east lobe of Lake Bonney and Lake Hoare exhibited significant viral induction at every depth assayed. The west lobe of Lake Bonney had significant induction in the two uppermost depths, whereas Lakes Fryxell and Vanda had significant induction at only one depth in each lake (6 and 55 m, respectively). Collectively, 63.2% of all the samples assayed contained mitomycin C–in-

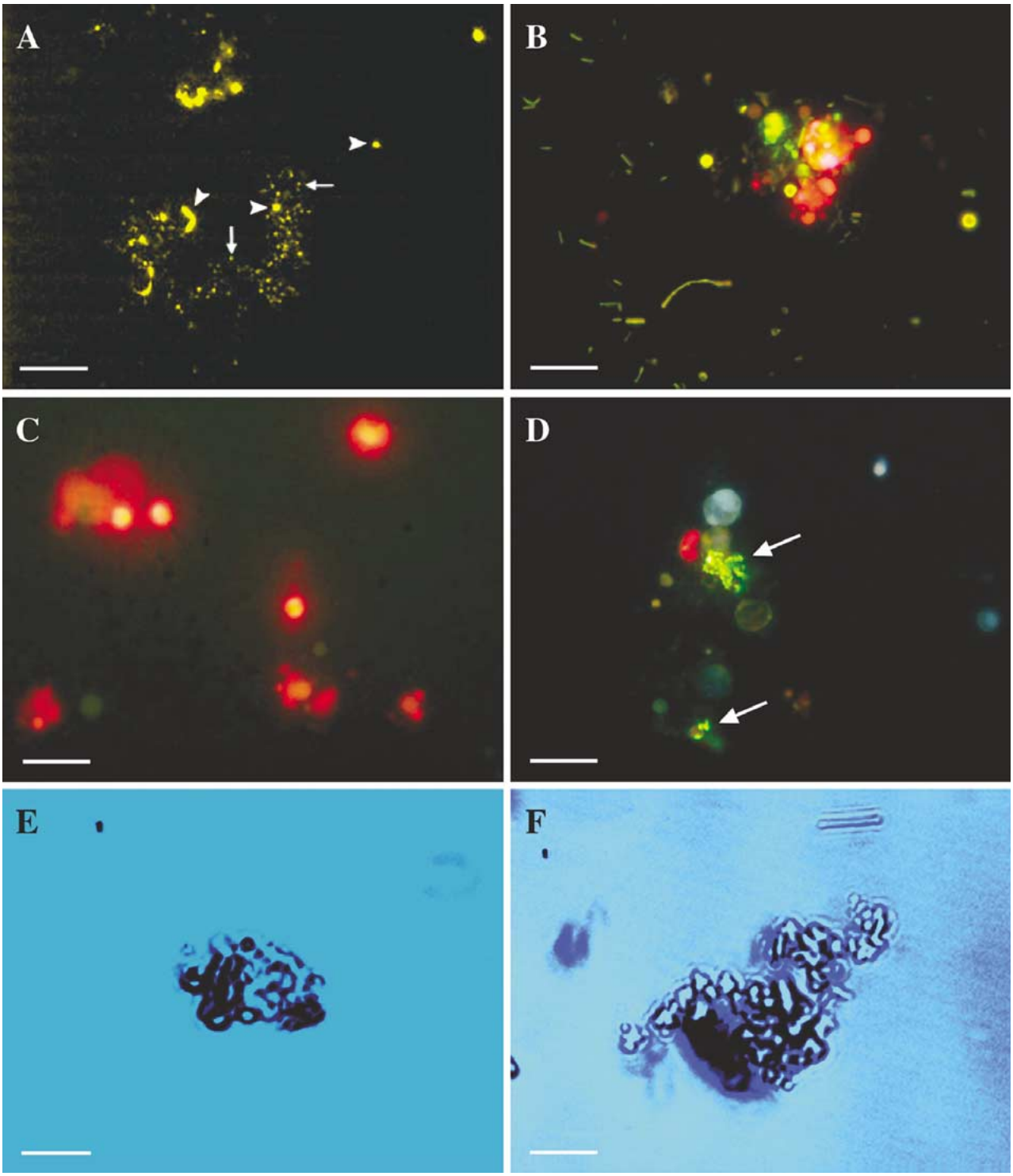

Figure 1. Lake aggregates stained with (a) SYBR Gold (► denotes VLP, → denotes bacteria), (b) SYPRO Orange, (c) no stain (phytoplankton autofluorescence only), (d) ELF 97 phosphatase substrate and DAPI (arrows denote ELF 97 expression), (e) alcian blue, and (f) Coomassie Brilliant Blue. Dark areas in (e) and (f) denote localization of polysaccharide and protein, respectively. Bar = 10 μm in all panels.

ducible lysogens. The range of VLP abundances in significantly induced samples ranged from 141.3 to 993.9% of the control samples. Total direct counts in these same samples ranged from 45.9 to 88.8% of the respective control samples. Only samples from 9 m in Lake Fryxell and 60 m in Lake Vanda did not respond to the inductant with either a significant increase in VLP or decrease in TDC. Of those samples that were significantly

Table 4. Results from mytomycin C induction experiments of indigenous lysogenic bacteria from selected depths within the study lakes[a]

Lake	Depth (m)	Max. induction time (h)	TDC control (mL^{-1})	TDC % of control	VLP control (mL^{-1})	VLP % of control	% of lysogenic bacteria based on: Bacterial mortality	% of lysogenic bacteria based on: Avg. burst size = 30
Lake Bonney (east lobe)	5	24	1.45×10^5	47.2**	4.09×10^5	259.0**	52.8	14.9
	13	8	1.53×10^5	87.9**	3.21×10^5	993.9**	12.1	62.5
	17	24	3.30×10^5	54.7**	2.72×10^5	179.8**	45.3	2.2
	20	24	5.70×10^5	88.8**	7.56×10^5	361.5**	11.2	11.6
Lake Bonney (west lobe)	10	24	1.22×10^5	55.2**	4.13×10^6	145.0**	44.8	50.8
	13	24	4.70×10^5	68.2**	1.98×10^6	176.8**	31.8	10.8
	15	8	7.64×10^5	90.6	1.87×10^6	139.4**	9.4	3.2
	20	24	4.27×10^5	92.1	1.03×10^6	134.6**	7.9	2.8
Lake Hoare	4.5	4	8.65×10^5	81.5**	6.48×10^5	179.6**	18.5	2.0
	10	24	7.81×10^5	50.0**	3.39×10^5	477.6**	50.0	5.5
	14	8	2.54×10^5	45.9**	3.68×10^5	485.3**	54.1	18.6
	19.25	24	1.65×10^5	78.9**	1.66×10^5	286.9**	21.1	6.3
Lake Fryxell	6	24	3.48×10^7	63.4**	4.17×10^7	210.4**	36.6	4.4
	9	24	8.42×10^5	128.4	4.00×10^7	113.1	0.0	0.0
	11	24	2.46×10^6	108.6	4.64×10^7	176.9**	0.0	0.0
	12	24	1.99×10^6	87.8	3.68×10^7	127.6**	12.2	17.0
Lake Vanda	50	8	2.90×10^5	127.0	3.39×10^5	294.0**	0.0	0.0
	55	24	3.04×10^4	69.4**	1.06×10^6	141.3**	30.6	48.0
	60	8	1.50×10^5	91.7	1.89×10^6	104.2	8.3	1.8

[a]TDC: bacterial abundance; VLP: viral-like particle abundance; **: statistically significant at $P < 0.05$. See text for details.

induced, 8.3%, 16.8%, and 75.0% reached their maximum rates of induction after 4, 8, and 24 h of incubation, respectively.

The percentage of lysogenic bacteria in the samples before mitomycin C addition can be estimated by assuming that lysogenic induction is the only source of VLP and bacterial lysis resulting from production and release of mature virus is the only mechanism responsible for the observed reductions in TDC. Based on these criteria, lysogenic bacteria in these lakes ranged from 11.2 (east lobe Lake Bonney, 20 m) to 54.1% (Lake Hoare, 14 m) of the total bacterial populations in the respective samples (Table 4). Another method of estimating the number of lysogenic bacteria assumes an average viral burst size per bacterium followed by normalization of the VLP abundance using this average. Estimates of burst size were calculated by dividing the net increase in VLP counts by the net decrease in the bacterial counts [18]. The calculated burst size in those samples that had significant induction rates averaged 26.4 (median: 9.6; range: 1.5–155) viruses bacterium^{-1}. Using this calculated average burst size we estimate that lysogenic bacteria composed 1.9 to 55.0% of the bacterial populations assayed, which is within the range reported by Jiang and Paul [18], who used a burst size of 30 viruses bacterium^{-1} in their calculations. If we use a burst size of 30 viruses bacterium^{-1}, a commonly used average, 2.0% (Lake Hoare, 4.5 m) to 62.5% (east lobe Lake Bonney, 13 m) of the bacterial populations in these lakes can be assumed to be lysogenic.

Discussion

The bacterial abundances in Lakes Bonney, Hoare and Fryxell in this study were approximately an order of magnitude less than the most recently published data from these same lakes [53], even after adjusting our data for potential cell loss during storage as described by Takacs and Priscu [53] and Spinrad et al. [51]. We caution that the general application of these decay equations to our study may yield inaccurate results because the decay constants for each study were obtained under different conditions and on different microbial assemblages. For example, the studies by Spinrad et al. [51], Turley and Hughes [58], and Takacs and Priscu [53] used acridine orange to fluorescently label cells preserved with formalin and glutaraldehyde. Though acridine orange has been shown to be more appropriate for labeling bacterial cells in marine samples [52] it has also been demonstrated to nonspecifically label a wide range of nonbiological substrates, including the membrane filter, and to quench quickly [21]. These characteristics collectively increase the probability of underestimating bacterial abundance counts in stored samples because of reduction in cell size and preferred intracellular target concentration (i.e., rRNA), which reduces the fluorescent signal per cell over time. This condition may allow the cell's reduced fluorescent signal to be overwhelmed in the high-background nonspecific fluorescence, leading to a false reduction in abundance estimates. The fluorescent label, DAPI, was used by

Gundersen et al. [15] to assess bacterial abundance reductions during storage. Though DAPI is more specific in its labeling of target molecules (i.e., AT-rich regions of DNA) and has a relatively lower background fluorescence than acridine orange [21], it also rapidly quenches, and recent studies have shown this label not to be as specific as previously thought [21, 59, 73]. Therefore, the same general precautions that should be considered when using acridine orange as a fluorescent label to assess bacterial abundance decreases during storage should be applied to DAPI as well. We used one of a new generation of high-affinity nucleic acid labels (i.e., SYBR Gold) that has been shown to have a relatively low background fluorescence when not bound to nucleic acids, to have a slow quenching rate in a wide range of salinities and water types, and to provide a fluorescent yield that is between 1.5 and 2.0 orders of magnitude greater than that from DAPI [7, 65]. The use of SYBR Gold allows the detection of bacterial cells that may have gone uncounted when using acridine orange or DAPI due to the problems associated being able to detect weakly stained, quickly quenched cells in a high background fluorescence. We acknowledge that there may have been some loss of bacterial abundance in our study during the storage period, but are not convinced that decay coefficients from other studies can be extrapolated accurately to our study or others that focus on different bacterial systems and use different fluorescent stains. Clearly, the detection and enumeration of bacteria in natural systems using commercially available stains is a semi-quantitative science.

The abundances of extracellular viruses, enumerated as virus-like particles (VLP), we detected in Lake Fryxell are similar to those reported in a previous study of Lake Fryxell, but 3-, 21-, and 28-fold lower than those previously reported in the west lobe of Lake Bonney, east lobe of Lake Bonney, and Lake Hoare, respectively [20]. It should be noted that the Kepner et al. study expressed the VLP abundance and VBR values as means of 10 to 20 depths per lake rather than the four depths per lake in our study, leading to some incompatibility in comparison. As with the bacterial abundance values, VLP abundance has been shown to decrease during storage [10, 29, 72]. Danovaro et al. [10], using fluorescent label SYBR Green I to label VLP released from marine sediments preserved with formalin or glutaraldehyde, found that VLP abundances were reduced by 21% and 23%, respectively, after 24 h of storage. VLP loss in the Danovaro et al. study increased to 30% and 40%, respectively, after 7 days of storage, but remained stable up to the end of the experiment at 90 days. Xenopoulos and Bird [72] found that VLP abundances decreased by up to 75% after 4 weeks of storage in marine and freshwater samples preserved with formalin or glutaraldehyde and subsequently stained with Yo-Pro-1. Neither study attempted to derive an equation to describe the kinetics of VLP decay. When our VLP abundance data are adjusted for loss during storage using the more conservative estimate of Danovaro et al. [10], the range of VLP abundance values listed in Table 2 could be 30% to 40% lower than they were at the time of collection. We did not apply the VLP reduction value (i.e., 75%) from the Xenopoulos and Bird study because they used a microwave-enhanced staining method, which we felt may predispose the viruses to deterioration and lysis and could possibly have contributed to the relatively high VLP reduction rate they observed. This possibility was not addressed in their publication. The two studies referenced above that have assessed VLP decay in stored samples used SYBR Green I and Yo-Pro-1, which like SYBR Gold, are categorized as high-affinity nucleic acid labels. However, SYBR Gold has been found to be more stable than and to have a fluorescent yield that is approximately twice that of SYBR Green I [3, 7]. Yo-Pro-1 has a fluorescent yield that is similar to that of SYBR Green I but requires an incubation period of 48 h, which makes it less attractive for routine use than SYBR Green I and SYBR Gold, which have effective labeling times of 15 min. As discussed for bacterial abundance losses during storage, we acknowledge there may have been reduction in our VLP abundance values during storage. However, we are cautious in the application of a VLP reduction value to adjust our VLP abundances derived from data that used a fluorescent label of lower quantum yield and quenches more rapidly than SYBR Gold. As with the application of fluorescent stains to enumerate bacterial cells, we believe that the determination of VLP in natural systems using labile stains should be considered semiquantitative.

Kepner et al. [20], found that VLP abundances varied significantly during the austral summer between and within each lake. Significant variability in VBR values within and between lakes is also evident in our study (Table 2). The average VBR for Lake Bonney's east lobe and Lake Hoare is similar to that found in open oceans, while Lake Bonney's west lobe value is closer to VBR ratios in coastal and estuarine waters [28, 71]. Conversely, VBR values in Lakes Fryxell and Vanda fall within the range found in fresh waters. We have no simple explanation for the extremely high VBR value at 10 m (31.9) in the west lobe of Lake Bonney, which has background conditions similar to that in the east lobe of the same lake. Similar temporal trends in bacterial and VLP abundances have been documented in estuary studies [9, 68] and in lysogeny in autotrophic marine picoplankton [31].

The significant correlations between VLP abundance and bacterial abundance (TDC), Chl *a*, bacterial productivity (TdR), and SRP (Table 3) support a common theme in the ecology of aquatic viruses: VLP production is positively influenced by factors that enhance the productivity of the host bacterial populations [71]. Inter-

estingly, there was a significant correlation between SRP and VLP abundance, but not SRP and bacterial abundance. Studies on the effect of phosphate limitation and the occurrence of lysogeny have shown that phosphate-depleted conditions favor the establishment of lysogeny over the lytic pathway in bacteria and phytoplankton [57, 69, 70]. Additionally, Williamson, et al. [68] have proposed that lysogeny may be a preferable option for temperate bacteriophage under nutrient-poor conditions, and that phosphate concentrations may modulate the lysogenic response in natural populations. Bratbak et al. [5] proposed that viruses might be more sensitive to phosphorus availability than other nutrients because of their high ratios of nucleic acid to protein, relative to their bacterial hosts. This enhanced requirement for phosphorus, relative to the host bacterium, may provide a mechanism through which the prophage is prompted to initiate a lytic cascade, with the synthesis of new viral genomes, following the detection of elevated phosphorus levels by the host bacterium. Our data support these observations, as the bacterial productivity rates and phosphate concentrations in the dry valley lakes are low and the microbial populations have been shown to be phosphorus deficient, particularly in Lake Bonney [11].

The discovery of microbial aggregates in all of the lakes and demonstration of phosphatase activity within the aggregates from the east lobe of Lake Bonney (Fig. 1) indicates that there are localized and heterogeneous sources of phosphate being released within and from these aggregates that are readily available to bacteria within and immediately surrounding the aggregates. The source of phosphate and other nutrients within the aggregates may be through the active release of cellular exudates, degradation of the phytoplankton and bacteria within the aggregates, and viral lysis of microbial inhabitants of the aggregates. Middelboe et al. [34] found that virus-induced bacterial lysates of a marine *Vibrio* sp. significantly increased bacterial nutrient uptake and alkaline phosphatase activity in a phosphorus-limited system, supporting our contention for the dry valley lakes. Bacterial lysates produced by viral induced lysis have also been shown to stimulate the recycling of bacterial carbon [35]. Alldredge [1] found that interstitial DOC concentrations within marine aggregates were one to two orders of magnitude greater than in the surrounding seawater and increased with decreasing aggregate size. This inverse relationship was attributed to the reduced porosity of the aggregates resulting from the fractal geometry of the interstices, which reduces diffusion rates from the aggregate. Additionally, the slow release of nutrients derived from cellular sources provides transient but elevated microscale patches of complex nutrients that can be immediately utilized by planktonic bacteria [4].

The biogeochemical processes implied by our data, in concert with high aggregate density in all of the lakes screened in this study, indicate that aggregates play major roles in the cycling of nutrients in the dry valley lakes, a conclusion also made for aggregates in marine systems [4, 16, 37, 40]. Specific interactions between bacteria and viruses within organic particles are also known to occur in marine waters [48]. These processes effectively short-circuit the flow of carbon by retaining and cycling dissolved organic matter within the microbial loop before being transferred to a higher trophic level [2, 6, 14, 36, 67].

Priscu et al. [47] calculated that water column integrated (5–20 m) viral carbon in the east lobe of Lake Bonney was 32 mg m^{-2} or 87% of the bacterial carbon integrated over the same depth. This relatively high percentage indicates that the viral component is a significant carbon pool in Lake Bonney and presumably other dry valley lakes. Importantly, owing to the absence of upper trophic levels commonly found in other fresh and marine waters [47], the viral populations in the dry valley lakes will have a greater influence on bacterial abundance, diversity, and associated biogeochemical processes within these lakes.

Several studies have determined the occurrence of lysogens in fresh [55] and marine waters using the inductant mitomycin C [8, 18, 63]. Using average burst size as the criterion for calculating the percentage of inducible lysogens in the marine studies, an average of 15.2% (median: 7.0; range: 0.7–92.9) of the bacterial populations from coastal waters were found to be lysogenic while 7.0% (median: 4.3; range: 0.2–15.8) were lysogenic in more oligotrophic, open waters. When substituting bacterial mortality for average burst size these same studies reported 28.8% (median: 29.5; range: 21.0–38.0) and 26.2% (median: 25.7; range: 17.3–36.0) of the bacterial populations were lysogenic in coastal and open waters, respectively. The lysogenic population ratios in the east and west lobes of Lake Bonney (30.4% and 38.3%, respectively), Lake Hoare (35.9%), Lake Fryxell (36.6%), and Lake Vanda (30.6%) were similar to those found in more temperate coastal waters when based on bacterial mortality. When using average burst size to calculate the lysogenic populations, Lakes Fryxell (4.4%) and Hoare (8.1%) were similar to lysogen percentage values found in oligotrophic waters, and both lobes of Lake Bonney (22.8% and 30.1%, respectively) and Lake Vanda (48.0%) were in the range of lysogen values for coastal waters.

The range of values discussed above from other studies that used burst size to calculate the percent lysogenic bacteria in coastal waters is large and includes the majority of the values published for oligotrophic waters. Most studies that have assessed burst sizes based on *in situ* observations have found virus:host cell ratios that are both significantly lower and significantly higher than the commonly used average burst size of 30 viruses bacteri-

um^{-1} [71]. *In situ* conditions that have been shown to significantly influence burst size include temperature and trophic state of the water [30, 61, 62]. When bacterial mortality is used to calculate percent lysogenic bacteria in these same studies, the majority of values also span both coastal and oligotrophic categories. Such variability suggests that the systems being sampled have highly variable inducible lysogen populations, and that data from a single sample or an average of many samples taken at different depths and times may not be appropriate for categorizing a water body as being "coastal" or "oligotrophic." Examples of the variability in lysogenic populations are provided by two studies of lysogen abundance in a eutrophic estuarine system [9, 68]. Both studies analyzed samples collected from the same site on a biweekly basis, using mitomycin C as the inductant, during separate 13-month periods. When using burst size to calculate the lysogenic populations in both studies, the values ranged from 1.5% to 210.0% of the total bacterial population. Within this range, 43.5% of the lysogen values were <10.0%, a value commonly assigned to oligotrophic waters. In these same studies, similar trends occur in the lysogen data when using bacterial mortality in the calculation. Based on bacterial mortality, lysogenic populations ranged from 4.1% to 51.2% of the bacterial population being lysogenic, with a small fraction (13.0%) of the values being <10.0%. Both studies showed a seasonal trend in lysogen abundance, with the authors suggesting that these trends were the result of changes in water temperature, nutrient concentration, and primary and bacterial productivity.

This same range of variability in the inducible lysogen values occurs in the data from our study on Antarctic lakes, with some depth strata falling in the "coastal" and others in the "oligotrophic" category within each lake over a relatively short vertical distance (Table 4). In open systems, one of the factors that significantly influences temperature and nutrient distributions, and thereby primary and secondary productivity, is physical mixing. The permanent ice cover and low stream flow in the dry valley lakes results in physical mixing processes at the molecular scale [50]. The extremely hydraulically stable water columns in our study lakes provide a vertical series of unique environmental niches that are stable over time. The variability in viral dynamics between and within the dry valley lakes is presumably due to differences in diversity within the bacterial host populations, and the quality and quantity of nutrients within each stratum. In comparison to well-mixed open systems, the dry valley lakes provide unique environments to address questions concerning the ecology of lysogens because many distinctively different temperature, nutrient, oxygen, and productivity gradients can be sampled in a single lake. Moreover, owing to the lack of vertical mixing, these gradients have persisted for thousands of years, allowing the same water to be sampled over long time scales.

Both the burst size and bacterial mortality methods for calculating the occurrence of lysogens have caveats. The burst-size approach uses an average number of viruses $bacterium^{-1}$, though burst sizes in similar types of water have been shown to vary significantly [71]. The bacterial mortality approach assumes the decrease in bacterial counts is totally dependent upon viral induced lysis; losses through grazing are assumed to be negligible. Fortunately, grazing of bacteria in the dry valley lakes is low because of the paucity of higher trophic levels [17, 22, 24, 33, 47, 49]. To ensure that bacterial grazers did not complicate our results we prefiltered (2.7 μm) all samples before beginning our experiments; no organisms larger than bacteria were observed in our microscopic observations, indicating the successful removal of potential grazers. In addition to grazing, infection by lytic viruses, cell death and toxicity of the inductant (i.e., mitomycin C) can also contribute to decreases in bacterial abundance values during the induction experiments [18]. The impacts of these factors were not assessed in this study.

VLP abundances may increase during an induction experiment without being the result of lysogen induction and host cell lysis. Bacterial host cells that had been infected with lytic viruses before sample collection could be a source of free VLP if they lysed the hosts during the experiment. These events, if they occurred during the induction experiment, would be indistinguishable from the lytic events resulting from the induction of a prophage. Though we could not directly address the impact of this factor on bacterial and VLP abundances during the induction experiments, their influences are minimized by our use of untreated controls to normalize the respective induction data for each lake.

The relative contribution of lysogenic viral production (V_L%) to total viral production can be calculated using the equation of Jiang and Paul [19]:

$$V_L\% = \frac{S_r G L B}{D_r V_t} \times 100$$

where S_r ($bacterium^{-1}\ G^{-1}$) is the prophage spontaneous induction rate, G (d^{-1}) is the number of bacterial generations, L is the fraction of lysogens in the total bacterial population, B (mL^{-1}) is the total bacterial abundance, D_r (d^{-1}) is the viral decay rate, and V_t(VLP mL^{-1}) is the total viral abundance. We used published data from other systems in concert with our present data to compute V_L% for Lake Hoare in our study. The range of prophage spontaneous induction rates (10^{-2} to 10^{-5}) was taken from Jiang and Paul [19]. The values for the number of bacterial generation day^{-1} (0.007 d^{-1}) and viral decay rate (0.3 d^{-1}) were calculated from previously published

data from Lake Hoare [20]. The bacterial and VLP abundance values (4.58×10^5 and 3.73×10^5 mL^{-1}, respectively) were averages of our counts from the 10-m and 14-m samples in Lake Hoare (Table 4). The decimal fraction of lysogens in the bacterial population are averages of the percent lysogenic bacteria given in Table 4, based upon bacterial mortality (0.521) and average burst size (0.121) from these same depths. When using bacterial mortality as the method for estimating percent lysogens, lysogenic viral production contributed 1.49×10^{-7}% to 0.015% of the total viral population at the average depth of 12 m in Lake Hoare. Using the average burst size method these values decreased to 3.47×10^{-8}% to 0.003% of the total viral population. Though we did not measure viral production directly in our study, Kepner et al. [20] estimated the total viral production rate in Lake Hoare at 12 m to be approximately 4.9×10^7 mL^{-1} d^{-1}. Using this viral production rate, we compute that viruses released from induced lysogenic hosts would add from 7 to 7350 (based on bacterial mortality) or 2 to 1470 (based on average burst size) viruses mL^{-1} d^{-1} to the total viral population. Assuming that similar viral production occurs at other depths of Lake Hoare and in the other study lakes, induced lysogens are a very minor contribution to the total viral production in the perennially ice covered lakes of the McMurdo Dry Valleys.

We reemphasize that the percent lysogen values from our study and other studies that have used mitomycin C as the inductant may overestimate the number of inducible lysogenic bacteria and thereby their contribution to total viral production. Though mitomycin C is a mutagenic agent, it is not commonly found in nature [56]. When using a natural inducer, such as sunlight, several groups found that indigenous lysogenic bacterial populations in temperate marine waters were not significantly induced [18, 19, 63, 66]. Results from these studies led the authors to conclude that *in situ* lysogenic phage production is not a significant source of virus production or bacterial mortality in marine waters, a conclusion we also make for the lakes of the McMurdo Dry Valleys. It should be noted, however, that mitomycin C may affect only a minor percentage of lysogens, possibly leading to underestimations of lysogeny in natural bacterial populations [64].

If the percentages of inducible lysogenic hosts can represent up to 63.2% of the total bacterial population but contribute such a small percentage to the total viral production in these lakes, what are the advantages of lysogeny? Proposed advantages include maintenance of a viable viral population as prophage when bacterial host abundance decreases below a critical level due to lytic viral infections, establishment of immunity from infection by other homoimmune viruses, acquisition of new phenotypic characteristics via gene exchange (e.g., specialized transduction), and enhanced reproductive fitness for the host [71].

Interestingly, the bacterial and free VLP abundances determined in our study and the study of Kepner et al. [20] are similar to those found in temperate fresh and marine waters despite the apparent inhospitability of the environment (e.g., extreme salt, temperature, and gas levels, large changes in solar radiation, and associated primary production and overall oligotrophic conditions) of the dry valley lakes. The absence of solar energy entering these lakes during winter effectively eliminates primary productivity for 6 months per year [47]. A major question that has been posed [22, 47] concerns the fate of bacterial and viral populations in the absence of winter primary productivity and little annual allocthonous organic carbon input (the surrounding area is devoid of vegetation and glacial melt is low). To date, there have been no experimental studies conducted in these lakes during the winter months because of safety and logistic constraints. The lack of phytoplankton production in the winter months could promote a maintenance physiological state in the bacterial population and thereby reduce lytic virus replication and lysogen induction. A corresponding reduction in bacterial and viral abundances would then be expected at the end of the winter. Interestingly, when bacterial abundance data from either side of the winter months (i.e., January and September) are compared, there are no clear differences in bacterial abundance or activity [53, 54]. Although the bacterial productivity rates are between 1 and 2 orders of magnitude lower than those found in more temperate waters, they do closely approximate those found in other high-latitude lakes and deep oceanic systems [53].

The percentages of the total lysogenic bacterial populations in the dry valley lakes are similar to that reported for temperate fresh and coastal and pelagic marine waters. Assuming that diminished physiological activity in the host cell population promotes the establishment of lysogeny, we expected there to be a relatively increased occurrence of lysogeny in the dry valley lakes. In fact, this was the case when using the average percent lysogenic bacteria values for each lake, based on bacterial mortality, as all values were >30.0%. When using average burst size to calculate the percent lysogenic bacteria, lakes Hoare and Fryxell averaged <10% of the total bacterial population, while the average values for both lobes of Lake Bonney and Lake Vanda ranged from 16.6 to 22.8%. We feel it more appropriate to assess the occurrence and significance of lysogeny in the dry valley lakes at each respective depth and not by using whole lake averages. We computed, using the significant induction event criteria, that 63.2% of all samples contained lysogenic populations that were inducible with mitomycin C, implying elevated lysogeny in the extreme environment posed by our study lakes.

Other studies using mitomycin C and the same criteria to determine significant lysogen induction have found lysogenic populations in 39% [18] and 60% [8] of their samples, respectively. Recently, studies on lysogeny in a subtropical estuary have used only statistically significant increases in VLP abundance values to determine significant induction events when using mitomycin C [9, 68]. This decision was based on the authors' opinions that current methods for enumerating bacteria may not be able to detect relatively small, but statistically significant, changes in TDC. Using this criterion for determining a significant induction event in the dry valley lakes, the number of samples with inducible lysogens in our study increases to 89.5%. Collectively, our data imply that lysogeny may be preferred over the lytic pathway in extreme environments, though lysogeny may contribute little to total viral production. Additional studies on this and other factors known to promote or inhibit the establishment of lysogeny in the dry valley lakes must be conducted during the austral winter before the overall ecological contribution of viral activity can be assessed.

How do the bacteria and viruses in the dry valley lakes obtain the required nutrients for maintenance and reproduction during the winter months when primary production stops? We propose that in addition to the slow upward diffusion of nutrients from deep water pools [42, 54], the aggregates found in all of the lakes we studied provide microenvironments with elevated concentrations of complex nutrients derived from active, dead, and lysed microorganisms that are slowly released or extracted from the matrix of the aggregate. The formation of these aggregates would continue into the winter months as phytoplankton populations die and degrade because of absence of solar energy to drive photosynthesis. The interactions among bacteria, phytoplankton, and viruses within these aggregates and the recycling of the bacterial carbon could provide a ''hot spot'' [2] that provides nutrients for resident and planktonic bacteria. The vertical distribution of the aggregates is dependent upon their size and mass and, in those lakes with salinity gradients, would accumulate at a depth of neutral buoyancy. The aggregates depicted in Fig. 1 reveal the complex microbiological consortia that form in these lakes, which presumably play a key role in microbial survival in these extreme environments. A similar contention was made for the microbial consortia that exist within the permanent ice covers of these same lakes [39, 46].

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