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Temperate viruses and lysogeny in Lake Superior bacterioplankton

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Abstract

The morphology and abundance of free viruses were measured in spring, summer, and fall at one site in Lake Superior. Free viral head sizes ranged from 10 to 70 nm and tail length ranged from 10 to 110 nm. The vast majority (98%) of free viral head sizes were ≤60 nm, smaller than reported in most freshwater habitats. Most of these free viruses (70%) had polyhedral heads and tails, indicative of bacteriophage. Free viral abundance only ranged from 0.1 to 9 × 10^6 viruses ml^{-1} in the surface microlayer (top 20 μm) and subsurface water (20 m) in Lake Superior, but viruses were 2–15 times more abundant in the surface microlayer. This difference may be due to the enrichment of bacterial hosts, higher levels of UV light that induce temperate phage, or differences in viral burst sizes in the surface microlayer relative to subsurface water. Bacterioplankton were always more abundant than free viruses in both the surface microlayer and subsurface water, which resulted in some of the lowest virus-to-bacterium ratios reported for marine or freshwater environments. Temperate viruses from both habitats responded equally to mitomycin-C and UV light treatments used to induce prophage into lytic cycles. An estimated 0.1–7.4% of the bacterioplankton from this site in Lake Superior contained temperate prophage depending on viral burst sizes that were assumed. Three times more bacteria in the surface microlayer may contain temperate viruses compared to bacterioplankton in subsurface waters. In the western arm of Lake Superior, bacterioplankton infected by temperate phage may be more important for the survival of bacteriophage populations than as future carbon sources for new microbial production.

Until recently, aquatic virology has been a neglected part of microbial ecology. Torrella and Morita (1979) demonstrated that viruses were present in seawater at higher concentrations than had been previously reported (>10^4 viral particles ml^{-1}). Several researchers have corroborated these observations by measuring viral abundances as high as 10^{10} viral particles ml^{-1} in seawater and lakes (Børsheim et al. 1990; Paul et al. 1991; Suttle et al. 1991). Seasonal variations in the size of aquatic viral populations have also been observed with viral abundances reported to be the most abundant during the spring and summer in some lakes and marine environments (Bratbak et al. 1990; Demuth et al. 1993; Shortreed and Stockner 1990). These observations have sparked a renewed interest to determine what types of viruses are present in various aquatic environments, identify host cells, estimate the possibility of viruses transferring genetic material among microbial communities, and understand the roles viruses play in nutrient cycling and the control of bacterioplankton populations (Proctor and Fuhrman 1990; Suttle et al. 1994; Jiang and Paul 1995).

Bacterioplankton abundances are usually lower in oligotrophic Lake Superior than in other warm, nutrient-rich lakes (Hicks and Owen 1991). The abundance of bacteriophage in Lake Superior or the other Laurentian Great Lakes has not been estimated even though host bacteria in these lakes may be dense enough to support active viral populations.

The surface microlayer is a habitat enriched in organic molecules, mineral nutrients, and metals relative to subsurface water (Duce et al. 1972; Hardy 1982). These factors probably contribute directly to the higher bacterioplankton abundances found in the surface microlayers compared to subsurface water in Lake Superior (Crawford et al. 1982). The dense bacterial communities in surface microlayers may indicate that viral populations are also large in this habitat.

Temperate bacteriophage can cause lytic or lysogenic infections. Prophage in lysogenically infected bacteria may be induced into a destructive lytic cycle by many environmental factors, including UV light, temperature, or chemicals (Roberts and Roberts 1975; Barksdale and Ardan 1976). The dose of UV light reaching the earth's surface has increased because of ozone destruction above polar regions, with increases in the UVB region (290–320 nm) being the greatest (Crutzen 1992). UVB light causes the most damaging effects to DNA in natural environments.

Although questioned by one study (Wilcox and Fuhrman 1994), environmental factors like the expected increase in UV light incident on aquatic environments may cause the composition of bacterioplankton communities with a high percentage of temperate viruses to change by inducing prophage into lytic infections. Bacterial populations containing temperate prophage may be reduced or eliminated, allowing other bacterial populations that may have been minor parts of a community to increase (Ogunseitan et al. 1992). Bacterioplankton are an integral part of normally functioning aquatic systems. Changes in the abundance or composition of bacterial communities may influence entire aquatic ecosystems.

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The percentage of aquatic bacteria that contain temperate prophage has only recently been investigated (Wilcox and Fuhrman 1994; Jiang and Paul 1996; Weinbauer and Suttle 1996). Lysogeny has been studied in some bacterial isolates, but extrapolating these results to communities of aquatic bacteria may be inaccurate because only a small percentage of bacteria in aquatic environments have been cultured. The extent of lysogeny in bacterioplankton communities can be estimated by inducing temperate prophage into lytic cycles with UV light or the antibiotic, mitomycin-C.

This study investigates the morphology and abundance of free viruses in the western arm of Lake Superior. One goal was to compare the density of free viruses in the surface microlayer and subsurface water. The second goal was to induce prophage in natural bacterioplankton communities into lytic infections. The objective was to estimate the percentage of bacterioplankton that contain temperate bacteriophage in the surface microlayer and subsurface water of Lake Superior.

Materials and methods

Site description and sampling—Water samples were collected from one site in Lake Superior during 1993 when the water column was thermally stratified, and in the spring and fall when the water column was not thermally stratified. This site, LR-3, was ~3 km offshore from the Lester River (46°48.22′N, 91°58.18′W) near Duluth, Minnesota. Water from the surface microlayer and subsurface water (20 m) was taken at LR-3 on 2 June, 20 July, 24 August, and 6 October. Water temperature measurements were made throughout the water column with a SeaBird CTD.

The surface microlayer (top 20 μm) was sampled with sterile Teflon sheets (15 × 15 cm; Kjelleberg et al. 1979; Schubauer-Berigan 1990). After placing the sheets on the surface of the lake, attached particles were rinsed with sterile water containing phenol red (0.00025%; Sigma Chemical, St. Louis) into a sterile 500-ml Nalgene container to form a single composite sample. A standard curve was calculated from known dilutions of lake water and phenol red to determine the dilution of surface microlayer samples. A negative control was created by performing the same procedure except that sterile Milli-Q water was substituted for lake water. Subsurface water (20 m) samples were collected with a 5-liter Niskin bottle (n = 1). Before sampling, sterile water was placed into the Niskin bottle and then emptied into a sterile container to serve as a negative control. Water samples were subsampled for viral and bacterial measurements and the remaining portions were used in induction experiments.

Viral morphology and abundance—Water samples for transmission electron microscopy (TEM) were fixed with glutaraldehyde (2% final concn; EM grade, Polyscience, Warrington, Pennsylvania) and stored at 4°C until analysis. Two portions of each sample (5–6 ml) were ultracentrifuged in a swinging bucket rotor (100,000 × g for 1 h, Beckman SW37) onto carbon-formvar coated 400-mesh Cu grids (Polyscience) to concentrate free viruses (Børsheim et al. 1990). Following centrifugation, the supernatant water was removed from each tube and the grids were air dried overnight. The grids were rehydrated for 15 min at 20°C with Milli-Q water (0.22-μm filtered). The specimens on the grids were stained with 2% uranyl acetate for 3 min and then washed for 5 min with Milli-Q water (Tandler 1990). Following air drying for 24 h, the specimens were viewed and counted with a Phillips 201 transmission electron microscope at 60 kV and 45,000× magnification. Although we did not measure virus losses during centrifugation, other researchers have demonstrated that 96% of phages in a sample can be recovered using this concentration technique (Maranger and Bird 1995).

Particles were classified as a virus if they stained with uranyl acetate, were the appropriate size (generally <200 nm in diameter), and if the head had a polyhedral shape (Bradley 1967). Particles with irregular or nonpolyhedral shapes were not counted as viruses. Filamentous viruses were also searched for in all samples. Infection by filamentous phage takes place through the pili of male bacteria and does not result in the lysis of their host (Johnson et al. 1993). Therefore, free filamentous viruses were not expected to be numerous. Viruses were divided into different categories by head size and the presence or absence of a tail. Viral dimensions were estimated from measurements of images on photographic negatives and corrected for magnification. Although calibrated microbeads were not used to confirm the size of the viral particles, the magnification of the TEM was calibrated prior to this study.

Only free viruses, those that were free in the water or attached to bacterioplankton, were counted. The abundance of free viruses was estimated by counting viral particles in 10–12 fields of four grid squares. Two grids were prepared and counted for each sample. All statistical comparisons were completed with the Statview SE+ Graphics statistical program (Abacus Concepts) ANOVAs were used to compare the data.

Bacterioplankton abundance—Water subsamples (n = 3, 10 ml each) were preserved with 37% formaldehyde (2% final concn; Sigma Chemical) and refrigerated until analysis (always within 5 d). Abundances of total bacterioplankton were determined by epifluorescence direct counts of DAPI-stained cells (Hobbie et al. 1977; Porter and Feig 1980). DAPI (final concn 10 μM) was added to 2 ml of each subsample and incubated at room temperature for 5 min. A water sample was then filtered onto a black polycarbonate membrane filter (25 mm, 0.22 μm; Poretics). The filters were placed on a microscope slide with a drop of nonfluorescing immersion oil (Cargille type A) and viewed with a Zeiss epifluorescence microscope (1,200× magnification). Two or three slides were prepared from each water sample. The number of bacteria in the negative control was subtracted from the number of bacteria in the lake samples to estimate the abundance of bacterioplankton.

Mitomycin-C treatment—Temperate bacteriophage were induced into a virulent state by adding mitomycin-C (final concn 1 μg ml^{-1}; Sigma Chemical) to duplicate 250-ml flasks containing 50 ml of water (Roberts and Roberts 1975). The flasks were incubated at 20°C on a shaker for the du-

Table 1. Morphology of free viruses observed in the surface microlayer and subsurface water from site LR-3 in the western arm of Lake Superior on 2 June, 20 July, 24 August, and 6 October 1993. Standard errors of the mean are shown in parentheses.

Head diam (nm)	Total viruses (%)	Tailed viruses (% of size class)	Nontailed viruses (% of size class)
<30	53(±1.4)	65	35
30–60	45(±0.4)	74	26
>60	2(±1.0)	100	0
Total	100	70	30

ration of the experiment. Subsamples were removed from the flasks at different times and fixed for bacterial and viral counts. Control flasks were prepared, each containing 50 ml of lake water and 25 μl of 0.22-μm Milli-Q water in place of the mitomycin-C solution. These control flasks were incubated and subsampled in the same manner as in the experimental flasks.

UV light treatment—UV light was also used to induce temperate phage into a virulent state. Water samples (50 ml) in duplicate 250-ml flasks were exposed to short-wavelength UV light (254 nm) for 30 s at a distance of 10 cm (energy output at sample surface was 24.5 ± 2.7 μW cm^{-2} s^{-1} between 245 and 335 nm; model UVG-54 mineralight lamp, 115 v, 60 Hz, 0.16 amp; UVP, San Gabriel, California). The flasks (n = 2) were gently and continuously shaken to ensure maximum exposure of the cells to UV light. Unexposed water samples in flasks were used as negative controls. The UV-treated flasks were incubated and subsampled in the same manner as the mitomycin-C–treated flasks.

Estimating burst size and the extent of lysogeny—The excess production of free viruses after treating water with mitomycin-C or UV light was used to estimate the average burst size of viruses infecting bacterioplankton and the percentage of bacterioplankton that contained temperate phage. Data taken 24 h after treatments were used to calculate these estimates.

Burst size is the number of viruses liberated from a bacterium as the result of a lytic infection (Hennes and Simon 1995). Viral burst sizes vary among different types of bacteria. Viral burst sizes were estimated from differences in viral and bacterial abundances in treated and control flasks:

$$\text{calculated burst size} = \frac{(\text{viruses}_{\text{treated}} - \text{viruses}_{\text{control}})}{(\text{bacteria}_{\text{control}} - \text{bacteria}_{\text{treated}})}.$$

Because calculated burst sizes were unrealistically small (*see discussion*), burst sizes of 20 and 120 viruses per bacterium were used as minimum and maximum estimates of burst size in further calculations (Hennes and Simon 1995). This resulted in two estimates of the bacteria containing temperate phage for each sample type and treatment:

Table 2. Viral and bacterial abundances and the virus-to-bacterium (VBR) ratio for water from site LR-3 in Lake Superior during 1993. Means are shown (n = 2–3) with standard errors in parentheses.

	Free viruses (10^6 ml^{-1})	Bacteria (10^6 ml^{-1})	VBR ratio
Surface microlayer (top 20 μm)			
2 Jun	2.78(±0.51)	5.20(±0.06)	0.53(±0.07)
20 Jul	9.24(±0.49)	18.27(±1.17)	0.51(±0.08)
24 Aug	0.69(±0.37)	1.65(±0.33)	0.42(±0.15)
6 Oct	1.65(±0.24)	9.15(±3.29)	0.18(±0.10)
Subsurface water (20 m)			
2 Jun	0.18(±0.01)	2.23(±0.14)	0.37(±0.04)
20 Jul	0.86(±0.10)	1.19(±0.61)	0.72(±0.09)
24 Aug	0.32(±0.07)	1.74(±0.04)	0.16(±0.05)
6 Oct	0.15(±0.07)	4.61(±0.68)	0.03(±0.01)

$$\text{bacteria containing temperate phage (\%)} = \frac{[(\text{viruses}_{\text{treated}} - \text{viruses}_{\text{control}})/\text{burst size}]}{\text{bacterial abundance}_{\text{initial}}} \times 100$$

Results

Description of water column at site LR-3—The water column at LR-3 was isothermal when sampled in June. The temperature at 1 m was nearly 7°C and the subsurface water was 5°C. The lake was thermally stratified when sampled in July and August. In July, the water temperature was 13°C at 1 m and 8°C at 20 m, with the thermocline between 15 and 17 m. The temperature at 1 m was 19°C and the subsurface was 10°C, with the thermocline between 12 and 15 m in August. The lake was again isothermal (6°C) in October.

Morphology of free viral particles—Many different morphological types of free viruses were seen in samples from surface microlayer and the subsurface water of Lake Superior. Viral head sizes ranged from 10 to 70 nm and tail sizes ranged from 10 to 110 nm. Tailed viruses accounted for 70% of the total free viruses observed. The majority of viruses had a head size <60 nm and were tailed (Table 1). Not enough photographic measurements were available to accurately determine if viral sizes or morphologies varied seasonally or were statistically different in the surface microlayer and subsurface waters. No free filamentous or intracellular viruses were observed.

Abundance of free viruses and bacterioplankton in the surface microlayer and subsurface water—The abundance of free viruses in the surface microlayer ranged from 0.7 to 9.2 × 10^6 virus ml^{-1} (Table 2). Free viruses were most abundant in July and least abundant in August and October (P < 0.05). Bacterioplankton were also more abundant in the surface microlayer during July but were least abundant in August (P < 0.05). Bacterioplankton were more abundant than free viruses in the surface microlayer on all dates (P < 0.05).

Table 3. Enrichment of free viruses and bacterioplankton in the surface microlayer compared to subsurface water at site LR-3 in the western arm of Lake Superior in 1993. Means are shown (n = 2–3) with standard errors in parentheses.

	Surface microlayer-to-subsurface ratio	
	Free viruses	Bacterioplankton
2 Jun	3.4(±0.6)	2.3(±0.1)
20 Jul	10.8(±0.8)	15.3(±1.0)
24 Aug	2.1(±0.8)	1.0(±0.1)
6 Oct	15.1(±3.5)	2.0(±0.5)

The abundance of free viruses in subsurface water ranged from 0.1 to 0.9 × 10^6 viruses ml^{-1} (Table 2). Free viruses were most abundant in June and July and least abundant in October ($P < 0.05$). As with the surface microlayer, bacterioplankton were more abundant than free viruses in subsurface water ($P < 0.05$). Bacterial abundance ranged from 1.2 to 4.6 × 10^6 cells ml^{-1} in subsurface water (Table 2). Bacterioplankton were most abundant in the subsurface water during October and least abundant in July ($P < 0.05$).

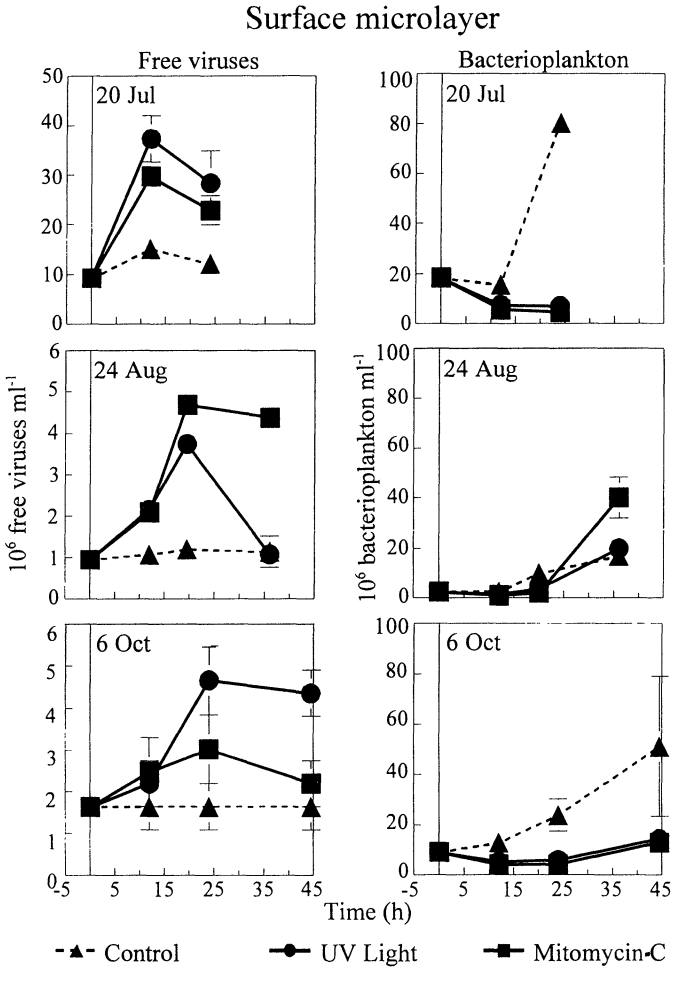

Fig. 1. Changes in viral and bacterioplankton abundances in surface microlayer water following treatments with mitomycin-C and UV light. Error bars are the standard error of the mean (n = 2).

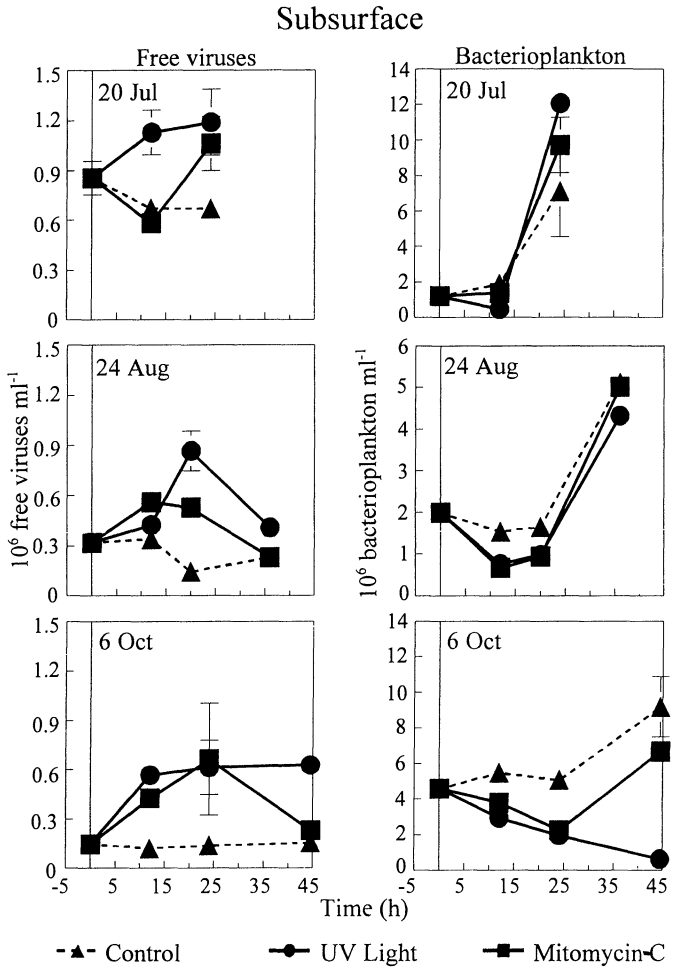

Fig. 2. Changes in viral and bacterioplankton abundances in subsurface water following treatment with mitomycin-C and UV light. Error bars are the standard error of the mean (n = 2).

The virus-to-bacterium ratio (VBR) for all surface microlayer and subsurface samples from site LR-3 was <1 (Table 2). This ratio was higher in the spring and early summer than in the fall at both depths ($P < 0.05$). The VBR was higher in the surface microlayer than in the subsurface water, except during July ($P < 0.05$). Free viruses and bacteria were more abundant in the surface microlayer than in the subsurface water ($P < 0.05$; Table 3) with the exception of August, when bacterioplankton abundances were similar ($P \geq 0.05$). This surface microlayer enrichment ranged from 2 to 15 for both free viruses and bacteria (Table 3).

Effect of mitomycin-C and UV light on free viral and bacterial abundances—Viral abundances increased relative to the controls in all surface microlayer and subsurface water samples following treatment with mitomycin-C or UV light (Figs. 1, 2). The maximum change in viral abundance was similar in the mitomycin-C and UV light treatments. However, the change in viral abundance following either treatment fluctuated from month to month in the surface micro-

Table 4. Maximum percentage change in free viral and bacterioplankton abundances following treatment with mitomycin-C (MC) and UV light (UV). Controls were not treated and were used to estimate the influence of confinement on viral and bacterial abundances. The greatest change between initial and post-treatment abundance was used to calculate the percentage change. Mean percentages from two replicate flasks are shown with standard errors in parentheses. Both treatments were always different from the control ($P < 0.05$).

	Free viruses (% change)						Bacterioplankton (% change)					
	Surface microlayer			Subsurface			Surface microlayer			Subsurface		
	Control	MC	UV	Control	MC	UV	Control	MC	UV	Control	MC	UV
20 Jul	64.2	222.1	304.2	−21.7	23.9	38.8	340.2	−74.9	−61.7	59.2	15.4	−60.6
	(±6.6)	(±32.9)	(±14.8)	(±2.6)	(±9.0)	(±12.9)	(±8.0)	(±15.0)	(±9.0)	(±18.9)	(±22.5)	(±1.0)
24 Aug	26.7	393.1	293.1	−57.8	64.9	170.9	2.5	−59.9	−43.8	−17.2	−52.7	−50.7
	(±4.2)	(±54.3)	(±88.1)	(±17.8)	(±14.7)	(±33.3)	(±0.0)	(±2.9)	(±10.1)	(±0.7)	(±2.5)	(±14.9)
6 Oct	0.3	83.6	183.6	−6.2	480.7	463.3	161.0	−54.6	−34.6	10.5	−51.1	−57.3
	(±0.1)	(±13.6)	(±50.0)	(±1.2)	(±44.0)	(±92.7)	(±42.9)	(±4.7)	(±1.1)	(±0.4)	(±2.4)	(±2.1)

layer and subsurface water (Table 4). The largest increases in viral abundance and often the largest decreases in bacterial abundance occurred 24 h after treatment, except in July. In July, the largest viral increase in treated surface microlayer water compared to control water occurred 12 h after treatment (Fig. 1). Similarly, the largest decrease in bacterial abundance in the treated subsurface water compared to the control occurred at 12 h in July (Fig. 2).

Bacterial abundance decreased in treated water compared to the controls concurrently with increases in free viral numbers. Bacterioplankton appeared to respond equally to both inducing agents (Table 4). Mitomycin-C or UV light caused bacterial numbers to decrease in both the surface microlayer and subsurface water compared to the controls (Table 4).

Average viral burst sizes calculated from changes in free viral and bacterioplankton abundances in the experimental treatments were extremely small (Table 5). Small percentages of bacteria in the surface microlayer and subsurface water were estimated to contain temperate prophage (Table 6). This percentage ranged from 0.1 to 7.4% in the surface microlayer and from 0.1 to 1.8% in subsurface water depending on the viral burst size that was assumed. A slightly larger percentage of bacteria taken in July and August appeared to contain temperate prophage than in October. At least three times more bacteria in the surface microlayer were estimated to contain temperate prophage compared to subsurface water at site LR-3 in Lake Superior.

Table 5. Burst size of viruses from site LR-3 in Lake Superior during 1993 calculated from measured changes in viral and bacterial abundances following exposure to mitomycin-C or UV light.

	Burst size (viruses bacterium^{-1})	
	Mitomycin-C	UV light
Surface microlayer (top 20 μm)		
20 Jul	1.5	2.7
24 Aug	0.4	0.4
6 Oct	0.1	0.2
Subsurface water (20 m)		
20 Jul	0.3	1.0
24 Aug	0.5	1.1
6 Oct	0.2	0.2

Discussion

Viral morphology and abundance—Free viruses found in Lake Superior were slightly smaller than viruses observed in other lakes and marine environments. In the western arm of Lake Superior, 53% of the free viruses had heads <30 nm in size, which did not vary much during the study (Table 1). In other environments, 50–95% of the viruses were >30 nm (Mathias et al. 1995; Wommack et al. 1992; Weinbauer et al. 1993).

Only bacteriophages are known to have complex (i.e. tailed) morphology, so when tailed viruses with polyhedral heads were observed they were assumed to be bacteriophage (Bradley 1967; Torrella and Morita 1979; Wommack et al. 1992). Therefore, at least 70% of the viruses enumerated in Lake Superior water were bacteriophages. There are a number of possible origins for the remaining nontailed, free viruses. They may have been nontailed bacteriophage or tailed bacteriophage that lost their tails during ultracentrifugation. Alternatively, they may have been viruses from eukaryotic organisms including algae, zooplankton, fish, other animals, or humans. The nontailed viruses may have also been bacteriocins. The production of some bacteriocins is induced by UV light (Patterson 1965).

The majority of viruses (57%) were nontailed in one Chesapeake Bay study (Wommack et al. 1992). Two hy-

Table 6. Percentages of bacterioplankton containing temperate prophage in the surface microlayer and subsurface water. All samples were collected during 1993 from site LR-3 in the western arm of Lake Superior. Percentages were estimated for bacteria using two inducing agents, mitomycin-C and UV light. The minimum and maximum burst sizes used in the calculations were 20 and 120, respectively (*see* equation in text).

	Mitomycin-C				UV Light			
	Surface microlayer		Subsurface		Surface microlayer		Subsurface	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
20 Jul	0.6	3.8	0.2	1.0	1.0	5.8	0.2	1.3
24 Aug	1.2	7.4	0.2	1.0	1.0	5.4	0.3	1.8
6 Oct	0.1	0.8	0.1	0.5	0.3	1.7	0.1	0.5

potheses may explain the higher percentage of nontailed viruses in Chesapeake Bay compared to Lake Superior—viruses of nonbacterial origin or a higher susceptibility of bacterioplankton to nontailed viruses. The higher percentage of nontailed viruses in Chesapeake Bay may be caused by the presence of more eukaryotic viruses from algae, fish, agricultural runoff, or human waste (Wommack et al. 1992; Puig et al. 1994). Alternatively, bacterial populations in Chesapeake Bay may be more susceptible to nontailed viruses while bacterioplankton in Lake Superior may more be susceptible to tailed phages. The different percentages of tailed viruses observed from these aquatic habitats may also indicate the presence of different bacterial populations in these aquatic environments.

Free viral abundances in subsurface water at the Lake Superior site were lower than viral numbers reported for most aquatic environments (Maranger and Bird 1995). Unlike Lake Superior, viruses were usually more abundant than bacterioplankton in the other aquatic habitats (Table 2). The VBR ratio has been reported to range from <2 to >100 in aquatic habitats, usually being larger in freshwater than in marine environments (Maranger and Bird 1995). The low free viral abundances measured in the Lake Superior samples ultimately led to the low VBRs that were calculated, considering that bacterioplankton abundances measured in Lake Superior were similar to bacterial abundances reported for other aquatic habitats.

Methodological, habitat, and microbial community differences between this and other studies could all explain why fewer viruses were observed in the subsurface water of Lake Superior than at other sites. While many previous studies counted both free and intracellular viruses, only free viruses were counted in the Lake Superior samples. Warm temperatures and high nutrient concentrations can cause the VBR to increase (Lenski and Levin 1985; Ogunseitan et al. 1990; Caldas et al. 1992). Encounter rates between host cells and viruses, differences in viral burst sizes, as well as differences in host cell abundances in various aquatic habitats could also influence the number of free viruses observed. We cannot conclude which of these factors contributed to the low viral abundances observed in Lake Superior because the sources of variability in viral numbers were not investigated.

Free viruses were 2–15 times more abundant in the surface microlayer than in subsurface water of Lake Superior (Table 3). While Crawford et al. (1982) demonstrated that the surface microlayers of many lakes including Lake Superior are enriched with bacteria, no one has shown that it is also enriched with free viruses. The VBR was usually higher in the surface microlayer compared to subsurface water (Table 2). These observations may be explained by two factors. First, bacterial enrichment in the surface microlayer may provide more potential host cells for viruses to infect. Second, high levels of UV light incident on the surface microlayer may induce temperate prophage into their lytic cycles, resulting in the production and liberation of progeny viruses.

Lysogeny—Bacterioplankton communities in the western arm of Lake Superior seem to harbor lysogenic bacteriophage. In both the surface microlayer and subsurface waters, prophage responded to both the mitomycin-C and UV light treatments (Figs. 1, 2). Free viral numbers increased and bacterial abundances concurrently decreased relative to the control treatments (Table 4). There seemed to be little difference in the response of temperate prophage and bacterioplankton to these two inducing agents (Table 4).

Unlike mitomycin-C, UV light is an environmentally relevant inducer of temperate viruses. The type of UV light used in this study (UVC, 254 nm) was probably less environmentally relevant than UVB because most UVC is absorbed in the atmosphere before reaching the earth's surface (Smith et al. 1992). UVC light has the most damaging effects on DNA of all the types of UV radiation. This spectrum of UV light was chosen to help ensure that DNA repair mechanisms would be activated and temperate prophage would be induced into lytic cycles. UVB light, however, has been shown to damage bacterial and algal DNA in natural environments, suggesting that it may also induce temperate prophage (Smith et al. 1992; Bothwell et al. 1994).

It is possible that all the bacterioplankton mortality relative to the controls was not due to viral lysis. Some bacterial cell mortality could have been directly due to the UV exposure. The mitomycin-C concentration used was known to induce temperate lambda phage but have a small direct effect on its host bacterium, *Escherichia coli* (Roberts and Roberts 1975). However, this mitomycin-C concentration may have a larger influence on different bacterial species. Even if the UV light or mitomycin-C treatments did not cause the death of bacterial cells but only reduced bacterioplankton productivity relative to the controls, then the decreases in bacterial abundances observed during these experiments may not have been due to viral lysis alone.

Although these hypotheses were not examined, the possibility of lethal or sublethal effects of the treatments on bacteria in these experiments cannot be disregarded. These type of treatment effects may have been responsible for the small viral burst sizes that were calculated from the experimental data (Table 5). Similarly, using the change in the abundance of bacterial cells in a treated flask compared to the control would overestimate the percentage of cells containing lysogenic prophage because some of the change in bacterial abundance would be due to a direct treatment effect on cells and not to cell loss due to viral lysis.

The induction experiments did indicate that a higher percentages of bacterioplankton were lysogenically infected in the surface microlayer than in the subsurface water at the Lake Superior site (Table 6). This result was contrary to our original hypothesis that a lower percentage of bacterioplankton from the surface microlayer would contain temperate prophage because of more constant induction by UV light in this habitat relative to subsurface waters. Two assumptions were made to estimate the percentage of bacteria that contained lysogenic prophage. First, we assumed that all temperate prophage were induced into lytic cycles by mitomycin-C and UV light. This assumption could not be verified, although similar induction by mitomycin-C and UV light suggests that a large majority of the prophage were induced (Table 4).

Perhaps a more critical assumption was the viral burst size chosen to estimate the percentage of cells that were infected

with temperate phage. The calculated burst sizes were much smaller than the range reported for most viruses (Hennes and Simon 1995). A minimum burst size of 20 and a maximum of 120 viruses released per lysed cell were chosen to estimate the percentage of bacterioplankton infected with temperate phage (Hennes and Simon 1995). This range is more likely to bracket the burst sizes of lysogenically infected bacterioplankton than the calculated burst sizes. This approach of using burst sizes reported in the literature resulted in more conservative estimates of the percentage of bacterioplankton that are lysogenically infected. There also may have been a difference between the burst sizes of surface microlayer and subsurface viruses. However, the true burst sizes of surface microlayer viruses would have to be at least 3 times higher on average than subsurface viruses for the percentage of lysogenically infected bacterioplankton in subsurface water to be as great as the percentage in the surface microlayer (Table 6).

Lysogeny is thought to be a strategy for survival of bacteriophage in harsh environments (Lenski and Levin 1985). The surface microlayer may be a more stressful environment than subsurface habitats in Lake Superior (Maki 1993). The surface microlayer is certainly less stable, where temperature, light intensity, and water turbulence usually fluctuate more than in subsurface water. The composition of bacterial communities in this habitat may be different from the composition of subsurface bacterioplankton and result in a larger diversity of potential host cells available to viruses capable of lysogenic infection (Fehon and Oliver 1979; Carlucci et al. 1985; Hermansson et al. 1987).

Lysogeny is also though to be a strategy for survival of bacteriophage in environments with low host cell abundances (Lenski and Levin 1985). Upon initial inspection, the results from this study do not seem to support this concept. Both total bacterial abundance and the estimated percentage of bacterioplankton containing temperate prophage were higher in the surface microlayer compared to subsurface water. However, the percentage of viruses in the surface microlayer that retain their infectivity while being continuously exposed to sunlight is unknown. The loss of viral infectivity in the surface microlayer may favor lysogeny in this habitat compared to subsurface waters.

Ultimately, bacterial abundances usually increased in both the control and treated flasks during the lytic induction experiments (Figs. 1, 2). Bacterial numbers often increase after being confined in bottles (Ferguson et al. 1984). This finding would help explain the increases in bacterial abundance observed in the control flasks. Occasionally, however, bacterial abundances in treated samples increased to densities equal to or greater than the controls, especially later in these experiments. Although unknown for these experiments, UV light or mitomycin-C may also damage heterotrophic nanoflagellates and thereby reduce predation pressure on bacterial populations. Reduced predation of bacteria may have allowed bacterioplankton to flourish in the treated flasks.

Dissolved organic matter (DOM) released from cells lysed by lytic viruses can support new bacterial production, which may explain increases in bacterial abundance observed in some investigations (Proctor and Fuhrman 1991; Thingstad et al. 1993). However, it is less likely that DOM released from bacteria lysed by temperate phage in the treated flasks significantly influenced bacterial production and led to the larger bacterial abundances compared to the control flasks. Even though the amount of DOM released from bacteria lysed by temperate phage in this experiment was not measured, an estimate can be made using marine bacteria as a model.

Assume there are 5×10^6 bacteria ml^{-1} (Table 2) and that a typical bacterium contains 2.5 fg DNA, 2.9 fg RNA, and 14.8 fg protein (Proctor and Fuhrman 1991). If 0.1–7.4% of the bacterioplankton cells contained temperate phage (Table 5), then 0.1–7.5 ng DOM ml^{-1} in the form of nucleic acids and protein would have been liberated when the lysogenically infected cells were lysed. Approximately 0.05–3.8 ng ml^{-1} of dissolved organic carbon (DOC) would have been released assuming that these compounds were 50% carbon by weight. DOC concentrations at site LR-3 are near 1.6 μg C ml^{-1} (unpubl. data). If all the bacterioplankton estimated to be infected by temperate phage lysed simultaneously, then the DOC concentration would have only increased 0.003–0.24%.

This relatively small quantity of additional DOC probably had a limited effect on bacterial production. By similar logic, DOC released by phytoplankton probably has a larger influence on bacterial production in Lake Superior compared to the DOC that would be released if all lysogenically infected bacterioplankton were lysed simultaneously. In Lake Superior's surface microlayer, bacterioplankton infected by temperate phage may be more important for the survival of bacteriophage populations than as carbon sources for new microbial production.

Low temperatures, oligotrophic conditions, the large size, and relatively small human influences make Lake Superior a unique aquatic environment. These characteristics may have indirectly contributed to the unique viral communities that were defined by small viral sizes, high percentages of tailed viruses, and relatively low abundances of free viruses. Similar to bacteria in Lake Superior and other aquatic environments, free viruses at this single site in Lake Superior were more abundant in the surface microlayer than in subsurface water. Few bacterioplankton in both the surface microlayer and subsurface water appeared to harbor temperate phage. The DOM that can be released from bacterioplankton in this area of Lake Superior by inducing temperate prophage is apparently small relative to the amounts of DOM released by phytoplankton and lytic viral infections. Lysogeny is probably more important as a strategy for viruses in Lake Superior to survive periods of low host availability in harsh environments.

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