

Abundance of Planktonic Virus-Like Particles in Lake Erie Subsurface Waters¹

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ABSTRACT. Abundance of virus-like particles (VLP) was determined in Lake Erie subsurface water. The relationship between VLP and the bacterial and phytoplankton communities were investigated. Viral and bacterial numbers were determined using nucleic acid stains and epifluorescent microscopy. Phytoplankton abundance was estimated by chlorophyll *a* extraction. Viral abundance averaged 1.05×10^6 VLP/ml and the ratio of viral to bacterial number was less than 1.0 across most sampling sites and dates. Viral abundance was not correlated with either bacterial abundance or chlorophyll *a* concentration. Viral abundance was found to be most similar to other Great Lakes and marine systems and dissimilar to other freshwater systems.

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INTRODUCTION

Traditionally, studies of viruses in natural environments have focused on human pathogens and public health. Increased emphasis has been placed recently on the role of viruses in the environment and their relationships to aquatic organisms and nutrient cycling. A majority of these recent ecological studies have focused on marine and estuarine systems; very little information is available on large freshwater lentic systems (Maranger and Bird 1995). Although high abundance of viruses in aquatic ecosystems have been reported (for example, Bergh and others 1989, Cochlan and others 1993, Weinbauer and others 1995, Maranger and Bird 1995, Steward and others 1996, Hara and others, 1996, Lemke and others 1997), the role of viruses in carbon and nutrient dynamics of aquatic ecosystems remains unclear. Several studies have suggested that viruses control bacterial production and phytoplankton populations thus affecting the microbial community structure and microbial loop (for example, Bratbak and others 1994, Suttle 1994, Hennes and others 1995). Other studies have attempted to uncover the ecological significance of viruses by investigating the relationship between viral abundance and selected biotic variables such as bacterial abundance and/or chlorophyll concentrations (Cochlan and others 1993, Weinbauer and others 1995, Hara and others 1996).

In this study, planktonic virus-like particles (VLP) were enumerated in Lake Erie using a relatively new method that provides accurate VLP abundance estimates (Hennes and Suttle 1995). The purpose of this study was to determine the abundance of viruses in Lake Erie pelagic waters and to compare these numbers to bacterial abundance and chlorophyll *a* concentrations.

MATERIALS AND METHODS

Lake Erie water was sampled from sites in the western basin on three dates; June, July and August 1997 (10 sites total, Table 1). At each site water samples were collected in triplicate at 3–4m depths using a 2.5 liter Kemmerer sampler, and processed, as described below, for number of VLP, total bacterial number, and chlorophyll *a* concentration. The relationships among viral abundance, bacterial number and chlorophyll *a* concentration were analyzed using linear regression.

VLP Samples: From each sample, 10 ml of water was filtered through a 0.2 μm low protein-binding polycarbonate syringe filter (Syrfil-mf, Costar Corp.) to remove bacteria and placed into sterile glass vials. Samples were kept on ice until further processing in the lab. Samples were processed in the manner described by Hennes and Suttle (1995); the accuracy of this method was confirmed by Weinbauer and Suttle (1997). Briefly, one ml of each sample was treated with 250 K units/ml DNase (to destroy free DNA) and filtered onto 0.02 μm Anodisc Al_2O_3 filters (Whatman). Filters were stained using the cyanine-based nucleic acid stain, Yo-Pro-1 (Molecular Probes) and incubated in the dark at room temperature for two days. Filters were then rinsed twice with deionized water and mounted in spectrophotometer-grade glycerol on glass slides. Virus-like particles were enumerated using epifluorescent microscopy. Twenty randomly selected fields per slide were viewed at 1,000X magnification.

Bacteria Samples: For each replicate, 10 ml of water was placed in a sterile plastic vial and preserved with 0.2 ml formaldehyde. Samples were kept on ice until further processing. To enumerate bacteria, 0.5 ml of sample was stained with 0.1 ml 15 $\mu\text{g/ml}$ DAPI (4',6'-diamidino-2-phenylindole) for three minutes, filtered onto 0.2 μm black polycarbonate filters (Poretics), and observed using epifluorescent microscopy (Porter and Feig, 1980). Bacteria in ten randomly selected fields were enumerated per slide at 1,000X magnification.

Chlorophyll *a* Concentration: Chlorophyll *a* concentrations were analyzed from each replicate sample

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TABLE 1

Lake Erie virus data collected summer 1997. Numbers in parentheses indicate standard error. Distance to shore indicates the closest distance to a mainland shoreline.

Date	Site	Location (Long. - Lat.)	Distance to Shore (km)	Viral Abundance (10 ⁶ particles/ml)	Bacterial Abundance (10 ⁶ cells/ml)	Virus:Bacteria Ratio	Chlorophyll <i>a</i> Concentration (ug/l)
June	1	41°47.5' - 83°05.1'	18.7	0.16 (0.05)	1.79 (0.06)	0.09	5.6 (0.0)
	2	41°50.0' - 83°00.0'	17.5	0.27 (0.01)	2.72 (0.31)	0.01	6.6 (0.2)
	3	41°39.8' - 82°49.3'	0.2	0.60 (0.15)	2.63 (0.28)	0.23	9.5 (0.6)
	4	41°39.0' - 83°01.0'	7.5	0.13 (0.01)	3.62 (0.82)	0.03	7.2 (0.7)
July	5	41°40.3' - 83°06.0'	7.0	1.12 (0.24)	1.76 (0.16)	0.64	2.0 (0.0)
	6	41°50.0' - 83°06.0'	23.0	0.53 (0.25)	1.39 (0.06)	0.38	17.0 (1.6)
	7	41°52.4' - 82°55.0'	12.0	0.46 (0.08)	2.14 (0.28)	0.21	4.5 (0.3)
	8	41°49.1' - 82°51.4'	20.0	0.92 (0.35)	2.51 (0.20)	0.37	6.3 (0.8)
August	9	41°42.0' - 82°41.3'	17.0	2.87 (0.14)	1.94 (0.13)	1.48	1.2 (0.0)
	10	41°38.2' - 82°40.7'	9.0	3.44 (0.72)	5.18 (0.47)	0.66	2.2 (0.2)

using Standard EPA Method 445.0 and served as an indicator of phytoplankton biomass. This method utilizes an acetone extraction procedure and fluorometric analysis (USEPA 1992).

RESULTS

The number of VLP detected in Lake Erie averaged 1.05×10^6 /ml across all sites and dates and ranged from 1.28×10^5 /ml to 3.44×10^6 /ml (Table 1). VLP abundances were generally highest in late August and there was considerable variation among replicates.

The number of bacteria was consistently greater than the number of VLP, averaging 2.57×10^6 /ml. There were no consistent temporal differences in bacterial abundance and on certain dates there were marked differences among sites. The VLP:bacteria ratio (VBR) for each site was <1.0 with one exception (site nine; Table 1). There was no significant linear relationship between VLP number and bacterial abundance ($r^2 = 0.222$).

Chlorophyll *a* concentrations also varied among sites and exhibited a seasonal trend opposite of that observed for VLP abundances. In spite of this seasonal pattern, there was no significant linear relationship between VLP abundance and chlorophyll concentration ($r^2 = 0.286$).

Sites that were nearest to shore tended to have the highest VLP abundance (Table 1). In contrast, bacterial numbers did not exhibit this same pattern.

DISCUSSION

The number of VLP detected in Lake Erie was two orders of magnitude lower than in most freshwater

systems. In other freshwater systems viral numbers averaged 1.1×10^8 per ml with a VBR of 20-25 (reviewed by Maranger and Bird 1995). VLP abundance in Lake Erie pelagic waters more closely resembled those in marine systems and other large scale freshwater lakes. Viral number in marine systems averaged 1.7×10^7 VLP/ml with some studies reporting abundances as low as 6.7×10^4 VLP/ml (reviewed by Maranger and Bird 1995). In Lake Superior, viral abundance in surface and subsurface waters were similar to those reported here for Lake Erie and ranged from 0.1×10^6 to 9×10^6 VLP/ml (Tapper and Hicks 1998).

Likewise, the VBR in some marine systems was less than 1.0, with an average ratio of 1-5 (Maranger and Bird 1995). In this study, the VBR ratio in Lake Erie was generally less than 1.0; similar ratios were reported for Lake Superior (Tapper and Hicks 1998). Similarities in VLP number and the ratio of virus to bacteria abundance between Lake Erie, Lake Superior, and oceanic-scale systems suggests that similar physical characteristics of these systems, such as littoral:pelagic ratios, may influence viral numbers in these systems, regardless of whether they are marine or freshwater.

One way researchers have sought to explain differences in viral abundance among sites is through correlation with other biotic variables. In this study, no relationship between viral number and either bacterial abundance or chlorophyll concentration was observed. In contrast, other researchers have reported such relationships. In oceanic and estuarine waters, viral abundance was correlated with bacterial numbers

(Boehme and others 1993, Cochlan and others 1993) while in freshwater lakes viral abundance was correlated with chlorophyll *a* concentration (Maranger and Bird 1995), suggesting that planktonic viruses may be derived from bacterio- or phytoplankton. In Lake Erie, the lack of relationship between VLP numbers and bacterio- and phytoplankton, coupled with the low VLP abundances, suggests that in oceanic-scale ecosystems other factors may control virus abundance.

One possibility that warrants further study, is that the primary source of generation of VLP may be the benthos. The discovery of large viral abundances in benthic freshwater habitats (Lemke and others 1997) supports the possibility that in systems where the benthos plays an important role in microbial processes, viral abundances may be higher than in systems such as Lake Erie. In addition, differences in viral decay rates, proportion of lytic versus temperate phage, rates and sources of bacterial and algal mortality, and other sources of virus generation (such as macroscopic plants and animals) may help explain differences among systems and warrant further investigation.

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