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Marine and Freshwater Cyanophages in a Laurentian Great Lake: Evidence from Infectivity Assays and Molecular Analyses of g20 Genes

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While it is well established that viruses play an important role in the structure of marine microbial food webs, few studies have directly addressed their role in large lake systems. As part of an ongoing study of the microbial ecology of Lake Erie, we have examined the distribution and diversity of viruses in this system. One surprising result has been the pervasive distribution of cyanophages that infect the marine cyanobacterial isolate *Synechococcus* sp. strain WH7803. Viruses that lytically infect this cyanobacterium were identified throughout the western basin of Lake Erie, as well as in locations within the central and eastern basins. Analyses of the gene encoding the g20 viral capsid assembly protein (a conservative phylogenetic marker for the cyanophage) indicate that these viruses, as well as amplicons from natural populations and the ballast of commercial ships, are related to marine cyanophages but in some cases form a unique clade, leaving questions concerning the native hosts of these viruses. The results suggest that cyanophages may be as important in freshwater systems as they are known to be in marine systems.

The distribution of viruses in marine systems and their potential impact on biogeochemical cycles has been well documented during the last 15 years (12, 45, 48). Moreover, research suggests that viruses play a role in the regulation of both microbial community structure and the transfer of genetic information among microbes (13, 20, 35). Although freshwater environments are more important than marine systems in terms of their influence on human activities, microbial communities (including viruses) in freshwater environments have received surprisingly little attention (9) relative to their marine counterparts. Invasive species, on the other hand, have received considerable attention in the Laurentian Great Lakes due to their potential impact on these waterways: e.g., the zebra mussel (*Dreissena polymorpha*) (28) and the spiny water flea (*Bythotrephes cederstroemi*) (32, 33). These two organisms, among others (23), are thought to have been introduced into the Great Lakes via ships' ballast water. Commercial shipping has been implicated as a possible dispersal mechanism in many nonnative introductions (29).

Cyanobacteria and cyanophages have been isolated from a variety of freshwater systems. Early work focused on cyanophages infecting the LPP (*Lyngbya-Plectonema-Phormidium*) group of cyanobacteria, in part due to the ease with which these viruses could be isolated (25, 30). Studies have also examined viruses of other cyanobacteria, including those that infect the freshwater organism *Microcystis* sp. (3, 4, 11, 19, 22). Early interest concerned the use of lytic viruses to control cyanobacterial blooms; the study of freshwater cyanophages waned when it became apparent that they would not be useful biological control agents (36). These studies concluded that coexistence of the cyanophages and their hosts occurs through oscillations in resistance and sensitivity of the populations to infection, a function of geochemistry and the mutation rates of the hosts and viruses (2). Similar conclusions have been drawn concerning virus-host co-occurrence in marine systems (40).

Since these early studies, insight into aquatic virus ecology has grown significantly as molecular methods have been adopted. While viruses infecting freshwater cyanobacteria can be divided into three major groups (the LPP group; the A, AN, N, and NP group; and the AS and AM group), the nomenclature of members of these virus groups is based entirely on the taxonomy of the host organisms. As noted by Suttle (36), this does not reflect any genetic relationship among these viruses. Currently, cyanophages across all habitats are classified into three major groups: *Cyanomyoviridae*, *Cyanopodoviridae*, and *Cyanostyloviridae*. These groupings are based on the morphologies and structures of the viruses, which are thought to imply some degree of genetic relatedness (36).

Perhaps the best-studied viruses in aquatic systems are the cyanophages that infect cyanobacteria of the genus *Synechococcus*. Although it has been only a decade since their discovery, these cyanophages have been intensively studied to evaluate their impact on cyanobacterial communities (20). Recent advances are primarily due to the availability of molecular markers (the g20 gene fragment) that can be employed to detect cyanomyoviridae in natural systems (47, 49) by PCR. During the last several years a number of researchers have employed these tools to look for samples in marine (31, 47, 49), brackish (21), and, most recently, freshwater (9) systems.

During the process of characterizing the activity of viruses in one of the Laurentian Great Lakes (Lake Erie), we discovered that populations of viruses that infect a marine cyanobacterium (*Synechococcus* sp. strain WH7803) were well

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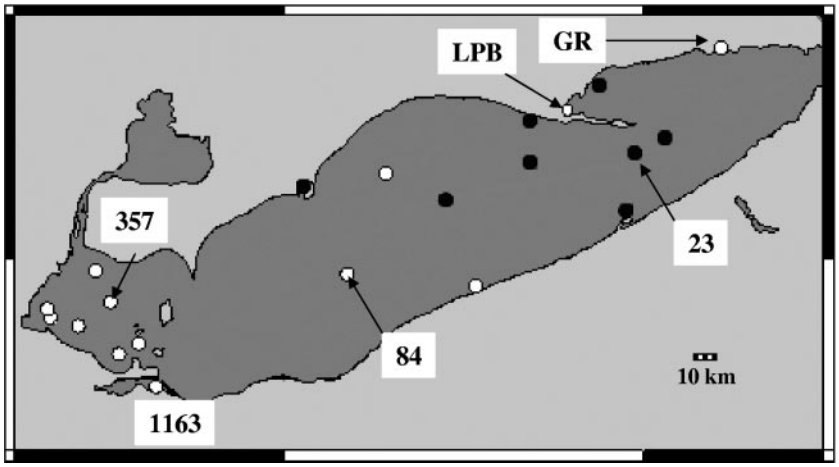


FIG. 1. Map of Lake Erie indicating stations sampled for this study. Black circles represent stations where no lytic activity was observed for *Synechococcus* sp. strain WH7803; white circles represent stations where lytic activity was detected. Stations discussed in more detail are labeled numerically (Environment Canada station coding) or with acronyms: GR, station located within the mouth of the Grand River; LPB, station located within Long Point Bay.

distributed throughout this lake. Ironically, phages capable of infecting this marine cyanobacterium are pervasive throughout the western basin of this lake and can also be found in areas of the central and eastern basin, even though the host cyanobacterium cannot persist in freshwater culture medium and no other hosts have been found. Sequence analyses of viral g20 genes amplified from isolated viruses, as well as DNA extracted from virus concentrates of whole lake water and ships' ballast water, demonstrate that delineations between samples originating in freshwater and marine environments may be possible.

MATERIALS AND METHODS

Site description and sampling. Sampling of Lake Erie surface waters was conducted during several research cruises aboard the *C.C.G.S. Limnos*. The locations of the 23 sampling stations are shown in Fig. 1. Surface water (5 to 10 m) was collected with a trace metal-clean pumping system comprising a Teflon double-diaphragm pneumatic pump (Husky 307; McMaster-Carr) and PFA Teflon tubing deployed off the port side of the ship. Water was pumped directly into an on-deck Class-100 clean room facility. The system was allowed to flush for 30 to 60 min at each station prior to water collection.

Water samples were also collected from a ship's ballast tank in the Ports of Hamilton and Windsor, in Canada, and Burns Harbor and Thunder Bay, in the United States, as the vessel transited the Great Lakes. Ballast water was collected at numerous locations within the tank with a Van Dorn sampling bottle released approximately 1 m below the surface. Water was pooled before subsampling.

Chlorophyll *a* determinations. As a proxy for algal biomass, chlorophyll *a* levels were determined from parallel filtration of samples collected on 0.2-μm polycarbonate filters (47-mm diameter; Millipore) after extraction (ca. 24 h, 4°C) in 90% acetone. Chlorophyll *a* retained on the different size class filters was quantified with a Turner Designs TD-700 fluorometer by the nonacidification protocol (41).

Concentration, screening, and isolation of infectious cyanophages. Lake water samples were serially filtered through 147-mm Whatman glass fiber filters and 0.2-μm-nominal-pore-size 142-mm-diameter filters (Gelman) to remove zooplankton, phytoplankton, and bacteria. The viruses remaining in the 0.2-μm filtrate were concentrated to ca. 50- to 200-fold by using an Amicon M12 ProFlux ultrafiltration system outfitted with an Amicon S10Y30 cartridge (44). Virus concentrates were stored immediately in the dark at 4° C until they could be returned to the lab for processing. Ballast water samples were processed similarly, being filtered serially through 20- or 5-μm-nominal-pore-size polycarbonate membranes (Osmonics) to remove relatively large particles and then through 47-mm, 0.2-μm membranes (Durapore); alternatively, in some cases they were directly filtered onto 0.2-μm membranes.

Potential viral activity was determined against liquid cultures, as most of the host cells used in our study do not form confluent lawns during plaque assays. To screen cultures for infectious cyanophage, 200 μl of virus concentrate was introduced into 5 ml of exponentially growing culture, with control cultures maintained for comparison. Growth and lytic activity were monitored in individual cultures with a TD-700 fluorometer (Turner Designs) as previously described (44). A series of cyanobacteria were screened for evidence of lytic activity. This included a number of freshwater strains, maintained in BG-11 medium (27), and marine strains, including *Synechococcus* sp. strain WH7803. The marine strains were maintained in A+ medium (46). All cultures were incubated at 25°C under constant light. The clearing of inoculated cultures (in vivo fluorescence drop) within 5 to 7 days and continued growth of controls indicated lytic activity. Virus-positive lysates were filtered through 0.22-μm-pore-size Durapore syringe filters to remove cell fragments, debris, and potentially lytic bacteria and stored in the dark at 4°C for use in dilutions or molecular work. Viruses were further purified via serial dilutions (44). For each lysate, triplicate dilution series (10-fold dilutions over 5 orders of magnitude) were screened for infectivity. In each case, the most dilute sample showing lysis was saved (theoretically containing a single particle) and the process was repeated. In all, the triplicate samples were processed through three sets of serial dilutions prior to characterization of the virus.

PCR amplification, cloning, and sequencing of *Cyanomyoviridae* g20. The oligonucleotide primers CPS1 [5′-GTAG(AT)ATTTTCTACATTGA(CT)GTTGG-3′] and CPS8 [5′-AAATA(CT)TT(AGT)CCAACA(AT)ATGGA-3′] were chosen based on previous work (14, 49); they amplify the region from ~826 to 1376 of the g20 capsid assembly protein. Two microliters of filtered lysate or virus concentrate was used directly as a DNA template for PCR amplification without DNA extraction or heat/cold treatment. For "particulate samples," DNA was extracted from glass fiber prefilters as previously described (26). Template was added to 50-μl Easy Start tubes (Molecular BioProducts), along with 0.4 μl of each primer (from 10 μM stock solutions), 0.5 μl of *Taq* DNA polymerase (2.5 units; Promega), and 5 μl of 1% Triton X-100 (Molecular BioProducts), and brought to a total volume of 50 μl with water. PCR amplification was carried out with an Eppendorf Mastercycler gradient thermocycler. The thermocycler was programmed to provide an initial denaturation step of 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 36°C for 15 s, with ramping at 0.3°C/s, and elongation at 73°C for 1 min, with a final elongation step of 73°C for 4 min. PCR products were screened by electrophoresis on a 1% agarose gel in 1× Tris-borate-EDTA buffer and visualized on a UV transilluminator after being stained with ethidium bromide for 30 min. Amplicons of the appropriate size were excised and purified with the QIAquick gel extraction kit (QIAGEN) and ligated into chemically competent *Escherichia coli* cells with the TOPO TA cloning kit (Invitrogen). Cells were plated onto agar plates and picked after an overnight incubation at 37°C. Clones were picked and grown overnight

TABLE 1. Station characteristics for locations used in *Cyanomyoviridae* g20 sequencing of samples

Sample designation	Date collected	Location	Chlorophyll *a* concn (μg liter^{-1})	Surface temp (°C)[a]
Virus concentrates				
UTK 63	10 July 2000	Sandusky Bay	14.0 ± 1.7	23.1
UTK 64	10 July 2000	Lake Erie Station 357	1.4 ± 0.2	22.7
UTK 145	17 July 2002	Lake Erie Station 357	13.5 ± 0.8	25.6
UTK 147	18 July 2002	Sandusky Bay	75.2 ± 7.9	26.3
UTK 167	23 July 2002	Grand River	62.5 ± 2.4	ND
Whole water samples				
MC 33	2003	Sandusky Bay	21.4 ± 1.3	23.6
Ballast samples				
MC38, MC42	10 July 2003	Burns Harbor[b]	0.08	22.6*
MC40	12 July 2003	Thunder Bay[c]	0.14	18.5*
Particulate samples				
MC9–MC11	10 July 2000	Sandusky Bay	14.0 ± 1.7	23.1

[a] ND, not determined; *, ambient outside air temperature when the ballast tank was sampled.
[b] The ship was in this port when its upper wing tank (port 5) was sampled.
[c] The water inside this tank had remained there since the previous sampling on 10 July.

at 37°C in Luria-Bertani medium with ampicillin (final concentration, 200 μg ml^{-1}). Plasmid DNA was purified with the Wizard *Plus* Miniprep DNA purification system (Promega). Sequence was obtained for amplicons at the University of Tennessee's Molecular Biology Resource Facility by using the Applied Biosystems ABI 3700 automated DNA sequencer.

Phylogenetic analysis of sequences. BLAST analyses of inferred protein sequences were conducted through the NCBI website (1), with the hits of strongest identity extracted for inclusion in phylogenetic analyses. Sequences were aligned with Clustal W (38) and then managed and manually edited in BioEdit (16). Neighbor-joining analysis of inferred protein sequences was conducted by using the Poisson correction model within the MEGA 3.1 software package (17). Only positions that were unambiguous and present for all sequences were utilized. Bootstrapping employed 5,000 replications, and percentages of ≥50 are reported. Phylogenetic reconstruction using the minimum evolution and maximum parsimony approaches in MEGA 3.1 yielded similar results (not shown).

Cyanophage morphology. Lysed cultures of *Synechococcus* sp. strain WH7803 were examined by transmission electron microscopy for the morphology of the cyanophage. After lysis, samples were filtered through 0.2-μm-nominal-pore-size Durapore filters to remove debris. Viruses were subsequently collected on carbon-coated collodion (2%; Electron Microscopy Sciences) films atop 400-mesh electron microscope grids by centrifugation. Grids were then rinsed with sterile water and stained with 0.75% uranyl formate. Samples were viewed with a Hitachi H-800 transmission electron microscope with an accelerating voltage of 100 keV.

Nucleotide sequence accession numbers. Nucleotide sequences determined in this study have been deposited in the GenBank database and are listed under accession numbers DQ318388 to DQ318432.

TABLE 3. Cyanobacterial isolates and growth media used in infectivity assays to screen for viruses in Lake Erie concentrates

Strain[a]	Medium	Activity
Synechococcus isolates		
WH 7803	A+	+
WH 5701	A+	−
WH 8007	A+	−
WH 8101	A+	−
WH 8102	A+	−
PCC 7002	A+	−
PCC 7942	BG-11	−
PCC 6803	BG-11	−
BO 8807	BG-11	−
S. rubescens	BG-11	−
Microcystis aeruginosa LE3	BG-11	−
Microcystis sp. UTEX 2386	BG-11	−
Anabaena sp. UTEX 2558	BG-11	−
Anabaena sp. UTEX 2576	BG-11	−
Oscillatoria luteus	BG-11	−

[a] Culture collections are denoted by the following acronyms: WH, Woods Hole; PCC, Pasteur Culture Collection of Cyanobacteria; UTEX, University of Texas Culture Collection of Algae. *Synechococcus* sp. strain BO8807 and *Synechococcus rubescens* were provided by Annaliese Ernst, while *M. aeruginosa* LE-3 (5) was provided by Wayne Carmichael. *O. luteus* was isolated and characterized in our laboratory.

RESULTS

Total chlorophyll *a* levels varied widely across the lake, ranging from 1.4 ± 0.2 to 75.2 ± 7.9 μg liter^{-1} across stations (Table 1). On board the ship transiting the Great Lakes, chlorophyll *a* levels in ballast water started at ~3.7 to 4.1 μg liter^{-1} (Hamilton) and dropped to ~0.1 μg liter^{-1} by the eighth day (Burns Harbor). As we documented previously (8), viruses are well distributed throughout the Lake Erie ecosystem. In parallel efforts, we reported that virus abundance in surface water ranged from 3.0×10^6 ml^{-1} to 4.9×10^8 ml^{-1} during the period of this study. Viruses in the ballast water were even more abundant, ranging from 1.5×10^8 to 5.5×10^8 ml^{-1} during sampling (Table 2).

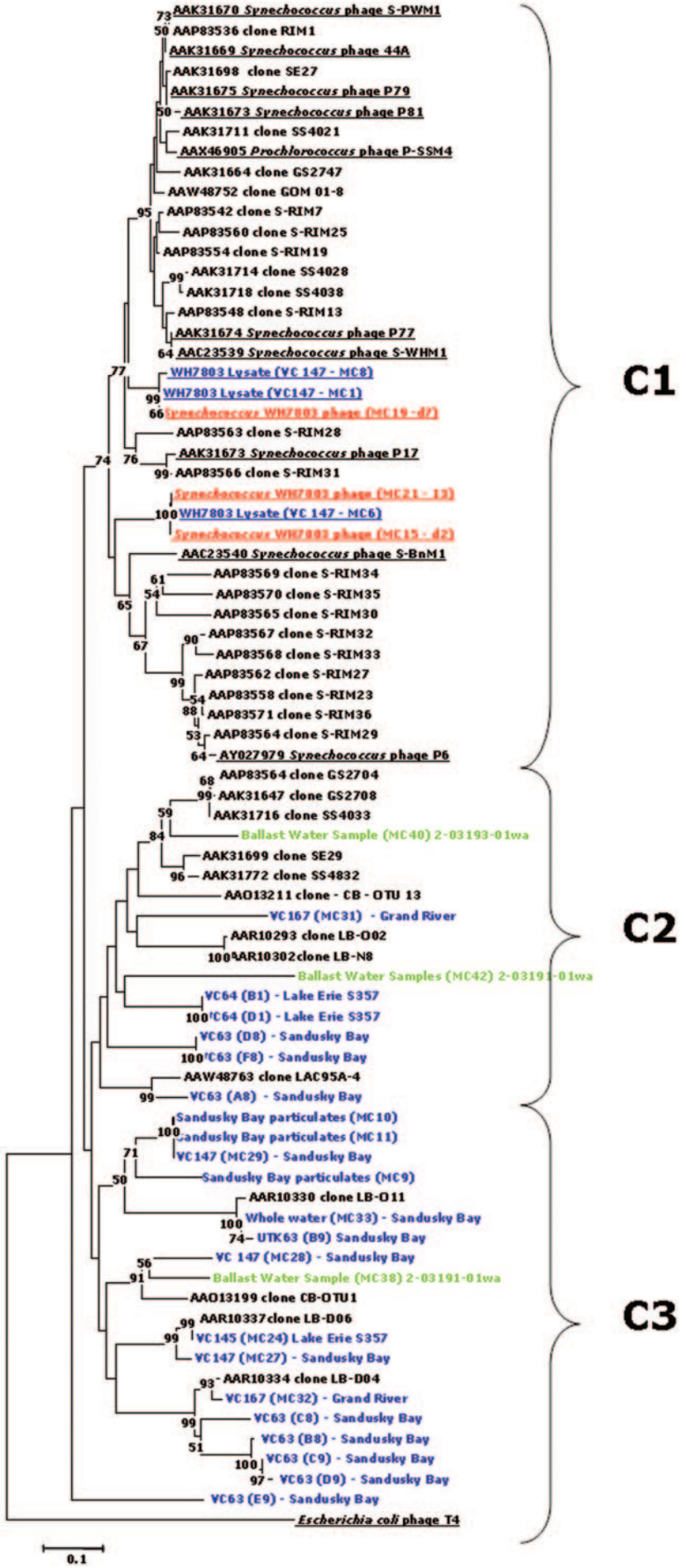
Infectivity assays carried out on concentrated virus communities from Lake Erie demonstrated the surprising yet consistent trend of only the marine cyanobacterium *Synechococcus* sp. strain WH7803 being affected by added viruses (Table 3). Stations from which we could find infectious cyanophage were primarily clustered in the western basin of Lake Erie (Fig. 1). Stations where virus concentrates were able to reproducibly lyse *Synechococcus* sp. strain WH7803 included two shallow-water (<5 m) stations (one behind Long Point Bay and one in the mouth of the Grand River).

The Lake Erie cyanophage isolates (amplified on *Synechococcus* sp. strain WH7803) do not appear to lyse other strains of marine or freshwater cyanobacteria to which they have been

TABLE 2. Changes in salinity, total virus-like particle and bacterial abundance, chlorophyll *a* concentration, and the ratio of functional to degraded chlorophyll collected from ship's ballast during transit from Hamilton to Thunder Bay[a]

Date	Port	NOBOB identification no.	Salinity (OD units)	Total VLP (10^8/ml)	Total bacteria (10^6/ml) (SD)	Chlorophyll *a* concn (μg/liter)	Chlorophyll *a*/phaeo ratio
2 July	Hamilton	2-03183-H	0.5	5.47	2.33 (0.06)*	3.69	7.24
2 July	Hamilton	2-03183-T0	0.5	4.54	2.32 (0.70)	4.07	0.92
6 July	Windsor	2-03187-T1	1	3.47	0.66 (0.00)	0.58	0.29
10 July	Burns Harbor	2-03191-T2	2	1.65	0.99 (0.33)	0.08	0.09
12 July	Thunder Bay	2-03193-T3	2	1.51	0.65 (0.02)	0.14	0.31

[a] NOBOB, no ballast on board; OD, optical density; VLP, virus-like particles; phaeo, degraded chlorophyll.


AAK31670 Synechococcus phage S-PWM1
AAP83536 clone RIM1
AAK31669 Synechococcus phage 44A
AAK31698 clone SE27
AAK31675 Synechococcus phage P79
AAK31673 Synechococcus phage P81
AAK31711 clone SS4021
AAX46905 Prochlorococcus phage P-SSM4
AAK31664 clone GS2747
AAW48752 clone GOM 01-8
AAP83542 clone S-RIM7
AAP83560 clone S-RIM25
AAP83554 clone S-RIM19
AAK31714 clone SS4028
AAK31718 clone SS4038
AAP83548 clone S-RIM13
AAK31674 Synechococcus phage P77
AAC23539 Synechococcus phage S-WHM1
WH7803 Lysate (VC 147 - MC8)
WH7803 Lysate (VC147 - MC1)
Synechococcus WH7803 phage (MC19 - d7)
AAP83563 clone S-RIM28
AAK31673 Synechococcus phage P17
AAP83566 clone S-RIM31
Synechococcus WH7803 phage (MC21 - 13)
WH7803 Lysate (VC 147 - MC6)
Synechococcus WH7803 phage (MC15 - d2)
AAC23540 Synechococcus phage S-BnM1
AAP83569 clone S-RIM34
AAP83570 clone S-RIM35
AAP83565 clone S-RIM30
AAP83567 clone S-RIM32
AAP83568 clone S-RIM33
AAP83562 clone S-RIM27
AAP83558 clone S-RIM23
AAP83571 clone S-RIM36
AAP83564 clone S-RIM29
AY027979 Synechococcus phage P6
AAP83564 clone GS2704
AAK31647 clone GS2708
AAK31716 clone SS4033
Ballast Water Sample (MC40) 2-03193-01wa
AAK31699 clone SE29
AAK31772 clone SS4832
AAO13211 clone - CB - OTU 13
VC167 (MC31) - Grand River
AAR10293 clone LB-O02
AAR10302 clone LB-N8
Ballast Water Samples (MC42) 2-03191-01wa
VC64 (B1) - Lake Erie S357
VC64 (D1) - Lake Erie S357
VC63 (D8) - Sandusky Bay
VC63 (F8) - Sandusky Bay
AAW48763 clone LAC95A-4
VC63 (A8) - Sandusky Bay
Sandusky Bay particulates (MC10)
Sandusky Bay particulates (MC11)
VC147 (MC29) - Sandusky Bay
Sandusky Bay particulates (MC9)
AAR10330 clone LB-O11
Whole water (MC33) - Sandusky Bay
UTK63 (B9) Sandusky Bay
VC 147 (MC28) - Sandusky Bay
Ballast Water Sample (MC38) 2-03191-01wa
AAO13199 clone CB-OTU1
AAR10337 clone LB-D06
VC145 (MC24) Lake Erie S357
VC147 (MC27) - Sandusky Bay
AAR10334 clone LB-D04
VC167 (MC32) - Grand River
VC63 (C8) - Sandusky Bay
VC63 (B8) - Sandusky Bay
VC63 (C9) - Sandusky Bay
VC63 (D9) - Sandusky Bay
VC63 (E9) - Sandusky Bay
Escherichia coli phage T4
C1
C2
C3
0.1

exposed. We screened both lysates of *Synechococcus* sp. strain WH7803 and virus concentrates from throughout the lake, finding no other confirmable lytic activity. Moreover, in an attempt to screen *Synechococcus* sp. strain WH7803 in medium more representative of Lake Erie (BG-11), we found that this cyanobacterium was unable to survive (data not shown).

The lysis experiments provided preparations for DNA analysis and subsequent purification of cyanophage by dilution. The results demonstrate that the g20 gene sequences from these virus isolates cluster separately but among other marine cyanomyoviridae in group C1 (Fig. 2). Purified phage isolates (MC15, MC19, and MC21) fall within this clade, which also contains the cyanophage known to lyse marine *Synechococcus* spp.

Direct amplification of the cyanophage g20 gene from virus concentrates revealed several novel sequences that were not represented in previous studies (Fig. 2). Some of these sequences were distributed into a clade containing samples of both marine and freshwater origin (C2). This includes sequences from two of the three ballast DNA amplification efforts that were positive. It also includes an amplicon—VC167(MC31)—that was generated from a sample collected inside the Grand River.

The final major clade resolved by these data contains primarily sequences from this study, including amplicons from glass fiber filter-collected materials ("Sandusky Bay particulates"), virus concentrates from Sandusky Bay, one of the ballast water amplicons, and one sequence each amplified directly from the Grand River and Lake Erie Station 357. These sequences most closely identified with sequences from Lake Bourget, France (AAR10330 LB-011, AAR10337 LB-06, and AAR10334 LB-D04) (9), and a sequence from low-salinity waters of the upper Chesapeake Bay (AAO13199 CB-OTU1) (39).

Morphologically, all of the cyanophage particles purified as part of this study were similar and were consistent with those seen in marine samples (37). Tails of ~147.4 nm (±5.1; n = 12) in length were consistently observed attached to capsids with a mean diameter of 69.6 nm (±6.0; n = 20) (Fig. 3).

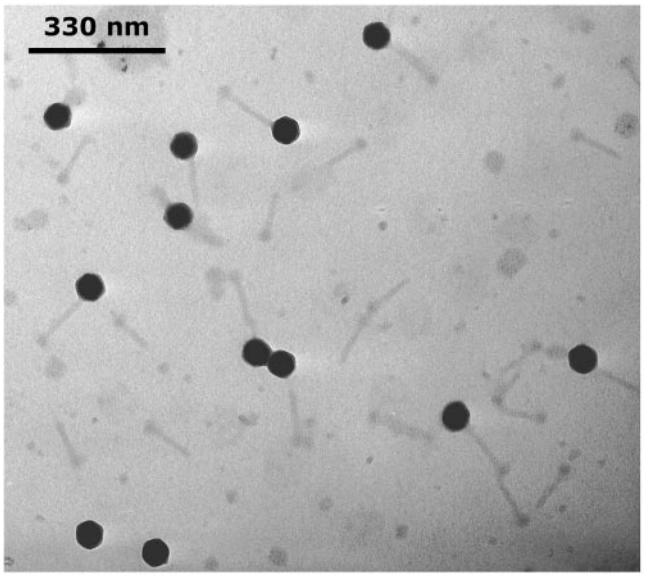


FIG. 3. Transmission electron micrograph of cyanophage MC15-d2 after passage through a culture of the marine cyanobacterium *Synechococcus* sp. strain WH7803. The image shows the dominant morphotype of intact, tailed bacteriophage, as well as the remnants of phage (primarily tails) damaged during the process of ultracentrifugation.

DISCUSSION

The presence of viruses in Lake Erie that infect a marine *Synechococcus* strain suggests that some mechanism(s) must be introducing these phages to the system (since we are unable to culture this host in freshwater), that there is a natural yet-to-be-cultured host in the lake that can be infected by this phage, thus allowing its continued existence, or both. Moreover, since the infectivity of cyanophage is known to be quickly destroyed by light (15), the introduction or production must be frequent, as we find these viruses in so many places. We consider both mechanisms plausible for the maintenance of the diverse population of cyanophages we have observed and address them in turn with consideration of their potential influence on this system.

Previous studies (29) have suggested that the ballast water of commercial ships can act as a mechanism for transport of small organisms, with estimated loads of 10^8 bacteria and 10^9 virus particles in each liter of ballast water, including high titers of the potential human pathogen *Vibrio cholerae*. Data from this study show that viruses taken on by ships along with ballast can persist in ships' ballast tanks and remain genetically intact in freshwater environments, leading to the potential both for changes in microbial community structure and for the transport and introduction of genetic elements into this system. We have also demonstrated the presence of cyanophage throughout the lake, as well as in the ballast of a commercial vessel, by using both molecular (g20-specific primers) and classical (infectivity assay) approaches. While we were unable to isolate viruses infecting *Synechococcus* sp. strain WH7803 from ballast water samples, we were able to PCR amplify g20-like sequences that are closely related to sequences associated with other putative cyanophages. The results suggest that ships' ballast water may transfer a variety of microbial agents and that marine-like agents (or at the least their genetic material) can survive movement into freshwater.

The other possible explanation for the presence of viruses particles (and g20-like DNA sequences) in Lake Erie surface

FIG. 2. Phylogenetic affiliation (neighbor-joining method) of inferred g20 amino acid sequences from Lake Erie phage isolates as well as direct amplifications from virus concentrates, whole water, ship's ballast water, and particulate samples collected during this study. Samples are color-coded to represent different source materials, and accession numbers are given to clarify sequences from other studies: black, natural samples (9, 18, 21, 31, 39, 49); black with underline, isolates (14, 34, 49); red, isolates (this study); blue, natural samples (this study); green, ballast water samples (this study). Bootstrap values of <50 are not shown. The scale bar equals 0.1 substitutions per site.

waters is that there exists a yet-to-be cultured potential host or hosts in the water column. Some stations where virus concentrates were able to lyse *Synechococcus* sp. strain WH7803 were distant from commercial traffic routes (e.g., the Grand River and Long Point Bay sites), suggesting that the population may be supported by potential hosts. Marine and freshwater *Synechococcus* spp. are typically readily distinguished from each other. Recent studies in the Laurentian Great Lakes have pointed to the potential importance of previously understudied picophytoplankton, predominantly cyanobacteria, in system production (7, 8, 43). Cyanobacteria of the genus *Synechococcus* appear to be abundant in Lake Erie, and to date the population in the lake has been thought to be typical of freshwater systems (24). However, data collected by employing eubacterium- as well as cyanobacterium-specific PCR primers and high-throughput DNA sequencing have suggested that a greater diversity of cyanobacteria persists in this system. Indeed, we have recently demonstrated that during seasonal (July to September) hypoxia in the central basin of Lake Erie, populations more typical of marine cyanobacteria (primarily marine *Synechococcus*) are frequently detected in clonal libraries by appropriate PCR-based molecular targets (42). Similar cyanobacteria have been well documented in other systems (10), and microscopic evidence documenting their presence in other Laurentian Great Lakes also exists (6).

The most likely explanation for the observations in this study probably arises from a combination of the above observations. As demonstrated in the phylogeny of g20 sequences, there appear to be amplicons in our library from the exclusively marine (C1) and exclusively freshwater (C3) clades. This observation is supported by the presence of sequence from other freshwater systems, including a number of sequences from putative cyanophage in Lake Bourget (9). Isolates collected in this study (using the marine cyanobacterium *Synechococcus* sp. strain WH7803) predictably fall within the marine group, suggesting that some mechanism may have introduced them to the lake. We remain, however, without isolates from the C3 clade; this implies that we may not be employing or have available the appropriate hosts.

One caveat to this is the suggestion that sequences outside of the clade containing known cyanophage isolates (C1) are potentially from viruses infecting other hosts (28). While this hypothesis cannot be rejected, it is surprising that within the abundance of sequences in informatics repositories the cyanophages remain the closest identity to these sequences. As researchers continue to explore new environments and new virus-host systems via genome sequencing, metagenomics, and targeted sequencing, we can anticipate that more insight into the identity of the source of these sequences will be provided.

Understanding mechanisms of mortality such as virus infection are critical in the development of functional models of ecosystem production and function. The transformation of nutrients from inorganic to organic, as they are assimilated and then released back into the water column after cell lysis, no doubt has a strong influence on competition in the water column among both primary and secondary producers. Although we do not imply any quantitative rates with our findings, this study nevertheless demonstrates that viruses are important and diverse members of this environment and impart mortality on at least a portion of the picophytoplankton. To this end, future studies of carbon cycling in this and other large lake systems need to account for the presence and activity of members of the virus community.

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