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I am submitting herewith a thesis written by Amanda Dean entitled "The Dynamics, Distribution, and Activity of Viruses in Lake Erie." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

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THE DYNAMICS, DISTRIBUTION, AND ACTIVITY OF VIRUSES IN LAKE ERIE

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ABSTRACT

As viruses-- specifically pervasive pathogens of aquatic microorganisms-- are now included in most studies of aquatic food webs and nutrient cycles, presented here is new information from samples collected in July 2003 which demonstrates that bacteria lysed by viruses released 122 - 1080 nM of phosphorus per day throughout Lake Erie, implying that viruses acted as a major mechanism of phosphorus recycling in the lake. Viruses have been shown to be ubiquitous throughout Lake Erie, with a weak correlation between their distribution and the distribution of bacteria. Moreover, evidence suggests that virus production rates are tightly tied to the growth rate of the microbial community, with changes in bacterial growth (brought on by amendments of PO_4-P) resulting in increases in virus production in the system. In combination with data compiled from 1997 through 2002, this information is presented to reinforce the importance of viruses as integral components of Lake Erie and other aquatic ecosystems.

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FIGURE LEGEND

LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
BS	Burst size
Chl-*a*	Chlorophyll *a*
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorus
FIC	Frequency of infected cells
FMVL	Fractional mortality attributable to viral lysis
FVIC	Frequency of visibly infected cells
GLFC	Great Lakes Fisheries Commission
HAB	Harmful algal bloom
KeV	Kilo- electron volt
MELEE	Microbial Ecology of the Lake Erie Ecosystem
NLET	National Laboratory for Environmental Testing
POM	Particulate organic matter
SRP	Soluble reactive phosphorus
TCA	Trichloroacetic acid
TEM	Transmission electron microscopy
VBR	virus-to-bacterium ratio
VC	Viral concentrate
VIM	Viral induced mortality

1. INTRODUCTION

For decades researchers have focused on bulk metrics such as oxygen, phosphorus and chlorophyll-*a* to resolve the spatial and temporal dynamics of lake productivity. In the Laurentian Great Lakes, this work has been primarily driven from a management perspective. Trophic dynamics in large freshwater lake systems remains a complicated area, and the development of linkages within any aquatic system ultimately requires more insight than can be achieved by these measures. Recently, renewed interest in the structure and function of these systems has lead to the re-examination of food web dynamics, with a particular focus on Lake Erie.

Although the smallest of the Laurentian Great Lakes, Lake Erie's central location, high human population density and impact on regional socioeconomic issues have made it an important focus during the last 40 years. Once termed "dead," the lake has enjoyed a successful revitalization due to the implementation of nutrient loading regulations (Nicholls and Hopkins, 1993; Makarewicz *et al.*, 1999), which have helped moderate changes in microalgal physiological processes such as photosynthesis and respiration (Millie *et al.*, 1999). However, invasion by exotic species, recurring potentially toxic cyanobacterial blooms, and the realization of seasonal hypoxia keep the lake in the popular press. To this end, researchers have begun to focus on developing a better understanding of the lake's function and dynamics. As part of this work, the Microbial Ecology of the Lake Erie Ecosystem (MELEE) consortium has been working to decipher the parallels and interactions between the grazing and microbial based food web components of the Lake (*e.g.,* Wilhelm *et al.*, 2003; DeBruyn *et al.*, 2004).

While the global importance of our freshwater resources continues to attract attention from researchers worldwide, it is surprising that the microbial component of these systems remains grossly understudied. Nowhere is this more obvious than in the arena of viral ecology. The role of viruses in marine microbial food webs has been shown to be significant (reviewed by Fuhrman 1999; Wilhelm and Suttle, 1999; Wommack and Colwell, 2000), but there have been few studies that address the dynamics of viruses in freshwater systems (*e.g.*, Table 1. All tables located in the appendix). Viruses can reach abundances of up to one hundred million particles per liter in the Great Lakes (Wilhelm and Smith, 2000; DeBruyn *et al.*, 2004) and may be responsible for as much as 20% of the microbial mortality. To continue to build on this knowledge, data from studies conducted in Lake Erie, including estimates of virus abundance and production, bacterial abundance and production, phytoplankton biomass and the results of mesocosm experiments have been collated for the past several years. The goal of this thesis is to not only document the presence of this group of obligate intracellular pathogens, but to also put their role in the ecology and biogeochemical cycles of this system into perspective.

Recent studies have focused on how viruses influence localized biogeochemical cycles. As agents of microbial mortality, viruses transform biologically active particles (cells) into a series of biologically inert but chemically active dissolved organic matter (DOM) and particulate organic matter (POM). The nitrogen and phosphorus in these pools are no doubt important components of the regenerated nutrients that drive food web dynamics and system production. Moreover, these compounds differ from nutrients regenerated by other processes (*e.g.,* grazing) in that the majority of these elements are

organically bound (whereas materials from grazing byproducts will be a mixture of organic and inorganic constituents). As was recently shown for virus-regenerated iron (Poorvin *et al*., 2004), the availability of these elements to planktonic communities varies relative to nitrogen and phosphorus from other sources.

In addition to the important role that viruses play in heterotrophic bacterial mortality, they also have a hand in cyanobacterial lysis. Toxic cyanobacteria can form harmful algal blooms (HABs), which are a current topic of interest because they contaminate drinking water and have socioeconomic impacts, with reports of toxic algal blooms on the rise (Chorus and Bartram, 1999). HABs can have rapid effects on thermal and chemical stratification, as well as causing severe fluctuations in oxygen, hydrogen, and carbon dioxide. In addition, HABs can cause prolonged changes in chemical budgets and trophic food chains (Millie *et al*., 1999). In the western basin of Lake Erie, including Sandusky Bay, bloom levels of cyanobacteria have been observed since the late 1990's, many of which have compromised water quality. Currently, work is being done to assess the relationships between cyanophages, their control of cyanobacterial populations, and the release of cyanotoxins into natural systems in both bloom and non-bloom conditions. Interestingly, some viruses that have been isolated from Lake Erie are closely related phylogenetically to marine viruses. It is thought that some of these viruses originated from ballast water (Carberry and Wilhelm, unpub.), which illustrates how wide the scope of impact is for invasive species in the lake. Other interests have touted viruses as potential biocontrol agents of harmful algal problems in both freshwater and marine systems.

1.1 Cyanobacteria

Cyanobacteria, also called blue-green algae, are photosynthetic prokaryotes that only require carbon dioxide, light, and an inorganic substrate to survive. Most cyanobacteria have the potential to form blooms, with some producing considerable toxins during these events. The factors that control their abundance and diversity include temperature, nutrient availability, sinking, and mortality due to grazing and viral lysis.

The focus of recent research has shifted from toxic marine cyanobacteria to those in freshwater systems. In part, this is due to the impact of bloom events on human health and socioeconomics. They can cause harm or even death to food sources such as fish during HABs. Increasing HABs in both freshwater and marine systems are blamed for a decline in tourism because beaches may be forced to close during bloom events. Moreover, toxic cyanobacteria can infiltrate drinking water and pass through treatment facilities undetected. A recent World Health Organization report on drinking water identified both cyanobacteria and their toxins as one of the most pressing areas in need of attention (Chorus and Bartram, 1999). In addition, the EPA is considering cyanotoxins for their Contaminant Candidate List and is proposing to add cyanobacteria and their toxins to the EPA's unregulated Contaminant Monitoring Rule.

Cyanobacterial toxins, or cyanotoxins, fit into one of three categories of related chemical structures: cyclic peptides (microcystins and nodularin), alkaloids [anatoxin-a, anatoxin-a(S), cylindrospermopsin and saxitoxins], and cytotoxins (including lipopolysaccharides) (Sivonen and Jones, 1999; Dow and Swoboda, 2000). The alkaloids, except cylindrospermopsin, are neurotoxins whereas microcystins, nodularin,

and cylindrospermopsin are hepatotoxins. Cytotoxins are generally less harmful, but do induce allergic reactions in humans and animals (Chorus and Bartram, 1999). Most cyanotoxins are known to cause significant illness (or even death) in relatively small (μg/kg) amounts (Jungmann and Benndorf, 1994). Another concern is that there can be long-term effects from chronic exposure to sublethal doses of cyanotoxins (Falconer, 1991; Chorus and Bartram, 1999; Dow and Swoboda, 2000).

There have been reports of toxic cyanobacterial blooms throughout the globe. Blooms of toxic *Microcystis* strains have been seen in the western basin of Lake Erie since 1995 (Brittain *et al.*, 2000). *Microcystis* spp. produce one or more types of toxin that belong to a family of potent hepatotoxins collectively known as microcystins. Understanding the regulation of growth and proliferation of *Microcystis* spp. in particular is an important issue in gaining insight into the components of the Lake Erie aquatic community.

1.2 Viral Components and Life Cycles

Viruses are 20 to 200 nm in length and consist of a nucleic acid genome (RNA or DNA) and a protective protein coat, called the capsid. Viruses are non-motile and are completely dependent on their host for biological activity and replication. In addition, most viruses are species-specific, therefore they require a host that can recognize their genetic material for replication. They can exist extracellularly as free particles or intracellularly within a host, where they either direct the host to produce virus progeny, or they integrate their nucleic acid into the host's genome (Brussaard, 2004).

There are three categories of the viral life cycles: lytic, lysogenic, and chronic (Figure 1. All figures located in appendix). In the lytic cycle, the virus attaches to the outside of the host and injects its nucleic acid into the cell. The host then makes copies of the viral nucleic acid and protein coat, and the viruses then self-assemble within the host. Finally, the host lyses and the progeny are released. In the lysogenic cycle, the nucleic acid of the virus becomes part of the host cell's genome and is replicated as genetic material in the host cell line. A switch to the lytic cycle may occur if the host experiences an induction event (Fuhrman, 1999). In the chronic cycle, the host makes copies of the viral genome as before. However, in this case, the virus does not lyse the host, but the host releases the progeny by budding or extrusion.

Because viruses have no means of motility, infection of hosts is probabilistic and dependent on direct contact and hence host density. As contact rates increase, infection rates rise, provided that the bacterial community is composed of permissive hosts.

Viruses are eliminated from the environment by solar irradiation, attachment to host cells that are then grazed, and attachment to fragmented particles. Solar radiation is the most common form of virus destruction in near-surface waters (Wilhelm *et al.,* 1998). The destruction of a viral community can occur very quickly, in a matter of hours, although it has recently been shown that viruses can persist in the water column for several days longer than previously thought (Wilhelm, *et al.*, 2003a).

1.3 Cyanophages

Cyanophages are viruses that infect cyanobacteria as their hosts. They belong to three families of tailed, double-stranded DNA viruses; Myoviridae, Styloviridae, and

Podoviridae. The Myoviridae have tails that contract, with a neck separating the tail from the capsid. The Styloviridae tail is long and non-contractile, whereas the Podoviridae tail is short and non-contractile (reviewed in Suttle, 2000).

The manner in which viruses are classified differs from conventional systematic methods for cellular organisms. They are classified according to whether they contain 1) DNA or RNA; 2) the nucleic acid is double- or single-stranded; 3) the presence or absence of an envelop; 4) the structure of the capsid; 5) host range; 6) how the host is affected; and 7) DNA fingerprinting (Brussaard, 2004). The most commonly isolated marine viruses are the Cyanomyoviruses, which are in the Myoviridae family.

The first isolation of a cyanophage was by Safferman and Morris (1963). The cyanophage, denoted as LPP-1, infects strains of *Plectonema, Phormidium,* and *Lyngbya.* Safferman and Morris (1967) isolated phages from waste stabilization ponds for potential use in biological control of algal blooms. The experiments were conducted by inoculating 35 different algal species with viruses in culture medium. Interestingly, none of the algae in the sample ponds were known to be host species for the viruses found there (Safferman and Morris, 1967). In addition, they monitored abundance of viral populations through time. The authors found that all of the viral isolates were algal-infecting viruses, and that 11 out of 12 viruses were taxonomically related to LPP-1. Taxonomic classification was based on each type of virus's ability to lyse *Plectonema boryanum.* The twelfth virus infected *Synechococcus elongates*, and was therefore considered unrelated. The conclusion that the authors drew from their study was that variations in LPP virus development are directly associated with fluctuations in its host population.

To follow up on Cannon and colleagues' (1976) finding that host-specific phage continued to multiply even after *Plectonema boryanum* became resistant to it, Barnet, Daft, and Stewart conducted a study to continue the investigation of virus-host kinetics by growing cultures of *P. boryanum* and *Aphanothece stagnina* with the phages LPP-DUN1 and Aph-1, respectively (1981). The results of the inoculations showed that major and minor oscillations between the growth of both species developed which damped-out oscillations after more than 40 days. After incubation, two strains of *Plectonema* were isolated from the cultures (strains PR1 and PR2) an additional mutant phage for *Plectonema*. It was shown that PR1 was resistant to the wild-type LPP-DUN1 phage, but not to the mutant phage. PR2 was unaffected by either cyanophage. Results also showed that most of the *A. stagnina* population was resistant to the Aph-1 phage. The resistance that some of the cyanobacteria showed toward formerly infective phages was explained by an alteration in the receptor sites on the cell wall surface of the hosts. Although PR2 was not affected by the phages, it was shown that if grown with PR1 in the absence of phages, it was competitively replaced by PR1, likely because it grew more slowly. The oscillation events were attributed to mutation effects. The explanation given for the Cannon *et al.* observation that phages continued to grow in the presence of resistant cyanobacteria was a differential sensitivity to infection (PR1 in this study) (Barnet *et al.*, 1981).

Waterbury and Valois (1993) came to the same conclusion when they exposed marine *Synechococcus* spp. to 75 viral isolates from inshore and open ocean waters. The 75 viral isolates were representative of all three viral families known to infect cyanobacteria, but most were Myoviridae. Their hypothesis was that bacterial

communities are resistant to co-occurring phages and are not greatly influenced by them. The study found that the annual abundance of *Synechococcus* spp. was driven by water temperature and not by phage-induced mortality. The authors ascertained that only a very small percentage of cyanobacterial mortality was due to cyanophage infection. This assertion was derived from calculations based on theoretical rates of phage adsorption and measured abundances of the host and its phages, in combination with the assumption that through electron microscopy, viruses can be seen in the last 50% of the latent period before cellular lysis. This is in contrast with the findings of Proctor and Fuhrman (1990, 1991), whose value for *Synechococcus* spp. mortality was calculated from the assumption that viruses within the host can only be detected in the final 10% of the latent period. By these calculations, Proctor and Fuhrman concluded that 30% of *Synechococcus* spp. mortality is attributable to virus lysis. Their estimation is the more widely-used in aquatic studies.

The study by Waterbury and Valois found that *Synechococcus* cells co-existed with phages, but were not affected by them. However, strains that were previously resistant to the phages became susceptible again after growing in phage-free conditions for about two years (Waterbury and Valois, 1993).

A recent study by Manage, Kawabata, and Nakano (1999) agrees with earlier conclusions that there is a tight host-pathogen relationship between cyanobacteria and cyanophages. The study was conducted in a hypereutrophic pond where *Microcystis aeruginosa* was a dominant algal species. They found that sharp decreases in *M. aeruginosa* densities were followed by significant increases in cyanophage densities.

They concluded that viruses were impacting the mortality of the cyanobacteria, although no viruses were ever isolated.

From these and other studies, it was determined that viruses would not be a viable biological control for HABs because of the resistance of potential hosts to the phages. This does not, however, discount the importance of understanding the relationship that viruses have with cyanobacteria and the rest of the microbial community.

1.4 The Microbial Loop and Nutrient Cycling

Within the last thirty years, the focus in aquatic ecology has shifted from macro-biology and the food web to include the role that microbes play in nutrient cycling (*e.g.* Pomeroy, 1974; Azam *et al.*, 1983). Even more recently, viruses have been recognized as key components in the microbial loop (Fuhrman, 1999; Wilhelm and Suttle, 1999; Weinbauer and Rassoulzadegan, 2004). The microbial loop is a pathway whereby dissolved organic matter (DOM) and particulate organic matter (POM), along with inorganic nutrients, are utilized and regenerated within the microbial community. In this loop, nutrients are recycled through the community by primary producers, heterotrophic prokaryotes, viruses, and grazers, with export occurring by predation on the larger grazers. When viruses lyse their hosts, DOM and cellular debris are released, making these nutrients available to other bacteria, and to a lesser extent, heterotrophic flagellates (Fuhrman, 1999). Because virus-mediated lysis is a major source of prokaryotic mortality in aquatic systems (on par with grazing-induced mortality), the impact of viruses in nutrient cycling is now clear (Weinbauer and Rassoulzadegan, 2004).

In Lake Erie, phosphorus is the limiting nutrient for primary production (Wilhelm *et al.,* 2003), and as such the virus-mediated lysis of cyanobacterial blooms could have a major impact on the availability of phosphorus to other organisms. In a model by Gobler, *et al.* (1997), virus-mediated lysis of a bloom of the marine phytoplankter *Aureococcus anophagefferens* enhanced dissolved phosphorus concentrations by as much as 15%. In addition, cell lysis by viruses was hypothesized to alter community structure by increasing nutrient availability to phytoplankton (Gobler, *et al.,* 1997).

There are two methods that phytoplankton can use to incorporate nutrients that have been lysed by viruses; 1) direct uptake of organic nutrients (DON, DOP) or, 2) assimilating bacterially regenerated inorganic nutrients, such as NH_4^+ and PO_4^+ (Gobler *et al.,* 1997). The chemical form of elements released by cellular lysis, either in the dissolved phase or within the particulate cellular debris, will influence the organisms that are able to assimilate the elements. Reinfelder and Fisher (1991) found that elements with the greatest enrichment in the cytoplasm of algae are generally assimilated with the greatest effectiveness by grazers. It stands to reason that those nutrients more associated with the cytoplasm would likely be in the dissolved phase upon cellular lysis, more so than nutrients tied up in cell walls, organelles, and membranes that exist in the particulate phase (Gobler *et al.,* 1997). Phosphorus is found to be more concentrated in algal cell walls and membranes (Reinfelder and Fisher, 1991), and has been shown to remain primarily bound to particles after lysis (Gobler *et al.,* 1997). However, some caution is needed when generalizing such results. For example, Fe from lysis-generated Fe-organic complexes released from bacteria in both particulate (>0.22 μm) and dissolved (<0.22 μm) form have both been shown to be highly biologically available to a series of model

marine organisms (Poorvin *et al.,* 2004). Hence, upon lysis some nutrients are readily available, while others are not. Coupled with the fact that different organisms utilize different elemental forms of nutrients, the effect of lysis in renewing nutrients differentially affects primary producers and hence community structure.

Gobler and colleagues (1997) found that the bacterial community in New York's Great South Bay assimilated almost five times more inorganic phosphorus (P) than virally-released P, which is consistent with Zweifel *et al*'s (1993) observation that bacteria preferentially use inorganic P to support growth because they would otherwise have to enzymatically cleave it from organic sources before uptake, an added metabolic cost (Ammerman and Azam, 1991). However, many bacteria have an ability to use P that has been released by virus-mediated lysis from bacteria, as alkaline phosphatase is prevalent in microbes, including those found in Lake Erie (DeBruyn, *et al.,* 2004). Further, phytoplankton can make use of elements released during bacterial decomposition, which is a major pathway of release for dissolved nutrients such as carbon (C) and P. This mechanism of reciprocal release and uptake of nutrients by bacteria and phytoplankton will lead to fractionation of nutrient elements so that they are highly conserved in the microbial community (Gobler, *et al.,* 1997).

One of the aspects of the current study conducted on Lake Erie was to identify the relationship, if any, between viruses and cyanobacteria in the lake community. Several experiments were conducted, many of which are similar to the above experiments, and some more recent applications that are more useful and efficient than in previous studies were used. Another focus of this study was to determine how viruses influence nutrient cycling, particularly the way in which viruses affect the release of the

limiting nutrient in Lake Erie. The experiments conducted to ascertain the viral effects on cyanobacteria and nutrient cycling included inoculating cyanobacteria with viruses and performing phosphorus addition experiments. In addition viral, bacterial, chlorophyll, and nutrient concentration data were collected throughout the lake for spatial distribution purposes.

2. MATERIALS AND METHODS

2.1 Location Description

Water samples for this study were collected in Lake Erie during July 2003 onboard the CCGS *Limnos.* The sites chosen correspond to sites previously visited during the summer months from 1997-2002 (Wilhelm and Smith 2000; Wilhelm *et al.*, 2003; DeBruyn *et al*., 2004). Stations were located throughout each of Lake Erie's three basins, including pelagic and near-shore areas (Figure 2). This provided a stratified sampling design of the lake's major regions.

2.2 Viral Concentrates

To test the infectivity of viruses in Lake Erie against certain strains of cyanobacteria, viral concentrates (VCs) were first produced. The premise is that viruses in a more concentrated sample will have a higher contact rate with host cells. In addition to the Lake Erie VCs, VCs were produced from Lake Loudoun (Knox Co., Tennessee) and a pond at the University of Tennessee Medical Center. Table 1 describes the site and date where water samples were taken. The sample numbers and UTK numbers are used for cataloguing purposes in the laboratory. These VCs were then compared to VCs from Lake Erie. For each VC, between 20 and 40 L of sample water were filtered through 142 mm diameter GF/F glass fiber filters (Whatman, average pore-size 0.7 μm). The sample was then filtered through 142 mm diameter, 0.45 μm pore-size, using low protein-binding Durapore polycarbonate filters (Millipore Corp.). Samples were all filtered using Millipore stainless steel filter towers driven by air pressure. After the two-fold filtration process, the sample was then concentrated with an Amicon M12 tangential flow

filtration system (Millipore), which was equipped with a <30,000 kDa spiral-wound ultrafiltration cartridge (S10Y30 Amicon, Inc.) as described by Wilhelm and Poorvin (2001). The permeate produced was ultrafiltered (<30 kDa), yielding virus-free water that was set aside and then utilized in other experiments. The volume of VC created varied between 500-850 mL. VCs were then stored at 4º C in the dark in either 0.5 L or 1.0 L HDPE polycarbonate bottles (Nalgene). After concentration, the S10Y30 cartridge was flushed with either 2 L Milli-Q water or ultrafiltrate, cleaned with 2 L of 0.1 M NaOH, and then cleaned with and stored in 0.05 M H_2PO_4 at 4º C. Before the next virus concentration, the cartridge was flushed again with either Milli-Q or ultrafiltrate.

Another concentration method employed was an ultracentrifugation technique. Whole water samples (~300 mL) were filtered through 47 mm diameter, 0.2 μm polycarbonate filters (Osmonics) on Nalgene Steri-filter units by vacuum filtration and placed into tubes (Nalgene Ultratube, thick wall polycarbonate). The tubes were placed on a Beckman SW28 free-swing rotor, which was positioned in a Beckman L8-80M ultracentrifuge and samples were centrifuged at 23,500 rpm for 3 h at 4º C. After centrifugation, 0.7 mL of supernatent was saved from each tube, discarding the rest. For each tube, the saved supernatent was used to resuspend the virus. The sample was then stored at 4º C in a 2.0 mL sterile microcentrifuge screw-cap vial (Fisher Scientific).

The two VC methods were compared by epifluorescence microscopy with no discernable differences in enumeration found (data not shown).

In addition to the VCs that were made, three known phages designated as N-1, AN-15, and AN-26 were obtained from American Type Culture Collection (ATCC). These phages are known to be infective to strains of *Anabaena* and *Nostoc* and were

obtained so that there would be known phages that would aid in determining the best experimental design for inoculating the cyanobacteria with the unknown phages.

2.3 Assays with Viral Concentrates

Viral concentrates from cruises from the summers of 2002 and 2003 on Lake Erie were used to inoculate strains of the cyanobacteria: *Anabaena, Microcystis, Nodularia, Oscillatoria, Lyngbya, Plectonema,* and *Synechococcus.* The cyanobacterial cultures were maintained in BG-11 medium (Kerry *et al.*, 1988) in either 35 mL capacity screw-cap glass culture tubes or in 250 mL Erlenmyer flasks. Cultures were then transferred to fresh BG-11 medium in either 9 mL screw-cap glass culture tubes or in the larger glass tubes described above. Depending on the tube size used, 0.1-1.0 mL of culture stock was added to 3 or 25 mL of BG-11, respectively. Phytoplankton biomass can be estimated by determining the *in situ* fluorescence of chlorophyll-*a* (Welschmeyer, 1994). A fluorescent molecule has the capability of absorbing light at one wavelength and emitting light at a new and longer wavelength. In a fluorometer, light goes through an excitation filter that transmits light of a wavelength range that is specific to a group of compounds. The light goes through the sample, which emits an amount of light energy that is proportional to the amount of fluorescent material present and to the intensity of the exciting light. The light that the sample emits passes through another filter that selects for the appropriate wavelength range, thus making detection by a photomultiplier tube possible (Turner Designs TD700 manual). To monitor cyanobacterial growth, chlorophyll-*a* concentration of the cultures was measured using fluorescence spectral units (FSU) on a daily basis with a Turner Designs TD-700 fluorometer. The bulb used

had an excitation wavelength between 340-500 nm and an emission wavelength > 665 nm, which are appropriate ranges for measuring fluorescence in samples containing chlorophyll-*a*. After establishing the cultures in the new tubes, they were inoculated with 100µL of VC, with duplicate tubes for each combination. In addition, control treatments consisting of each species alone, and grown in BG-11, were established. Cultures were considered lysed if FSU increased initially followed by a decline after the addition of the VC. When lysis occurred, the lysate was filtered through a 0.2 µm pore-size filter to remove any possible non-viral pathogens (*e.g.* bacteria). The filtered lysate was then re-screened with tubes of fresh culture and media to determine if infectivity persisted.

Spot assays were also used to test for virus infectivity. Two approaches were used: either cyanobacterial cultures were mixed with 0.6% top agar and placed on a BG-11 agar plate, or 4 mL of liquid culture were poured onto a BG-11 plate and allowed to absorb into the agar for 2-4 days. Afterwards, 10 µL of VC were spotted onto the agar in a grid fashion so that more than one VC could be screened on a plate. Two spots per VC were plated. If clearing occurred on the areas spotted, then lysis was assumed.

2.4 Viral, Bacterial, and Phytoplankton (chl-*a*) Abundance

Whole water samples were collected using the ship's Niskin /rosette system. For viral and bacterial enumeration, samples were preserved with glutaraldehyde (2.5% final concentration) and stored at 4°C storage until further processing. To enumerate virus-like particles (VLPs), samples were stained with SYBR Green I following the procedure of Noble and Fuhrman (1998). Using 100-µL of whole water, samples were diluted to a final volume of 800-µL with sterile Milli-Q water and filtered onto 0.02-µm nominal

pore-size Anodisc filters (Whatman Corp.). To determine if changes in viral abundance occurred with depth, we also collected water from discreet depths at several stations and enumerated viruses and bacteria. Bacterial abundance was determined using Acridine Orange staining (Hobbie *et al.*, 1977). Preserved samples (2 mL) were stained and filtered onto 0.2 -µm nominal pore-size black polycarbonate filters (Millipore GTBP). Bacteria and VLPs were enumerated using a Leica DMXRA epifluorescence microscope. For both viruses and bacteria, 200 particles or cells, or a minimum of 20 fields of view, were examined.

To estimate phytoplankton biomass and size-classifications, samples were collected onto 0.2-µm or 2.0-µm nominal pore-size polycarbonate filters (Millipore) or 20.0-µm nominal pore-size nylon membrane filters (Millipore Corp.). Chlorophyll-*a* was immediately extracted from the biomass using 90% acetone. Samples were allowed to extract at 4°C for 24 h and quantified using a Turner Designs TD700 fluorometer (Turner Designs) using the non-acidification protocol (Welschmeyer, 1994).

2.5 Virus Production

The rate of virus particle production was estimated using the dilution technique described by Wilhelm *et al.* (2002). Sample water (300 mL) was filtered under vacuum through a 0.2-µm nominal pore-size polycarbonate filter (Millipore). The volume in the retentate was constantly maintained through the addition of ultrafiltered (< 30 kDa), virus-free water. Ultrafiltered water was generated using an Amicon M-12 tangential flow unit equipped with a S10Y30 spiral cartridge as described above (Wilhelm and Poorvin, 2001). The final volume of the retained cell suspension was 300 mL. The end

result was a bacterial community that contained previously infected cells with a reduced population of free viruses remaining. The water was divided into three polycarbonate bottles and kept at *in situ* temperatures under dark conditions. Samples (5 mL) were taken every two hours for up to eight hours and preserved with glutaraldehyde as described above. Viruses in samples were subsequently stained with SYBR Green I and enumerated using an epifluorescence microscope, as above. Mean virus production rates (± 1 SD) were determined from the rate of increase of viral abundance in triplicate samples.

2.6 Estimates of Infection Parameters

Transmission Electron Microscopy (TEM) was employed to examine the frequency of visibly infected cells (FVIC) in the native bacterial community, as well as to estimate the abundance of viruses released per bacterium lysed (burst size). Whole water samples (40 mL) were collected and preserved with glutaraldehyde, as above, and stored in the dark in sterile polypropylene tubes at 4ºC. Samples were collected onto carbon-coated collodion films atop 400-mesh electron microscope grids by centrifugation (1 hour, 15 minutes at 16,600 x g). Grids were subsequently rinsed with sterile water, stained with 0.75% uranyl formate, and rinsed again. The FVIC and burst size were determined by TEM as previously described (Weinbauer and Suttle, 1996; Noble and Steward, 2001). Samples were viewed with a Hitachi H-800 TEM with an accelerating voltage of 100 KeV. For each sample, two grids were prepared, and at least 1,000 bacterial cells were examined for infection in each. Burst size was defined as the average number of virus particles in all visibly infected cells. This is likely the minimum burst

size as some cells would have developed further particles prior to lysis (Weinbauer *et al*., 2002).

It has been estimated that viruses inside bacterial cells can only be seen during the last ~10% of the lytic cycle (Proctor *et al*., 1993). To account for this, conversion factors (3.7 to 7.17) are multiplied by the FVIC to estimate the frequency of infected cells (FIC). This provides a range (as a percentage) of the entire population of infected cells.

The fractional of bacterial cell mortality attributable to virus lysis (FMVL) has previously been found using the factor-of-two rule, which assumes 1) steady-state conditions, 2) that latent period is equivalent to generation time, and 3) that infected cells are not grazed (Proctor *et al*., 1993). However, since a proportion of the infected population is also removed by grazers, FIC and FMVL were determined according to Binder (1999), where bacterial infection (also as percentages of the total bacterial population) is derived from the following:

(a.) $FIC = 7.1 \bullet FVIC - 22.5 \bullet FVIC^2$

(b.) $FMVL = (FIC + 0.6 \bullet FIC^2) / (1 - 1.2 \bullet FIC)$

2.7 Impacts of Viral Activity on Localized Biogeochemical Cycles

To determine the impact of viruses on system geochemistry, the rate of phosphorus remobilization from bacteria to the DOM/POM pool by viral activity was calculated. Viral induced mortality rates (VIM) were determined by dividing virus production rate by the burst size (as determined by TEM), resulting in an estimate of the bacterial cells lysed mL^{-1} h^{-1} (Wilhelm *et al*., 2002). From this inferred mortality, the

amount of phosphorous released as a result of virus lysis was estimated from quotas of phosphorus from heterotrophic bacterial cells, which range from 0.5 fg $cell^{-1}$ (Heldal *et al.*, 1996) to 4.7 fg $cell^{-1}$ (Makino and Cotner, 2004).

2.8 Bacterial Production

Bacterial production rates across the surface waters of Lake Erie were estimated from ^{3}H-leucine incorporation rates using the previously described microcentrifugation approach (Kirchman, 2001). Briefly, triplicate samples were amended with 20µL ^{3}H-Leu stock solution (173 Ci $mmol^{-1}$, Perkin Elmer Life Sciences, Inc., final concentration of 40 nM) and incubated at *in situ* temperatures for one hour. Two controls were killed with 100% trichloroacetic acid (TCA) at T=0. After incubation, the live samples received 100% TCA and all samples were treated with subsequent washes of 5% TCA and 80% EtOH, with centrifugation and liquid extraction between each treatment. After drying overnight, 1 mL of scintillation cocktail (Perkin-Elmer, Wallac OptiPhase 'SuperMix') was added to each tube. A Wallac 1450 Microbeta Trilux scintillation counter was used to measure ^{3}H-Leu incorporation by the bacteria to provide gross production estimates for all samples. Where bacterial carbon production estimates are provided, the conversion factor (3.1 kg C mol^{-1} ^{3}H-Leu) of Wetzel and Likens (2000) was employed.

Bacterial biomass turnover rates can be derived from bacterial production estimates and bacterial abundances provided that the total amount of carbon per cell is known. Simon and Azam (1989) have found that cells with a volume between 0.050 and 0.100 μm^3 have between 15.2 and 23.3 fg of carbon per cell. Hwang and Heath (1997) found similar amounts in Lake Erie in July 1994. Sandusky Bay's bacterial cell size

averaged 0.084 μm^3 and contained 22.43 fg C $cell^{-1}$ in July of 1997, whereas the average offshore cell size was 0.063 μm^3 with a cellular carbon content of 16.82 fg C. Total carbon in the bacterial assemblage can be found by multiplying the bacterial abundance by the amount of carbon per cell in one liter of water. From this, the bacterial biomass turnover rate is established by dividing bacterial production by the amount of total carbon found above. The turnover rate gives an estimate of how quickly the bacterial population is replaced by new cells in a given time (Hwang and Heath, 1997).

2.9 Phosphorus Addition Experiment

To determine the effects of changes in trophic status on viral activity, amendments with the limiting nutrient in Lake Erie (PO_4) were carried out as previously described (Wilhelm *et al*., 2003) and virus-specific parameters were monitored. Surface water was collected, prefiltered through 210-µm Nytex and placed in 2.7 L sterile polycarbonate bottles. Phosphorus was added using a 1:4 mixture of KH_2PO_4 : K_2HPO_4, generating triplicate treatments of 0, 50,100, 250, and 500 nM above ambient concentrations. Once sealed, bottles were kept at *in situ* conditions (37% ambient solar irradiance and lake surface temperatures) for 72 hours. A set of dark bottles (n = 3) containing 500 nM amounts of phosphorus were also incubated at ambient surface temperatures. Following incubation, samples were screened for bacterial, viral, and phytoplankton (chl-*a*) abundance, bacterial burst size, bacterial production, and frequency of virus infection.

2.10 Nutrient Measurements

Nutrient concentrations [total and dissolved P, soluble reactive phosphorus (SRP), total and dissolved N, NO_2, and $NO_3 + NO_2$] were determined for the phosphorus addition experiment. Measurements of concentrations were made at the National Laboratory for Environmental Testing (Environment Canada) using standardized techniques (NLET 1994). "Dissolved" concentrations refer to the dissolved material passing through a 0.20-μm polycarbonate filter (Millipore) whereas "total" concentrations refer to whole water (unfiltered) samples. Sample filtration and pre-processing were conducted aboard ship and stored at in the dark at 4°C. NO_3 was determined by subtracting NO_2 from the NO_3+NO_2 estimates.

3. RESULTS

3.1 Cyanophages

Of all the VCs used to inoculate the cyanobacterial strains tested, only UTK 112 and UTK 113 showed a decline in chlorophyll-*a* in *Nodularia spp.* and *Oscillatoria luteus*, which indicated that lysis had occurred. Upon 0.2 μm pore-size filtration and re-inoculation, UTK 112 still lysed the two cyanobacteria, but UTK 113 no longer lysed *Nodularia* (Figure 3). Additional tests with the two VCs failed to produce any further lysis in the cyanobacteria. The two VCs were taken from Lake Loudoun in May 2001. The three stock phages, N-1, AN-15, and AN-26, did not cause lysis, even in the *Anabaena* hosts for which they were supposed to be specific.

3.2 Viral, Bacterial, and Phytoplankton Abundance

During the July 2003 survey, a lake-wide average of 7.50×10^7 mL^{-1} of VLP was found (Table 3). As expected, VLP abundance increased from the oligotrophic eastern and central basins to the eutrophic western basin. The eastern basin VLP quantity averaged 3.97×10^7 mL^{-1} (n=4), while in the central basin, 5.51×10^7 VLP mL^{-1} (n=9) were enumerated. The western basin had noticeably more particles, with the average per mL being 1.04×10^8 (n=13).

The number of bacteria across the lake ranged from 6.3×10^5 to 5.89×10^6 mL^{-1} (Table 3). The abundance of bacteria in the western basin averaged 3.03×10^6 cells mL^{-1} (n=13), while in the eastern and central basins, there were fewer bacteria [2.28 (n=4) and 2.06×10^6 mL^{-1} (n=9), respectively].

At the Maumee River, we measured a total chlorophyll-*a* concentration of 22.7 μg L^{-1}, with lake wide amounts ranging from 0.13 – 22.4 μg L^{-1}.

No significant changes in viral abundance were seen across water column depths at any of the stations, regardless of water column depth, *in situ* fluorescence, or volume of the hypolimnion (Figure 4). Minor trends in bacterial abundance were noticed with depth, although this was not consistent among stations.

3.3 Bacterial Production

On the whole, the western basin experienced higher bacterial production rates than the rest of the lake, although littoral stations in all basins had high rates of productivity. The ^{3}H-leucine uptake in the samples taken from the western basin ranged from 17.5 - 168 pmol leucine, L^{-1} h^{-1} (5.43 – 52.1 x 10^{-8} g C L^{-1} h^{-1}), central basin from 0.268 - 36.4 pmol leucine L^{-1} h^{-1} (8.31 – 1130 x 10^{-10} g C L^{-1} h^{-1}), and the eastern basin from 8.34 - 47.1 pmol leucine L^{-1} h^{-1}(2.58 – 14.6 x 10^{-8}g C L^{-1} h^{-1}). The daily bacterial biomass turnover rate in Sandusky Bay was 0.02 – 0.09 d^{-1}, the central basin from 0.001 to 0.09 d^{-1}, and the eastern basin between 0.02 and 0.05 d^{-1}.

3.4 Viruses and Phosphorus Cycling

Burst sizes and FMVL measurements did not vary across the lake (Table 4). The dilution assay showed that between 0.79 x 10^{6} and 13.5 x 10^{6} bacteria mL^{-1}were lysed each day in these waters. Frequency of infected cells ranged from 6.9 to 10.1%, while FVIC ranged from 1.02 to 1.49% across the lake. Depending on the estimated bacterial PO_4 quota (using either 0.5 fg $cell^{-1}$ or 4.7 fg $cell^{-1}$ as described above), these results lead

to estimated releases of phosphorus from cells that range from 13 to 222 nM and 122 to 1080 nM each day. Using the same estimates for Wilhelm and Smith (2000), whose abundance for Lake Erie bacteria lysed by viruses per day was between 0.24 and 0.46 x 10^6 mL^{-1}, the amount of phosphorus released ranged from 3.87 to 7.43 and 36.4 to 2162 nM each day in August 1997.

3.5 Effects of Phosphorus Addition on Viral Infection

Upon the addition of 500 nM amounts of phosphorous, a significant increase in viral abundance was seen, as well as an increase in phytoplankton abundance and bacterial production, which was also seen with the addition of phosphorous in as little as 50 nM amounts (Fig. 6). A change was not seen in bacterial abundance, burst size, and, with the exception of chl-*a* and bacterial growth rates, there was not a significant difference between the dark controls and the bottles containing 500 nM additions of phosphorus [significance based on two-tailed t-tests ($p < 0.05$)] for any of the parameters that were measured.

There was a statistically significant increase ($p < 0.05$) in ^{3}H-leucine assimilation by bacteria after the addition of 50 nM of P [(a) P_{add}=0 nM, 10.5 ± 3.0 pmol L^{-1} h^{-1}, and (b) P_{add}=50 nM, 20.8 ± 1.2 pmol L^{-1} h^{-1}]. The addition of 100, 250, and 500 nM aliquots of phosphorus yielded lower assimilation rates [18.2 ± 4.77; 17.6 ±. 1.20; and 16.0 ± 5.92 pmol ^{3}H-leucine L^{-1} h^{-1}, respectively]. At 72 hours, the dark samples displayed an uptake of 328 ± 9.09 pmol ^{3}H-leucine L^{-1} h^{-1}, which is significantly higher ($p < 0.05$) than the samples that were kept on deck at *in situ* light.

4. DISCUSSION

There now remains little doubt that viruses are important players in aquatic microbial food webs, especially those dominated by pelagic nutrients. As regulators of community production (Wilhelm and Suttle, 1999) and species diversity (Fuhrman and Schwalbach, 2003), viruses affect the pools of biotic carbon as well as the fluxes between these pools. As has been shown here, viruses also act on these processes indirectly, by altering the fates of required nutrient elements, as in the case of the current study through the shunting of phosphorus from microbes back into a dissolved organic pool through cellular mortality. We discuss these roles of viruses below in context of the available data.

4.1 Cyanobacterial Lysis by VCs

In several studies (e.g. Waterbury and Valois, 1993, Barnet *et al.,* 1981), cyanobacterial cells became resistant to viruses that they had previously been able to infect. This can happen after only a few days. In this study, the VCs UTK 112 and UTK 113 were able to infect strains of *Nodularia* and *Oscillatoria luteus* for two sets of experiments. Subsequent tests showed no lysis by the VCs. It is likely that the cyanobacteria became resistant to the viruses in the concentrates.

4.2 Viral, Bacterial, and Chl-*a* Abundance

On a temporal scale, VLP concentrations are relatively consistent (Table 3, figure 5a). In July of 2002, the VLP abundance varied from 0.3 - 4.1 x 10^8 mL^{-1} at pelagic stations (DeBruyn *et al.*, 2004). In 2000, station-wide VLP abundances averaged 3.48 x

10^7 mL^{-1} (Rinta-Kanto, unpublished data). In comparison, virus samples taken in August 1997 and July 1998 reached lake-wide amounts between 3.7 and 37.9 x 10^6 mL^{-1} (Wilhelm and Smith, 2000). In contrast, Leff *et al*. (1999) reported VLP amounts in the summer of 1997 as 1.05 x 10^6 mL^{-1}. The low VLP measurement could be due to their sampling technique, which was to filter samples through 0.2-µm nominal pore-size filters prior to staining and enumeration (Chen *et al.*, 2001).

Bacterial abundances in July of 2003 were similar to those found in other years. In 2000, bacterial abundances averaged 1.46 x 10^7 mL^{-1}, while in 2002 the numbers ranged from 1.1 - 5.9 x 10^6 cells mL^{-1} (DeBruyn *et al*., 2004). Wilhelm and Smith (2000) reported bacterial abundances from 1.8 x 10^6 to 4.6 x 10^6 mL^{-1} for summer 1997.

In 2002, chlorophyll-*a* concentrations in the pelagic zones ranged from 4.7 - 37.4 µg L^{-1}, while littoral stations were much greater, with a high of 179.2 µg L^{-1} at the mouth of the Maumee River. It should be noted that a cyanobacterial bloom later in the 2003 summer (August – September) drove chlorophyll-*a* levels in the Maumee to values more reminiscent of the 2002 sample (Rinta-Kanto and Wilhelm, unpub.).

4.3 Bacterial Production

Bacterial production in 2003 differed from production rates reported in other years. In 2002, production was higher, with rates as high as 405.8 pmol ^{3}H-leu L^{-1} h^{-1} at near shore stations and 29.0 - 212.7 pmol ^{3}H-leu L^{-1} h^{-1} at pelagic sample sites. In the central basin, rates varied from 6.4 - 27.2 pmol ^{3}H-leu L^{-1} h^{-1} and in the eastern, production was between 6.7 - 40.6 pmol ^{3}H-leu L^{-1} h^{-1}.

The 2003 bacterial turnover rate for Sandusky Bay was similar to that found by Hwang and Heath (1997), who reported a turnover rate of 0.08 d^{-1} in Sandusky Bay and 0.03 per day for the entire lake. Wilhelm and Smith (2000) reported a much higher biomass turnover rate of 0.6 to 0.8 per day. The authors also reported a bacterial production rate of 1.26 ± 0.05 µg C L^{-1} h^{-1}, which is two orders of magnitude higher than reported above.

4.4 Virus Abundance and Production

Although significant research over the last decade has focused on the distribution and activity of viruses in aquatic environments, questions remain about the factors that influence virus proliferation in aquatic systems. One correlation that received significant attention for aquatic viruses is the virus-to-bacterium ratio (VBR). Researchers in several papers have commented on the relative distribution of viruses to bacteria in different environments, commonly finding that viruses outnumber bacteria by approximately one order of magnitude. To determine if this trend exists, this paper examined data from the current study as well as data from previous studies in Lake Erie (Figure 5a) as well as in relation to other freshwater (Table 1a, figure 5b) and marine (Table 2b) environments. As illustrated in Table 2, viral and bacterial abundances tend to be higher in freshwater compared to marine systems. Arctic seas are productively robust; therefore the abundances in that region are higher than in other marine systems. Although the limited data set suggests some seasonality in Lake Erie (comparing the November results with those collected in July and August), a trend in the VBR does exist when we exclude the data from Leff *et al.* (as noted in the results section). This trend seems more consistent

when data from lakes of other trophic status are included (Figure 5b). At this point however the trend remains a correlation, with no explanation as to the factors that may drive the relationship. As illustrated in Figure 7, correlation of viral abundance to a series of different chemical and biological measurements within the lake demonstrates no conclusive driving forces. However, careful analysis of the results from the PO_4-P addition experiments seems to provide some insight.

Results from the mesocosm PO_4-P addition experiments indicate some of the factors that may be driving virus production in this system. As shown in previous work (DeBruyn *et al.*, 2004) as well as the current study, augmentation of PO_4 amounts by as small an adjustment as 50 nM lead to increases in phytoplankton abundance as well as bacterial production (Figure 6, also DeBruyn *et al*., 2004). As argued in DeBruyn *et al.* (2004) the light-dependent linkage between these parameters appears to be a stimulation of DOM production from phytoplankton as growth constraints by PO_4-P are reduced. Although constraints on the growth rate of the heterotrophic bacterial populations were reduced, no increases in abundance were seen. In a manner analogous to the ecumenical hypothesis for iron regulation of marine primary production (Morel *et al*., 1991; Price *et al.*, 1994) it appears that mortality mechanisms respond at a sufficient rate to regulate the abundance of heterotrophic bacteria in the system. In the case of our current study, the removal of grazers by 210-µm prefiltration suggests that control by viruses is the main factor regulating bacterial abundance.

Analysis of viral abundance, rates of FIC and burst size suggest that only changes in the abundance of virus particles occurred in our replicated treatments in relation to

added PO_4-P. To determine which parameters of infection may be leading to this increase, we need to clarify the factors that constrain viral abundance:

$$V' = V_o + (V_{prod} - V_{dec}) \bullet T$$

where V' is the abundance at the end of the experiment, V_o the abundance at the beginning of the experiment, V_{prod} and V_{dec} are the respective production and decay rates of viral particles, and T is the duration of the experiment. In terms of our experiments, we can also use this equation to begin to tease apart the effects of changes in nutrient concentrations on virus production. Assuming that the decay rate of viruses is constant, since it is primarily driven by light field, (Wilhelm *et al.*, 1998) then the difference between treatments +PO_4-P and the control are simply the difference in virus production rates (virus/mL/h) between the treatments. This rate itself is a function of three parameters:

$$V_{prod} = (T_{LC} \bullet FIC) \bullet BS$$

where T_{LC} (d^{-1}) is the length of the lytic cycle, FIC (cells) the frequency of infected cells (which can be inferred from the FVIC) and BS (viruses/cell), the burst size. In the current study both FIC and BS do not change due to added PO_4-P (Fig 6e and 6f), and as such changes in V_{prod} must be a function of the change in the length of the lytic cycle of the infection process. Although we have no current methods to measure this *in situ*, the parallel between the length of the lytic cycle and the growth rate of the host cells (which also changes, Fig 6c) has been previously noted in lab studies with *Phaeocystis* (Bratbak and Heldal, 2000). Given the caveat that community structure is constant (admittedly tenuous), it appears that increasing bacterial carbon production is held in check (in terms of bacterial cell density) as a function of a decrease in the length of the lytic cycle of

infected cells. It should be noted however that Wilson and colleagues (1996), in lab studies with marine *Synechococcus* (WH7803), observed that no change in the length of the lytic cycle occurred in cells grown under conditions of phosphate-limited relative to phosphate-replete controls. They also noted an 80% reduction in burst size under phosphate-stress, and a shift in susceptibility to virus infection (from 10% in phosphate-stressed cultures to 100% in phosphate-replete cultures). While other incongruities no doubt exist between the studies, perhaps the reason for the variation lies in that *Synechococcus* WH7803 was isolated from a region where limitation by PO_4-P would not be expected (the subtropical Atlantic Ocean). Regardless of the difference, the results of our study when contrasted with that of Bratbak and Heldal (2000) as well as Wilson *et al*. (1996) clearly suggest that a variety of factors are involved in the regulation of virus production.

While there is no doubt that *in situ* an increased grazing pressure may also keep bacterial abundance in check, the increase in viral abundance noted in this study (in the absence of any increases in burst size or FVIC) clearly suggests that viral lytic cycles can be altered to match the growth rate of host cells and, as such, hold the population at a relatively steady-state abundance.

This process may also be further ameliorated by an increase in the contact rates between host populations and infectious viruses that accompanies the increase in growth. Virus host contacts kinetics in aquatic systems have been shown to be regulated according to Murray and Jackson (1992) as follows:

$$\text{Contact rate} = (2S\pi \bullet \omega \bullet D_V)V \bullet B$$

where S is the dimensionless Sherwood number (1.06, Wilhelm *et al.*, 1998), ω is the diameter of the bacterium (*ca* 0.45 x 10^{-4} cm, Lee and Fuhrman, 1987), D_V the diffusivity of viruses (3.456 x 10^{-3} cm^2 d^{-1}, Murray and Jackson, 1992) and V and B the respective abundance of the virus and host populations. What becomes obvious from this equation is that any net change in the abundance of the viruses or hosts influence contact rates; as such increases in bacterial growth rates and abundances in our treatments would make this population susceptible to viral infection (with subsequent production of numerous virus progeny exacerbating the process). Although this coordinated control is also a function of viral and host diversity (as viruses must contact permissive hosts to lead to an infection) it provides an independent mechanism for the regulation of bacterial cell abundance in the face of increased growth rates. Known as the "kill the winner" hypothesis (Thingstad and Lignell, 1997), this control has implications for the ecology of the Lake Erie in general. In Thingstad's model (2000), the key component that controls viral abundance is that viruses act as a balancing factor, by which all species of bacteria that have differing growth rates can coexist in a steady state. If virus-host systems have similar adsorption coefficients, then viruses that have fast-growing hosts should be the most abundant. The high diversity of hosts leads to numerous pathways for biogeochemical transport of matter and energy through the food web. This process leads to a reciprocal controlling mechanism between viruses, bacterial diversity, and the biogeochemical future of bacterial production (Thingstad, 2000).

4.5 Characterization of the Role of Viruses in System Geochemistry

Gobler *et al.* (1997) found that, immediately following virus-mediated lysis of *A. anophagefferens*, nearly all of the C, N, P, Fe, and Se (76-100%) stayed in the particulate phase. Proctor and Fuhrman (1991) suggest that cells lysed by viruses release high-molecular weight proteins, nucleic acids, and polymers that can act as amassing agents, promoting particle formation. In deep water, these particles could sink out, but in shallow, homogeneously mixed waters such as Sandusky Bay, cellular debris and breakdown products remain throughout the water column, and as such are bioavailable to both bacteria and phytoplankton. Viruses are of critical importance in this process in that they release limiting nutrients back into the community after lysing cyanobacteria, such as *Microcystis*, that as a community contain large amounts of nutrients during blooms. The oscillation between phytoplankton release of particulate nutrients, bacterial uptake of POM, and subsequent release of dissolved nutrients by bacterial decomposition, grazing, and photochemical effects is a major component of the microbial loop.

To illustrate the scale of this process, we can consider data from previous studies that have examined the impact of PO_4-P additions on phytoplankton production. Those studies have shown that as little as a 50 nM increase in the amount of phosphorous stimulates primary production in all three basins of Lake Erie (Wilhelm *et al*. 2003; DeBruyn *et al*. 2004). As such, the amount of phosphorous (13 to 222 nM using the quotas of Heldel *et al*., 1996, or 122 to 1080 nM using the quotas of Makino and Contner, 2004) returned to the system each day by virus lysis is staggering. In Lake Ontario, Millard *et al*. (1996) estimated P uptake rates (using ^{32}P) ranging from 72 – 15000 nM d^{-1}, noting that the higher estimate was an extreme (Millard *et al*., 1996). Given our

knowledge of PO_4 impacts on system productivity, it is obvious that recycling of organic phosphates by virus activity must be driving a significant proportion of system production through the regeneration of dissolved organic-phosphates.

5. CONCLUSIONS

Although the results of this study were hampered by the inability to isolate a virus that would infect cyanobacteria found in Lake Erie, we have been able to demonstrate from *in situ* studies that viruses are an active component of the Lake system, and responsible for the regeneration of significant quantities of nutrients via the microbial loop.

Although there has been little explanation for the driving factors behind the consistent VBR in various systems, the VBR appears to be a qualitative relationship that persists in Lake Erie. Considering that heterotrophic bacteria and viruses are the most abundant species in the lake (~ 10^9 and 10^{10} L^{-1}, respectively), they form the basis for the food web and are important starting points in the microbial loop. There did not appear to be a strong correlation between viral abundances and phytoplankton or nutrient concentrations. And although there were no strong virus abundance-nutrient correlations, this study has shown that viruses play a hand in system geochemistry and nutrient remobilization. One can view the role of viruses in the Lake Erie microbial ecosystem in small parts, leading to a larger picture. The first part is the way in which viruses impact production when phosphorus limitation is alleviated. As described earlier, when adding phosphorus treatments reduced growth constraints on bacteria, which resulted in increases in bacterial production and viral abundances, but no significant changes in bacterial abundances, burst size, or FIC. If viral production is a function of FIC, burst size, and the length of the lytic cycle, and neither the FIC nor the burst size changes, then it becomes clear that the length of the lytic cycle is what is controlling viral production.

When coupled with the growth rate of the host cells, viral production can help keep the host population in a relatively steady state.

Another part of the picture is the importance of virus-host contact kinetics. If either viruses or hosts increase in quantity, then contact rates should increase (*vis a vis* Murray and Jackson 1992). With amplified contact rates, viruses have a better chance of infecting permissive hosts, which in turn causes increased viral production. This mechanism may maintain bacterial diversity because bacterial strains that are more abundant than others are held in check by the increase in viruses that infect them.

A third aspect of the function of viruses in the microbial community in Lake Erie is their importance in system biogeochemistry. As shown in this study, the release of the limiting nutrient, phosphorus, back into the community by viral lysis has a direct effect on phytoplankton abundance and bacterial production. This is where the larger picture comes into focus: as experiments showed in this study, phosphorus is being returned back into the community by viral lysis in significant amounts, and this was seen in samples taken from all parts of the lake. The recycling of organic phosphates is a major driving force behind system production (Figure 8). Indeed, by extrapolating beyond the data (since we have no way to experimentally "scrub" PO_4-P from samples, this is our only choice) we can see that in removing as little as 118 nM of PO_4-P, we would in theory halt chlorophyll accumulation. Although there is an inherit prudence that must be followed when extrapolating from this data, it nonetheless demonstrates the critical role that this regenerated organic PO_4 must play.

One goal of this paper was to examine how viruses affect, if at all, nutrient availability in Lake Erie. An increase in filter feeder populations (*Dreissena*) has resulted

in a dramatic decline in phytoplankton biomass to the extent that the amount of phosphorus in the lake has fallen below the target amount. Understanding the role of viruses in nutrient release is helpful in analyzing how the lower trophic levels are affected by nutrient limitation, which is a concern for the Great Lakes Fisheries Commission (GLFC), who is considering relaxing phosphorus limitations in Lake Erie, pending further study of lower trophic dynamics (DeBruyn *et al.,* 2004). In the larger picture, Lake Erie has seen huge impacts in its ecosystem caused by nutrient loading from agriculture and industry, followed by nutrient reduction from invasive filter feeders. The higher trophic levels have changed as the nutrient levels oscillate, with populations of food and sport fishes coming and going. Although Lake Erie cannot be reverted back to a lake unencumbered by anthropogenic influences, understanding each aspect of the ecosystem, down to the smallest particle, will help in deciding how to improve the community.

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APPENDIX

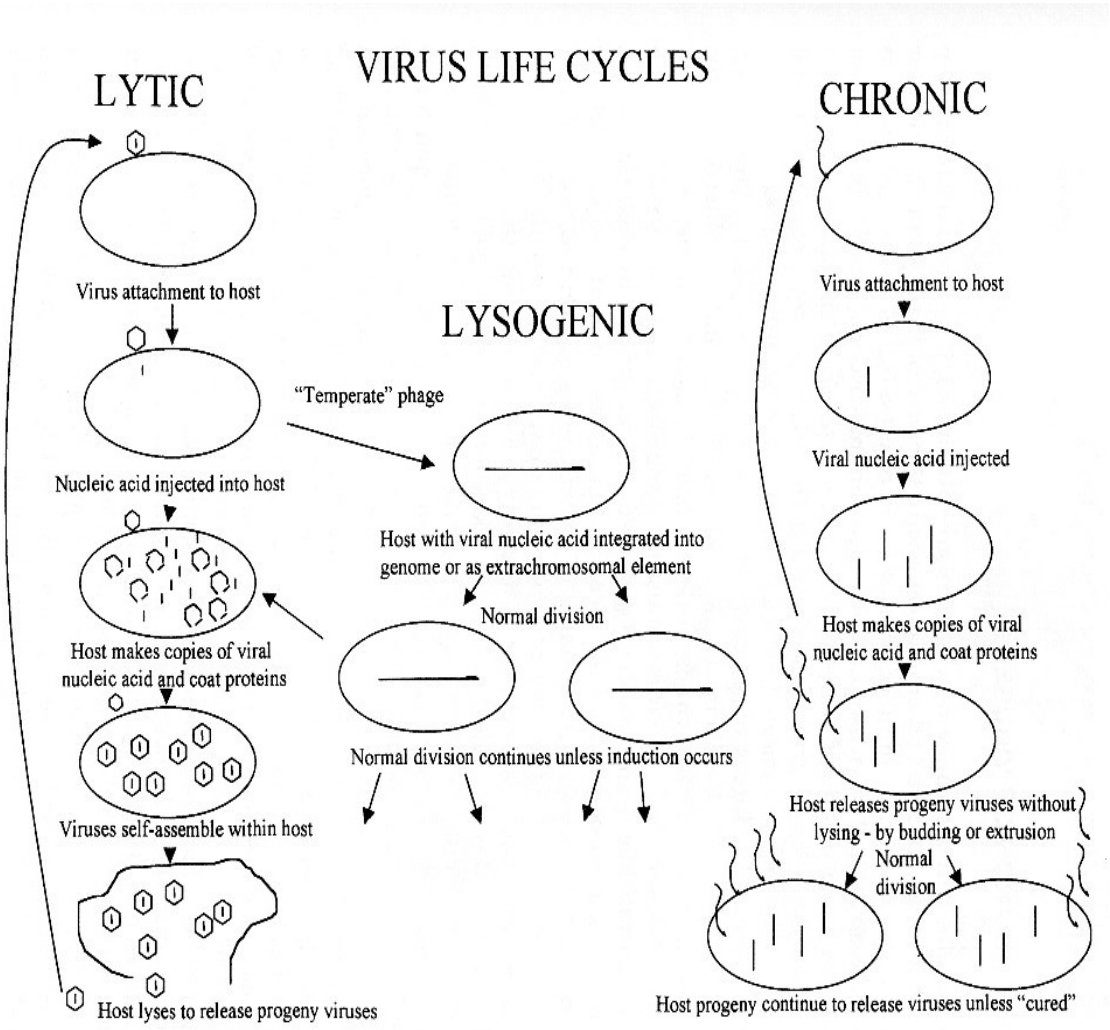

Figure 1. Virus life cycles.

Figure 1. Schematic of viral life cycles.

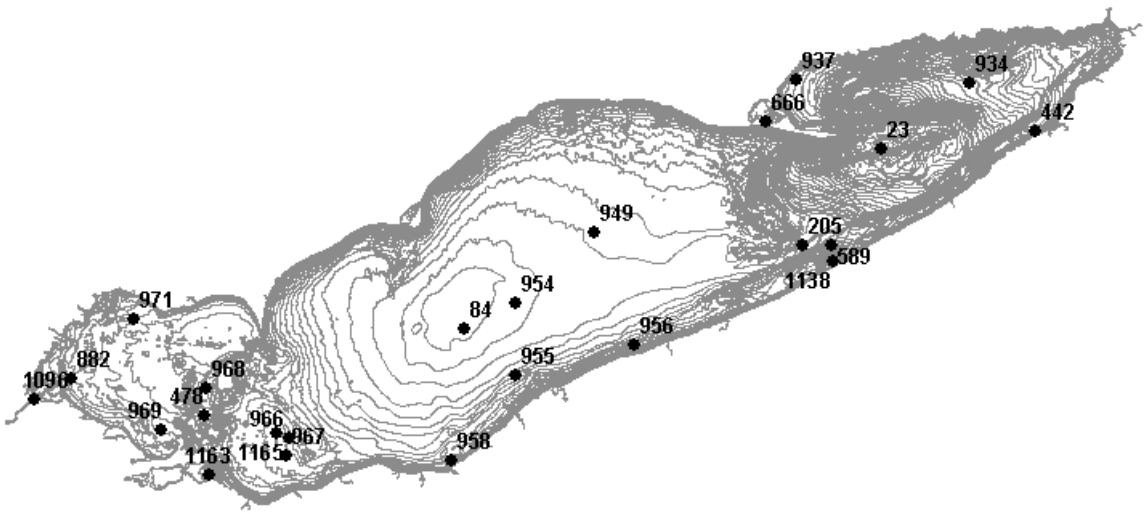

Figure 2. Map of stations in Lake Erie used for the current study.

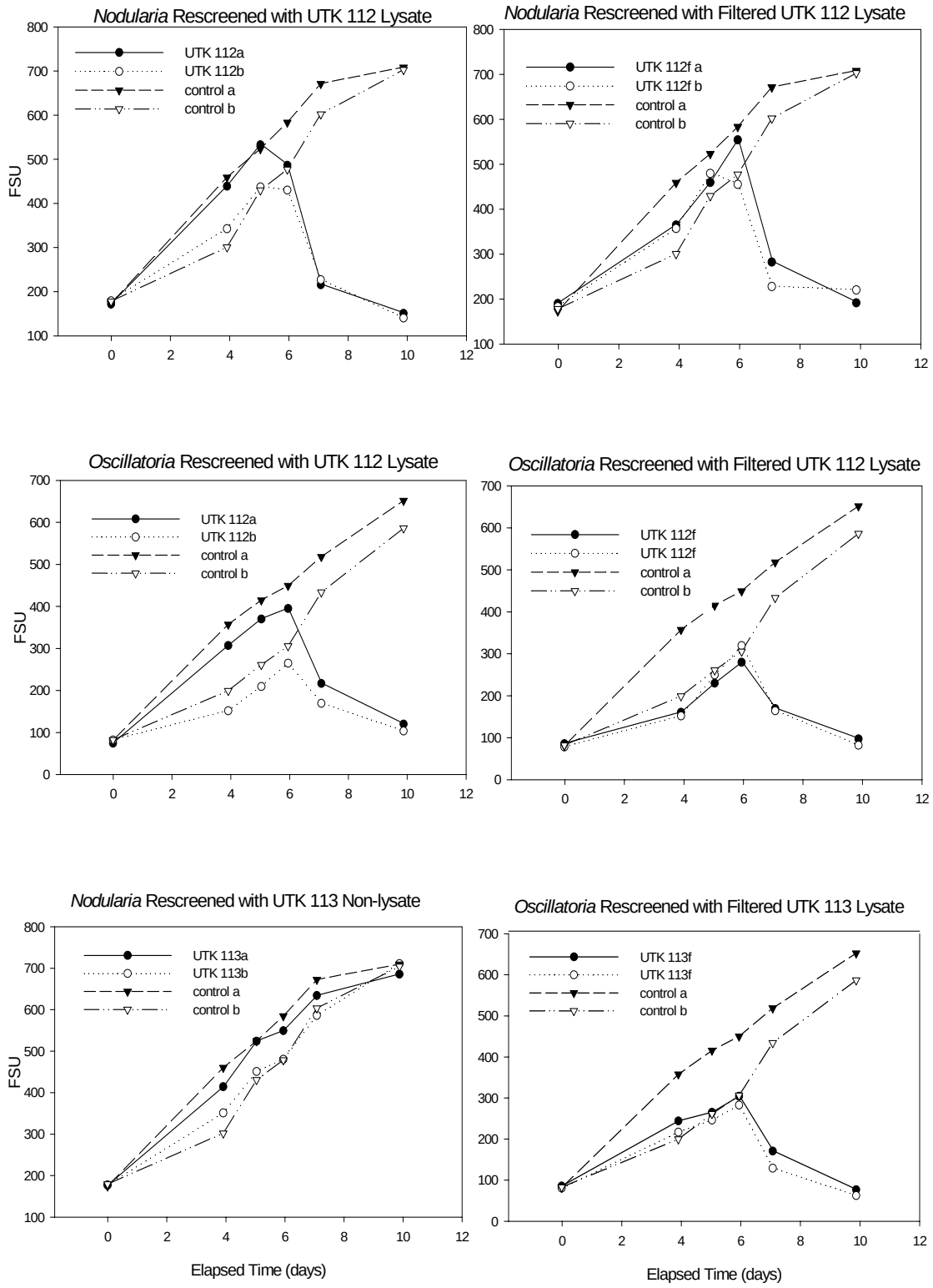

Figure 3. *Nodularia* and *Oscillatoria luteus* inoculated with VCs UTK 112 and 113.

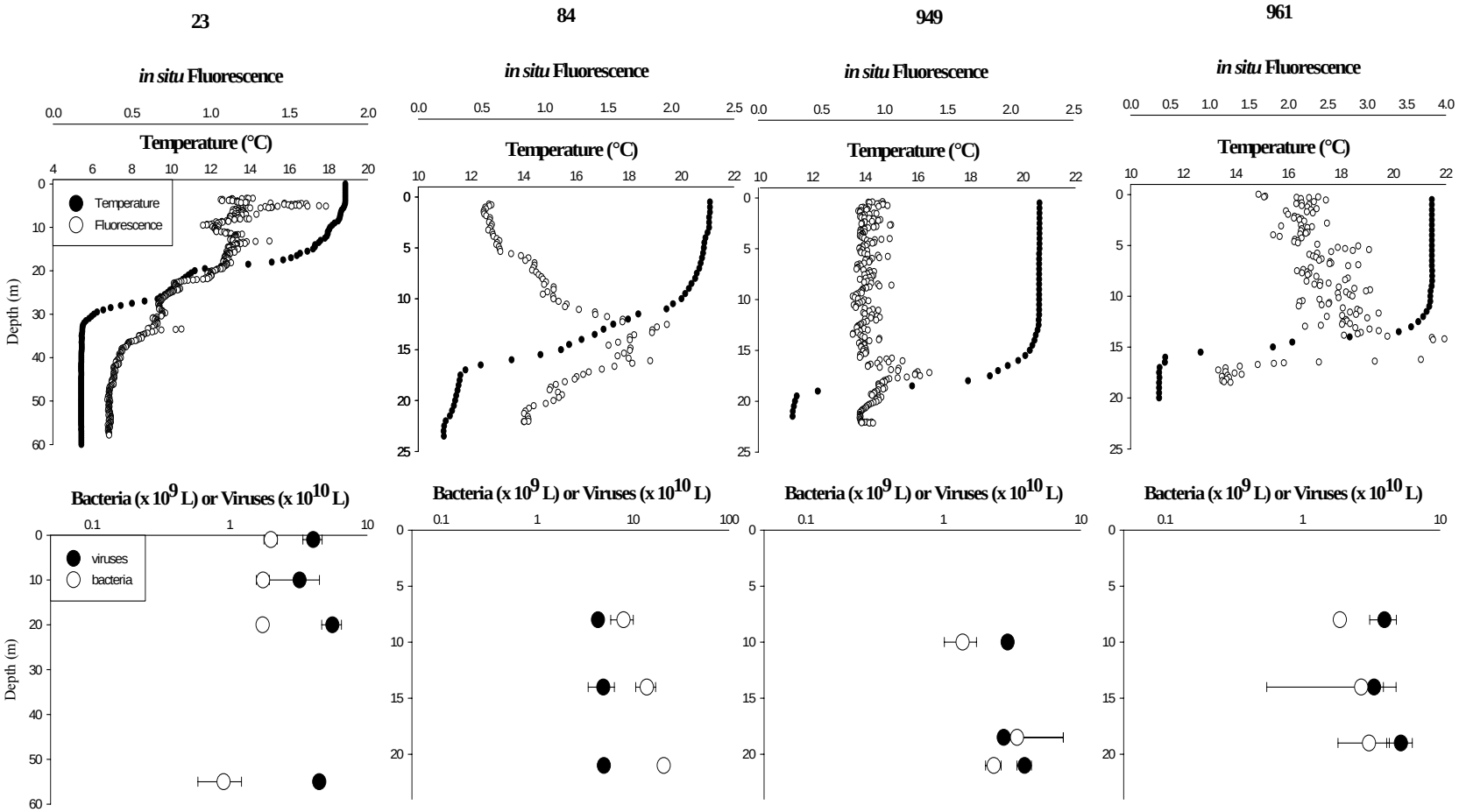

Figure 4. Depth profiles of temperature, *in situ* chlorophyll-*a* fluorescence, and bacterial and viral abundance (range ±, n = 2).

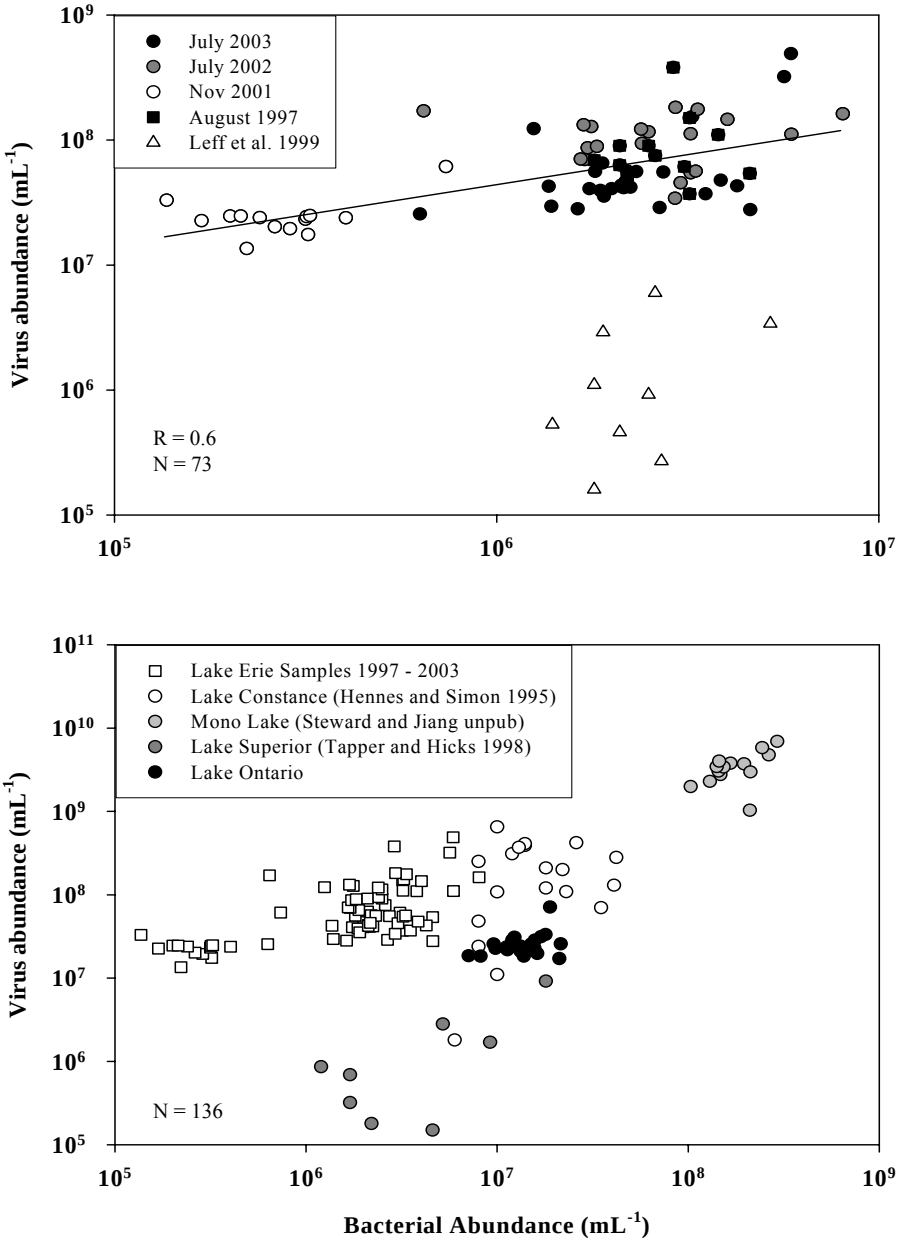

Figure 5. Correlations between bacterial and viral abundances.

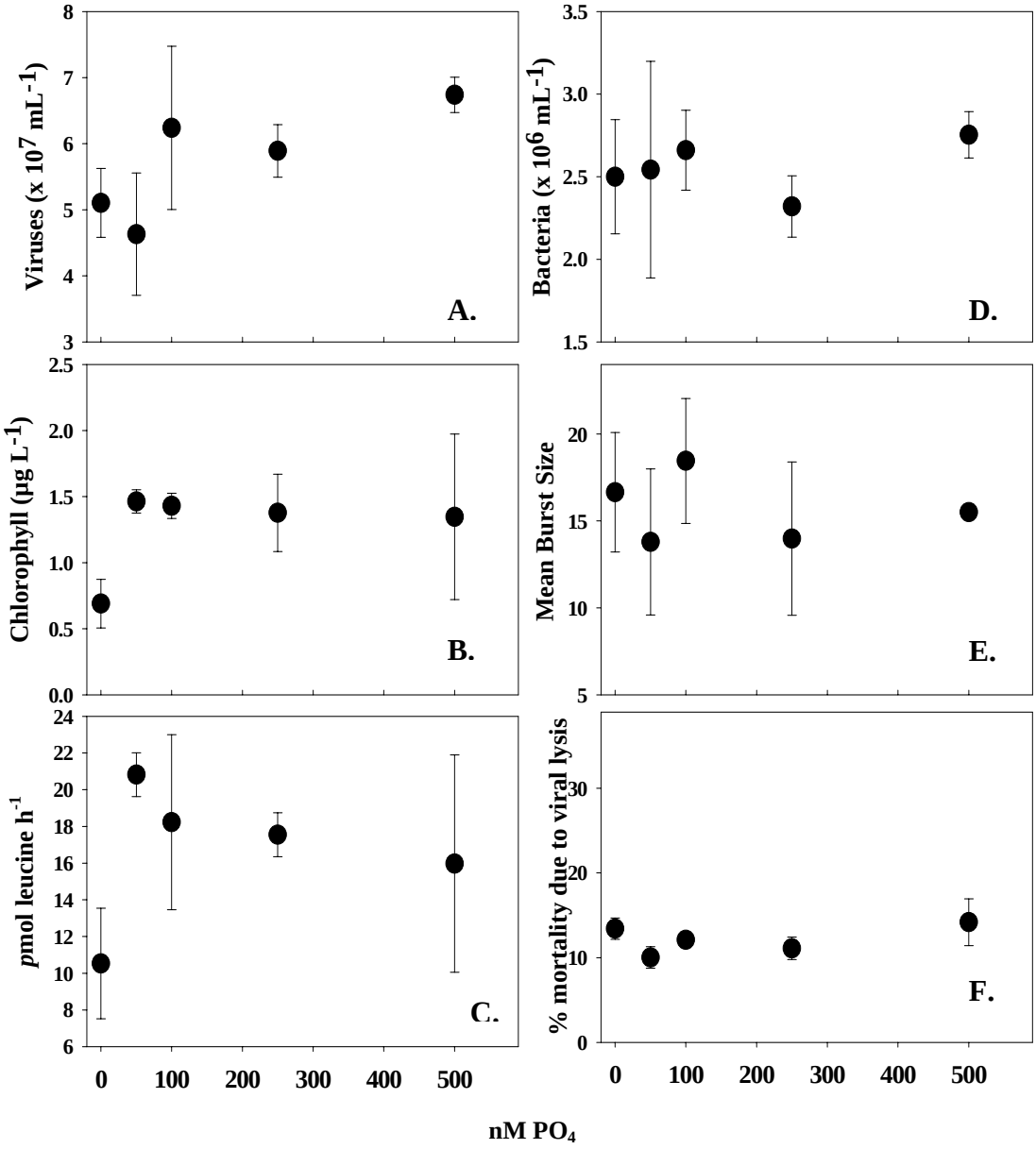

Figure 6. Response of the microbial community to changes in available PO_4.

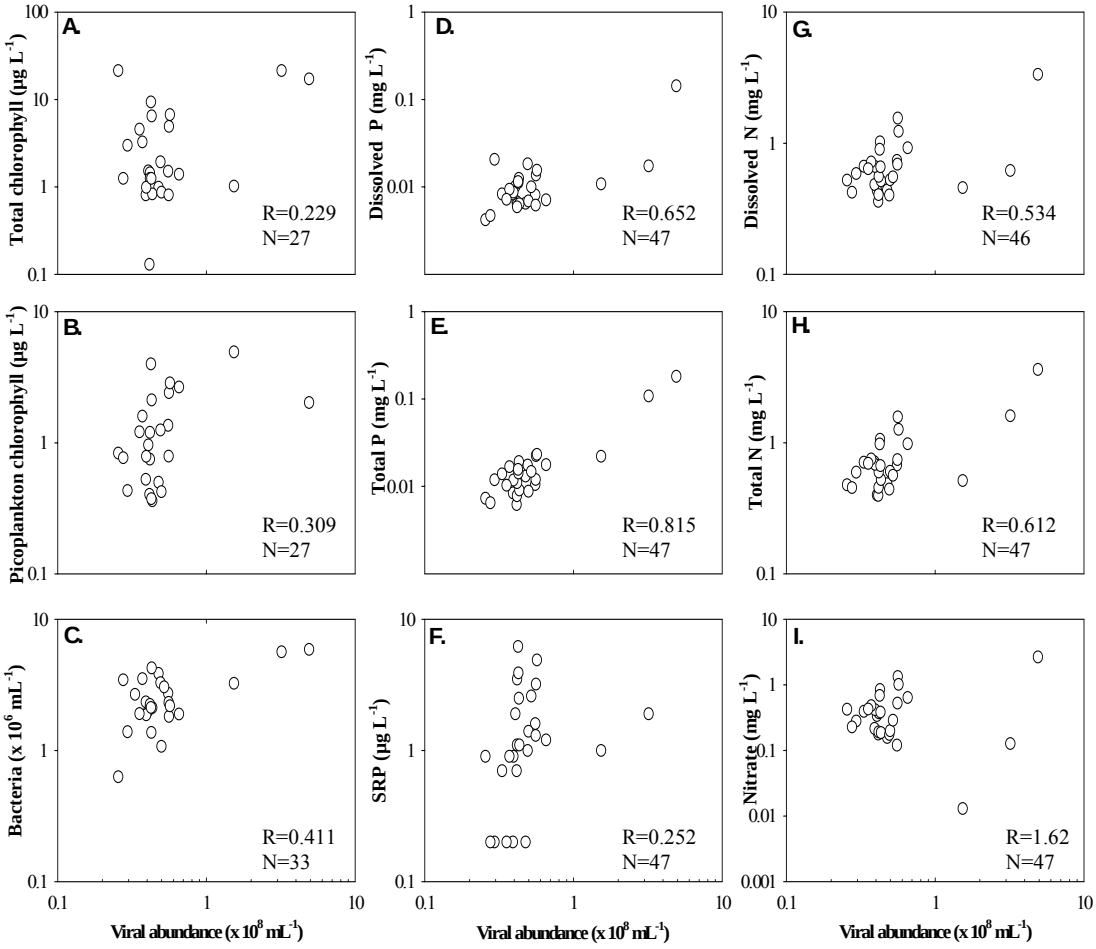

Figure 7. Correlations between viral abundance and surface water chemistry for stations in Lake Erie (July 2003; VLP n=33).

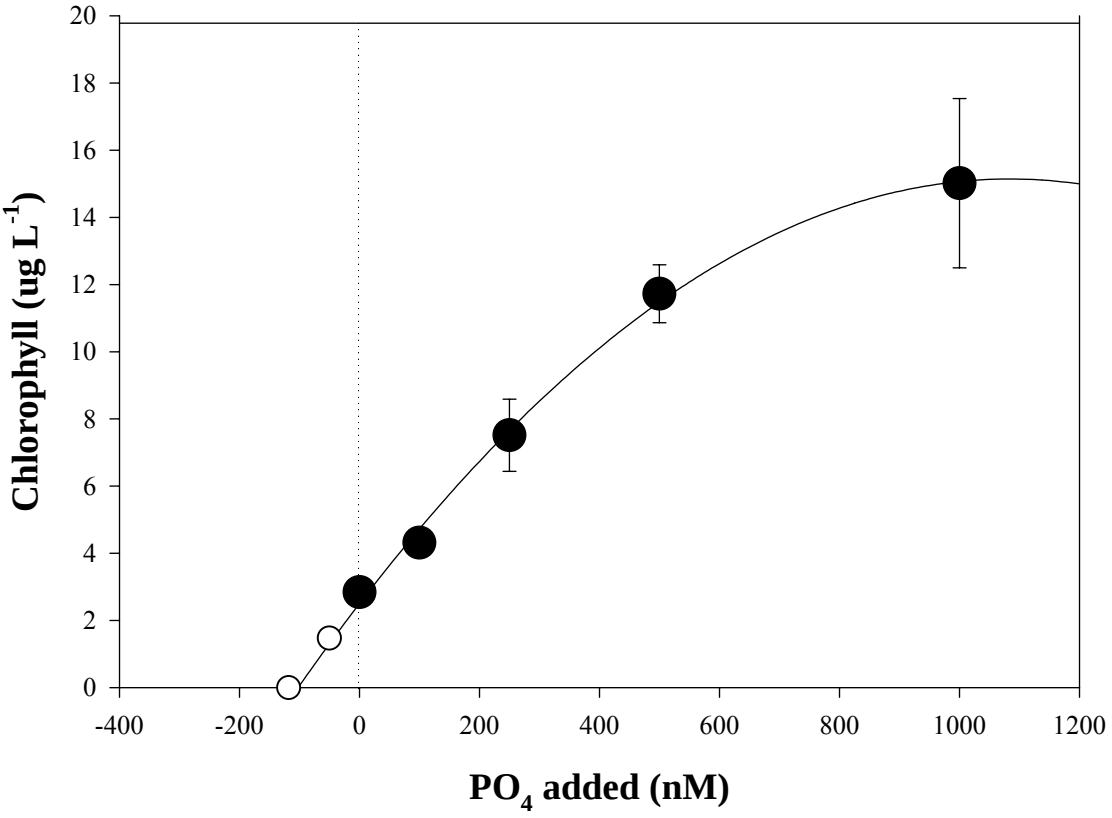

Figure 8. Extrapolation of influences of $P0_4$ impacts on water column chlorophyll.

Table 1. Sample sites for virus concentrates. PIB= Put In Bay; SB= Sandusky Bay; RB; Rondeau Bay; LPB=Long Point Bay; MGR= Middle Grand River; SHP= Sequoyah Hills Park.

Sample	Date	Station	Sample	Date	Station
Lake Erie 2003					
145	17-Jul	357	185	22-Jul	23
146	18-Jul	PIB	186	22-Jul	LPB
147	18-Jul	SB	187	22-Jul	589
148	18-Jul	969	188	23-Jul	949
149	18-Jul	882	189	23-Jul	84
150	18-Jul	973	190	23-Jul	958
151	18-Jul	971	191	24-Jul	478
152	18-Jul	84	192	24-Jul	1163
153	19-Jul	956	193	24-Jul	969
154	19-Jul	952	194	24-Jul	882
155	20-Jul	RB	195	13-Aug	21
156	20-Jul	84	196	13-Aug	494
157	21-Jul	84	197	13-Aug	84
158	21-Jul	943	198	13-Aug	84
159	22-Jul	945	199	14-Aug	449
160	22-Jul	23	200	14-Aug	936
161	22-Jul	940			
162	22-Jul	589			
163	23-Jul	LPB			
164	23-Jul	MGR			
165	23-Jul	23			
166	23-Jul	LPB			
167	23-Jul	MGR			
Lake Loudoun					
40	22-Jul 02	SHP			
46	20-May 02	SHP			
47	30-May 02	SHP			
UT Medical Center					
1	17-Aug 02	2			
2	22-Aug 02	1			
3	30-Aug 02	1			
4	30-Aug 02	2			
5	04-Sep 02	1			
6	04-Sep 02	2			

Table 1. Continued.

Sample	Date	Station	Sample	Date	Station
7	13-Sep 02	1			
8	13-Sep 02	2			
9	10-Oct 02	1			
10	10-Oct 02	2			
11	17-Oct 02	1			
12	17-Oct 02	2			
13	11-Nov 02	1			
14	11-Nov 02	2			
15	17-Jun 02	1			
16	22-Aug 02	1			

Table 2. **A)** Virus like particles and bacterial abundances in Lake Erie and other freshwater systems. [1]Weinbauer and Höfle, 1998, [2]Klut and Stockner, 1990, [3]Tapper and Hicks, 1998, [4]Carberry, Dean and Wilhelm, unpublished data, [5]DeBruyn, et al., 2004, [6]Wilhelm and Smith 2000, [7] Leff et al. 1999. **B)** Virus like particles and bacterial abundances in marine systems. [1]Balsom, unpublished data [2]Wommack *et al*, 1992, [3]Bratbak *et al*, 1996, [4]Jiang and Paul, 1996, [5]Noble and Fuhrman, 1997, [6]Proctor and Fuhrman, 1990.

A)

		VLP (x 10^7 mL^{-1})	**Bacteria (x 10^6 mL^{-1})**
[1]Lake Plußsee, Germany	(epilimnion)	1.37	4.6
	(metalimnion)	4.3	7.7
	(hypolimnion)	2.8	5.8
[2]Sproat Lake, BC	(epilimnion)	~ 0.15-0.2	summer 1-1.5
	(epilimnion)		winter 0.06
[3]Lake Superior	(surface microlayer, 0.2µm)	0.07-0.92	1.65-18.27
	(subsurface, 20m)	0.015-0.086	1.19-4.61
[4]Lake Ontario		2.61	13.8
Lake Erie, 2003	(eastern basin)	3.97	2.28
	(central basin)	5.51	2.06
	(western basin)	10.4	3.03
[5]Lake Erie, 2002	(eastern basin)	10.0	6.48
	(central basin)	12.1	2.29
	(western basin)	11.6	3.67
Lake Erie, 2000		3.48	14.6
[6]Lake Erie, 1997	(western basin)	3.7 – 37.9	1.8 – 4.6
[7]Lake Erie, 1997	(western basin)	0.13 – 3.44	1.39 – 5.18

Table 2. Continued.
B)

	VLP (x 10^7 mL^{-1})	Bacteria (x 10^6 mL^{-1})
[1]Gulf of Alaska	4.76	0.31
[1]Bering/Chukchi Sea	5.54	0.41
[1]Beaufort Sea	3.55	0.22
[2]Chesapeake Bay	0.26 - 1.4	
[3]Norwegian coast	4-9	
[4]Florida coast	0.27 - 1.15	
[4]Hawaiian Islands	0.07 - 0.12	
[5]Santa Monica Bay	1.0	
[6]Caribbean Sea	0.19 - 0.48	

Table 3. Comparison of chlorophyll a, bacterial abundance and virus abundance at stations in Lake Erie during July 2002 and July 2003. Locations for stations are given in degrees. Chlorophyll estimates (± range, n = 3) are given for total chlorophyll (> 0.2 µm) and for large phytoplankton (> 20 µm) to contrast productivities.

station	location		Chlorophyll *a* (µg L^{-1})				Bacteria (10^6 mL^{-1})		viruses (10^7 mL^{-1})	
			> 0.2 µm		>20 µm					
	lat	long	2002	2003	2002	2003	2002	2003	2002	2003
23	42.5	79.9	2.0 (± 0.1)	1.5 (± 0.3)	0.2 (± 0.0)	0.1 (± 0.0)	4.1	2.0	8.8	4.1
84	41.9	81.7	2.3 (± 0.1)	1.0 (± 0.1)	0.1 (± 0.0)	0.4 (± 0.0)	2.5	2.1	12.8	4.3
589	42.2	80.1	22.2 (± 2.7)	21.4 (± 0.2)	2.8 (± 0.3)	19.4 (± 0.2)	5.4	3.3	12.1	15.3
882	41.8	83.3	82.2 (± 5.0)	17.2 (± 2.7)	42.4 (± 6.0)	3.1 (± 0.3)	8.1	5.9	8.3	49.1
934	42.7	79.5	2.0 (± 0.1)	1.7 (± 0.0)	0.2 (± 0.0)	0.4 (± 0.0)	1.4	1.6	6.7	2.8
937	42.7	80.2	1.2 (± 0.0)	1.0 (± 0.0)	0.4 (± 0.0)	0.2 (± 0.0)	3.4	2.2	4.2	4.2
949	42.2	81.1	1.5 (± 0.1)	0.8 (± 0.2)	0.3 (± 0.0)	0.3 (± 0.1)	3.4	1.4	17.6	3.0
954	42.0	81.4	1.8 (± 0.1)	1.4 (± 0.0)	0.2 (± 0.0)	0.3 (± 0.0)	1.6	2.2	4.1	4.2
955	41.8	81.4	1.2 (± 0.1)	2.6 (± 0.1)	0.1 (± 0.0)	1.1 (± 0.0)	1.7	2.3	7.0	4.2
956	41.8	80.9	1.4 (± 0.4)	3.5 (± 1.0)	0.2 (± 0.0)	0.6 (0± 1)	1.6	2.3	8.9	5.6
958	41.5	81.7	3.6 (± 0.0)	8.4 (± 0.3)	0.2 (± 0.0)	4.7 (± 0.4)	2. 7	1.9	0.3	6.5
969	41.6	82.9	1.5 (± 0.6)	9.4 (± 0.3)	0.4 (± 0.0)	1.7 (± 0.0)	3.3	2.1	5.6	4.2
971	41.9	83.1	5.7 (± 0.3)	n/a	2.4 (± 0.0)	n/a	1.8	1.3	8.9	12.3
LPB	42.6	80.5	0.7 (± 0.1)	1.5 (± 0.0)	0.2 (± 0.0)	0.5 (± 0.0)	4.4	3.9	23.3	4.8
478	41.7	82.8	6.4 (± 0.6)	4.9 (± 0.2)	0.3 (± 0.1)	2.3(± 0.3)	3.6	1.8	10.7	5.6
1163	41.5	82.7	75.2 (± 7.9)	21.4 (± 1.1)	7.7 (± 0.3)	9.3 (± 0.9)	17.3	5.7	18.2	32.0
1096	41.7	83.5	15.5 (± 6.5)	22.7 (± 0.6)	1.4 (± 0.2)	3.5 (± 0.1)	17	2.2	12. 8	4.6

Table 4: Impact of viruses on microbial communities for select sites in Lake Erie during July 2003. Mean values ($\bar{x}$) and sample sizes (n, infected cells examined) and are included in parentheses. **A).** Burst sizes (viruses produced per lytic event), fractional mortality attributable to virus lysis (FMVL), and frequency of visibly infected cells (FVIC) estimated according to Binder (1999). **B).** Bacteria destroyed per day by virus-induced mortality according to Wilhelm *et al*. (2002). Estimated phosphorus released by virus activity is derived from the estimates of Heldal et al. (1996) and Makino and Cotner (2004) for bacterial PO_4 quota.

(A) Station	Burst size (mean)	FMVL	FVIC
LPB	8-22 ($\bar{x}$ = 13.3, n = 9)	11.33%	1.40%
934	5-27 ($\bar{x}$ = 12.6, n = 16)	9.36%	1.18%
23	5-27 ($\bar{x}$ = 13.3, n = 30)	7.97%	1.02%
949	5-42 ($\bar{x}$ = 12.7, n = 13)	12.60%	1.49%
84	5-27 ($\bar{x}$ = 17.5, n = 13)	12.49%	1.49%

(B) Station	Bacterial cells lysed by viruses **(**10^6 ml^{-1} day^{-1}**)**	P-released nM day^{-1}	
		Heldal *et al.*	Makino & Contner
23	1.25-6.98 (2.89)	20.5-120 (47.6)	139-1080 (636)
949	1.61-13.5 (5.32)	26.5-222 (87.6)	249-824 (536)
84	0.79-4.27 (1.22)	13.0-70.3 (28.7)	122-661 (391)

VITA

Amanda Leigh Dean was born in Knoxville, Tennessee on July 14, 1974. She attended Shannondale Elementary and Gresham Middle schools. She graduated from Central High School in 1992. From there, she went to the University of Tennessee, where she received a B.S. in biology with a minor in anthropology, as well as a M.Sc. in ecology and evolutionary biology.