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Department of Botany

The University of British Columbia
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Date 05/23/03
ABSTRACT

This study focuses on the infection characteristics (rate of adsorption, length of lytic cycle and burst size) of two cyanomyoviruses, cyanophage S-PWM1 infecting cultures of Synechococcus DC2 (Marine Cluster A) grown at a high irradiance (299 µmol quanta m\(^{-2}\) s\(^{-1}\)) and low irradiance (19 µmol quanta m\(^{-2}\) s\(^{-1}\)), and cyanophage S-PWM3 infecting Synechococcus DC2 and SNC1 (Marine Cluster B) grown at high irradiance. The adsorption coefficients for S-PWM1 adsorbing to DC2 under conditions of high and low light varied by a factor of 12 (1.26 x 10\(^{-8}\) and 1.03 x 10\(^{-9}\) mL min\(^{-1}\); SD = 1.88 x 10\(^{-9}\) and 3.75 x 10\(^{-10}\) respectively; n = 3). The coefficients of adsorption for S-PWM3 adsorbing to DC2 and SNC1 were 9.04 x 10\(^{-9}\) and 9.72 x 10\(^{-9}\) mL min\(^{-1}\) (SD = 4.33 x 10\(^{-10}\) and 3.34 x 10\(^{-10}\); n = 3) respectively. One-step growth experiments indicated that for S-PWM1 infecting Synechococcus DC2 the length of the lytic cycle was 17 h while the calculated burst size was 328 under high and 151 under low light, respectively. For S-PWM3 infecting Synechococcus DC2, the length of the lytic cycle was 13 h and the burst size was 135. For S-PWM3 infecting Synechococcus SNC1 the length of the lytic cycle was 17 h and the burst size was 8. The results of this study show that light limitation affects the rate of cyanophage adsorption and the burst size, and that there can be large differences in burst sizes among different cyanophage-host systems. This implies that in nature the physiological condition of the host will have a marked effect on phage replication, and that these effects will be dependent on the composition of the cyanophage and cyanobacterial communities.
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<tr>
<td>$C_d$</td>
<td>Adsorption coefficient</td>
</tr>
<tr>
<td>MOI</td>
<td>Multiplicity of infection</td>
</tr>
<tr>
<td>MPN</td>
<td>Most probable number</td>
</tr>
<tr>
<td>MPV</td>
<td><em>Micromonas pusilla</em> virus</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically active radiation</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>Maximum growth rate</td>
</tr>
<tr>
<td>VLP</td>
<td>Virus like particles</td>
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ACKNOWLEDGEMENTS

First and foremost I thank Curtis Suttle for his help throughout my years of graduate school. I also thank Amy Chan for her invaluable help in the laboratory. I thank all of the present and past members of the Suttle Lab for their help and constructive comments along the way. These include, but are in no way limited to: Jessie Clasen, Andre Comeau, Alex Cully, Cindy Frederickson, Janice Lawrence, Karen Reid, Jim Rossi, Steven Short, Tanya St. John, and Vera Tai. I also acknowledge the contributions of the two undergraduate students, Andrew Shum and Natalie Wolfe, who provided me with invaluable help.

My parents, Malcolm and Linda, and my sister, Natasha, also deserve a hearty acknowledgement. Their ongoing support kept me encouraged throughout the years.

Finally I thank all the friends that have helped me along the way. In particular, James Bowden and Andrea Verhagen kept me well fed with Sushi. Lionel Farmer and Jeremy Smith kept me in touch with Victoria. Rene Siguenza gets a huge mention here for being such a great friend and having such a comfy couch. I also thank Dao Le, Martin Payne, and Geoffrey Berkshire for some fun times. Finally I would like to thank all those others (who wouldn’t fit on this dwindling page and wouldn’t read this anyways) who understood how good (and bad) the music can be.

Gosh that was interesting but I don’t think I’ll ever do this again.
DEDICATION

This thesis is dedicated for my parents for their unfailing encouragement and understanding throughout my academic career.
INTRODUCTION

The first evidence of viral infections in photosynthetic marine organisms began to accumulate in the 1970's with a number of observations showing the presence of virus like particles (VLPs) in cultures and natural assemblages (Chapman & Lang, 1973; Pienaar, 1976). Shortly after, Mayer & Taylor (1979) reported the isolation of a virus (MPV) that caused lysis of the photosynthetic marine flagellate, Micromonas pusilla, but not 35 other species representing five classes of algae that were tested. Concurrently, transmission electron microscopy (TEM) provided the first estimates of viral abundance in seawater (Torrella & Morita, 1979). The reported abundances of >10^4 viruses mL⁻¹ in Oregon coastal seawater were acknowledged to be underestimates as they were based on VLPs caught on filters with a pore size of 0.2 μm. Despite the potential significance of viral infection to marine primary producers, little interest was shown in the results from these studies.

Subsequently, researchers became aware of the abundance and ecological importance of prokaryotes in the marine environment. Epifluorescence microscopy counts of seawater samples showed that there are, on average, >10^5 bacteria mL⁻¹ in the marine environment (Hobbie et al., 1977; Porter & Feig, 1989) and that these bacteria are actively growing (Fuhrman & Azam, 1980; 1982). Such findings led to speculation that microbes were major players in marine foodwebs and nutrient and energy cycles (Pomeroy, 1974; Conover, 1982; Azam et al., 1983). In fact, it became increasingly clear that prokaryotes constitute most of the biomass in the world’s oceans (Fuhrman et al., 1989; Cho & Azam, 1990). Because of the perceived significance of microbial processes in the ocean, subsequent observations of high abundances of
viruses in the sea (Bergh et al., 1989; Proctor & Fuhrman, 1990) stimulated a great deal of interest.

Observations of high abundances of viruses in seawater also stimulated interest in the role of viral infection in marine phytoplankton and shortly thereafter it was shown that viruses infecting phytoplankton such as cyanobacteria, prasinophytes, and cryptophytes could be readily isolated from seawater and the viral size fraction (2-200 nm) could suppress primary production by up to 78% (Suttle et al., 1990; Suttle, 1992). Based upon these experiments it was calculated that 2-3% of primary production might be lost due to viral production (Suttle, 1994). Such results clearly established the need to quantify viral mediated mortality in phytoplankton. Of viruses infecting phytoplankton, those infecting cyanobacteria belonging to the genus Synechococcus are most abundant (reviewed in Fuhrman & Suttle, 1993; Suttle, 2000). This is significant because populations of Synechococcus spp. can be extremely abundant (Waterbury et al., 1979; Johnson & Sieburth, 1979) and account for a significant fraction of carbon fixation in much of the world’s oceans (Li et al., 1983; Joint & Pomroy, 1983; Waterbury et al., 1986).

Rippka et al. (1979) placed all the small unicellular cyanobacteria with ovoid to cylindrical cells that reproduced by binary transverse fission on a single plane, and that lacked sheaths within the genus Synechococcus. Marine isolates of Synechococcus are a heterogeneous assemblage that falls into two distinct subgroups. One group, Marine Cluster B, lacks phycoerythrin, contains phycocyanin and does not have elevated salt requirements for growth. An example of this group is strain SNC1. Strains of this type have been isolated from coastal waters within the continental shelf margin but have not been reported or cultivated from the open
ocean (Waterbury et al., 1986). The possession of phycocyanin as their primary light harvesting pigment places strains of this type at a spectral disadvantage in clear oceanic water and is thought to account for their inability to compete successfully in the open ocean (Wood, 1985). It is thought these strains are halotolerant, terrestrial forms that have invaded the marine environment (Waterbury et al., 1986). The second group of marine Synechococcus, Marine Cluster A, contains phycoerythrin as its primary light harvesting pigment and has elevated salt requirements for growth. An example from this group is strain DC 2 (a.k.a. WH 7803).

The first isolation of marine cyanophages was from Romanian coastal waters of the Black Sea (Moisa et al., 1981). Water samples were collected from a variety of locations and simply screened against specific host cultures. Almost a decade passed until it was shown that cyanophages could be easily isolated from seawater samples taken from a variety of locations (Suttle et al., 1990). In this study the viral size fraction (2-200 nm) was concentrated before being screened against specific host cultures. Since then, cyanophages have been isolated from a wide variety of locations and are mainly found to belong to the double stranded DNA phage family Myoviridae, although Styloviridae and Podoviridae have also been isolated (Suttle & Chan, 1993; Suttle et al., 1993; Waterbury & Valois, 1993; Wilson et al., 1993; Lu et al., 2001).

The host range of marine cyanophages has been reported to be broader than that of freshwater cyanophages (Moisa et al., 1981). In this study they reported on nine marine cyanophage isolates. It has been noted that although the data seem convincing, little information was provided on the host strains or phage purification procedures (Suttle, 2000b). In a different study of seven marine cyanophages, Suttle & Chan (1994) found two (S-PWM3 and S-PWM4)
that were capable of infecting more than one host. It was also noted in this study that Synechococcus strain DC2 was the most permissive to infection by numerous viral isolates. Interestingly, Waterbury & Valois (1993), Suttle & Chan (1993), and Lu et al. (2001) have isolated cyanophages capable of infecting hosts from both Marine Cluster A and Marine Cluster B. In their study of cyanophages in estuaries, Lu et al. (2001) found that cyanophages infecting marine Synechococcus spp. were not able to infect freshwater Synechococcus spp.

It is now recognized that cyanophages can be extremely abundant in seawater with the highest abundance of cyanophages infecting Strain DC2 reported to be $\sim 10^6$ mL$^{-1}$ during a bloom of Synechococcus spp. in the Gulf of Mexico (Suttle et al., 1996). Working in the coastal waters of the Gulf of Mexico, Suttle & Chan (1993) found lytic cyanophages in every virus community screened with viruses infecting the red phycoerythrin-rich Synechococcus spp. strains (DC2, SYN 48) being particularly common. Cyanophages infecting DC2 were found to be temporally variable, ranging from undetectable levels to as high as $1.9 \times 10^5$ mL$^{-1}$; most often abundances were in excess of $10^5$ mL$^{-1}$. A further study, along a transect in the Gulf of Mexico, found that cyanophage abundances decreased with depth and distance offshore (Suttle & Chan, 1994). In studies done in Woods Hole Harbor, Waterbury & Valois (1993) found infectious cyanophage abundances at times exceeded $10^4$ mL$^{-1}$. Several studies on the coastal waters of British Columbia during summer reported abundances of viruses infecting DC2 that were frequently lower. Baur (unpublished data) found the abundance to be from $9 \times 10^{-3}$ to $9 \times 10^{-2}$ lytic agents mL$^{-1}$, Garza & Suttle (unpublished data) reported abundances between 10 and 5000 mL$^{-1}$, and Ortmann et al. (2002) found on average $3 \times 10^4$ mL$^{-1}$. Interestingly, the abundance of cyanophages that infect non-phycoerythrin containing Synechococcus spp. is typically
undetectable (Suttle, 2000b). One exception is strain SNC1 (= UTEX 2624) for which infectious cyanophages were found to follow a seasonal pattern similar to cyanophages that infect DC2, although the maximum abundance was only about 10% of that titered on DC2 (Suttle & Chan, 1994).

The high abundances and ecological importance of cyanophages led researchers to try and estimate the mortality caused by these viruses in nature. A number of methods were used which, reassuringly, produced similar results. The first method involved using TEM to determine the percentage of infected cells (Proctor & Fuhrman, 1990). In a study of seawater samples collected from a wide variety of locations they found, on average, 1.5% of cyanobacteria contained mature phage. Using the infection characteristics of the marine bacterium, *Cytophaga marinoflava*, in which the final visible stage of assembled phage represents 10% of the latent period, they calculated that 15% of cyanobacteria were infected at any given time. Waterbury & Valois (1993) claimed this number was an overestimate, and argued that since freshwater cyanophages infecting filamentous cyanobacteria are visible for half the latent period (Padan & Shilo, 1973; Sherman *et al.*, 1976), the actual number of infected cyanobacteria was closer to 3%. It is interesting to note that in a study of a virus infecting a freshwater unicellular cyanobacterium, the virus particles were visible within the infected cells for 25% of the lytic cycle (MacKenzie & Haselkorn, 1972b). If mature phage were visible for 25% of the lytic cycle and 1.5% of cells were visibly infected (Proctor & Fuhrman, 1990) it would mean that in total ca. 6% of *Synechococcus* cells would be infected in natural communities. These calculations emphasize the need to have appropriate conversion factors for
estimating the % of infected cells from data based on the frequency of cells containing visible virus particles.

Using a different method, Suttle and Chan (1994) based their mortality calculations on the assumption that the rates of removal and production of infectious cyanophages were balanced. Assuming a burst size (the number of viral progeny produced per lytic event) of 250 (Suttle & Chan, 1993) and an estimated decay rate of 2 d⁻¹, ca. 5 to 7 % of cells would be lysed daily by viruses. It was later discovered that the decay rate and burst size used in these calculations, while not changing the final result, were likely overestimated and underestimated by three-fold, respectively (Suttle, 2000b). In a different study of two stations in the Gulf of Mexico, decay rates for infectious cyanophages were reported to be 0.53 and 0.75 d⁻¹ and the burst size 81 (Garza & Suttle, 1998). These data implied that 1 and 8 %, respectively, of Synechococcus cells were lysed daily, to balance the decay rates.

A third method used is based upon the contact rates between viruses and potential host cells. Contact rates are based upon transport theory (Murray & Jackson, 1992) and are directly proportional to the product of viral and host abundances. As the infection rate cannot exceed the rate at which viruses encounter or adsorb to potential host cells, these rates provide maximum estimates of the viral infection rate (Suttle, 1994). Calculations based on contact rates showed that ca. 5 to 14 % of Synechococcus spp. were contacted daily by infectious cyanophages in offshore waters (Suttle & Chan, 1994). The similar results between this method and others also suggested that in offshore waters most Synechococcus cells were susceptible to infection and that most contacts resulted in infection. In contrast, in nearshore water ca. 80 % of Synechococcus
cells were contacted daily by infectious cyanophages. Consequently, only about 1% of contacts would have to result in infection in order to produce enough cyanophages to balance estimated virus removal rates. It was concluded that cyanophages are responsible for lysing a small but significant portion of the *Synechococcus* population on a daily basis.

While marine viruses, including cyanophages, have been shown to cause host mortality, there are many factors that influence the magnitude of these estimates. One important factor is the viral replication cycle. The method of viral replication by which most viruses in marine communities are produced is thought to be lytic as opposed to lysogenic (as reviewed in Suttle, 2000a). The lytic cycle describes the process by which the virus immediately begins to replicate following introduction of viral nucleic acid into the host cell. Viral nucleic acids and proteins are made, assembled into viral progeny, and released through lysis of the host cell. The length of the lytic cycle and the burst size (the number of viruses produced per lytic event) can significantly influence the inferred ecological effects of viral replication. The equation for viral production is: \( R = V \times P \times (B)^{-1} \), where \( R \) = # host cells lysed (No. mL\(^{-1}\) d\(^{-1}\)), \( V \) = viral abundance (No. mL\(^{-1}\)), \( P \) = viral production rate (d\(^{-1}\)), and \( B \) = burst size.

The length of the lytic cycle is also important because it affects the rate of viral production. As infected unicellular cyanobacteria photosynthesize and divide until cell lysis (Suttle & Chan, 1994), a lytic cycle that is longer than the division rate of the host will allow viruses to be passed to daughter cells and increase the number of viral progeny from a single infection event. Also the length of the lytic cycle is thought to serve as a strategy for the
optimization of infectivity of newly produced cyanophage particles in the marine environment (Suttle et al., 1996; Garza & Suttle, 1998; Suttle, 2000b).

Burst size is also important because the number of viruses produced represents the potential for other cells to be infected. Estimates of viral mediated mortality, calculated from estimates of viral decay rates or production rates are dependent upon reliable estimates of burst size. Burst-size data for marine cyanophages are very limited. *Synechococcus* strain BBC1 infected with cyanophage S-BBS1 resulted in a burst size of approximately 250 particles (Suttle & Chan, 1993). Weinbauer (cited in Garza & Suttle, 1998) reported a burst size of 81 based upon a TEM survey of *Synechococcus* cells completely filled with VLPs and Wilson et al. (1996) reported a burst size of 45 when infecting *Synechococcus* DC2 with cyanophage S-PM2.

Burst size and length of lytic cycle are sensitive to the physiological status of host cells and can be affected by factors such as growth rate, nutrient limitation, salinity, and temperature (Proctor et al., 1993; Wilson et al., 1996). There are very little data for *Synechococcus* on the effects of physiological state on viral replication. One of the most important factors affecting physiological state is growth rate and one of the most important factors controlling growth rate is light (reviewed in Suttle, 2000a).

Previously, there has been little work on the effect of light on viral replication in cyanobacteria and eukaryotic algae. Studies of freshwater cyanobacteria have shown that cyanophage replication requires ATP and, therefore, is reduced in darkness (Padan et al., 1970; Sherman & Haselkorn, 1971; Adolph & Haselkorn, 1972); however, the effect of darkness on
viral replication varies among cyanobacteria. In some filamentous freshwater cyanobacteria, such as *Plectonema boryanum*, the length of the lytic cycle is increased and the burst size is decreased in darkness (Padan *et al.*, 1970; Sherman & Haselkorn, 1971), while, in freshwater unicellular cyanobacteria (MacKenzie & Haselkorn, 1972c; Allen & Hutchinson, 1976) and the filamentous cyanobacterium *Nostoc* (Adolph & Haselkorn, 1972), viral replication has been shown to essentially stop in darkness. Viral replication is also affected by light in unicellular eukaryotic algae. Viral replication in the prasinophyte *Micromonas pusilla* is significantly reduced or prevented entirely in darkness (Waters & Chan, 1982; Cottrell & Suttle, unpublished data), whereas in the haptophyte *Phaeocystis pouchetii* viral replication proceeds in darkness, but the burst size is reduced (Bratbak *et al.*, 1998). Despite the body of work on light effects on viral replication, past studies have focused on contrasting viral replication at a single irradiance to viral replication in darkness. To date, there are no data on how viral replication is affected under light-limited growth.

The host range of cyanophage isolates can vary widely (Suttle & Chan, 1993; Waterbury & Valois, 1993). One interesting cyanophage described in Suttle & Chan (1993) is cyanomyovirus S-PWM3 that infects Marine Cluster A *Synechococcus* DC2 and Marine Cluster B *Synechococcus* SNC1. To date, there are no data on how replication differs in a virus propagated on different host strains. It was also noted by Suttle & Chan (1994) that *Synechococcus* DC2 was the most permissive host and that cyanomyoviruses S-PWM1 and S-PWM3 both infect it. There are also no data on how viral replication differs between different viruses propagated on the same host.
This thesis tackles several key issues in the relationship between *Synechococcus* spp. and cyanophages. Specifically, I examine how irradiance (and subsequently host-cell growth rate) affects the length of the lytic cycle and burst size in the cyanobacterium *Synechococcus* strain DC2. Second, I investigate the lytic cycle in two cyanophages (S-PWM1 and S-PWM3) when infecting the same host (*Synechococcus* DC2). Finally, I compare the lytic cycle and burst size of a cyanophage (S-PWM3) when infecting two different *Synechococcus* strains (*Synechococcus* DC2 and SNC1). Together these investigations shed light on viral-mediated mortality of *Synechococcus* spp. and provide important data for understanding the interactions between cyanophages and cyanobacteria in nature.
MATERIALS & METHODS

Cyanophage and *Synechococcus* spp. isolates

The cyanobacteria used in this study were *Synechococcus* strains DC2 (=WH7803) and SNC1 isolated from the North Atlantic (33° 45' N, 67° 30' W) and from hypersaline Laguna Madre, Texas (27° 38' N, 97° 15' W), respectively (Suttle & Chan, 1993). The cyanophages used in this study were the cyanomyoviruses S-PWM1 and S-PWM3 (Suttle & Chan, 1993) and were isolated near Port Aransas, Texas, USA. Based on previous host range studies, S-PWM1 can lyse *Synechococcus* DC2 while S-PWM3 can lyse both *Synechococcus* DC2 and SNC1 (Suttle & Chan, 1993). Stocks of these phytoplankton and virus strains are maintained in the laboratory of Dr. Curtis Suttle, at the University of British Columbia.

Growth rate versus irradiance

Growth rate versus irradiance curves were determined by growing the cyanobacterial isolates in triplicate 250 mL tissue culture flasks (Falcon) using 200 mL of SESAW (Cottrell & Suttle, 1993) an ESAW based medium (Harrison *et al*., 1980) supplemented with 5 mM Tris-HCl (pH 7.7) and 10 nM Na$_2$SeO$_3$. Samples were incubated in growth chambers at 20 °C and light was attenuated with neutral density screening to achieve quantum scalar irradiances of 913, 498, 232, 133, 40 and 12 μmol quanta m$^{-2}$ s$^{-1}$ photosynthetically active radiation (PAR). Irradiance was measured with a quantum meter (Biospherical Instruments Inc.) using a 4π sensor immersed in culture vessels filled with ESAW. Cells were pre-adapted to each irradiance for 8 doublings prior to experimentation. Maintenance stocks were kept in exponential growth at 250 μmol quanta m$^{-2}$ s$^{-1}$ and transferred before senescence.
Experiments were initiated by inoculating exponentially growing cultures into fresh media to a cell density of ca. $10^5$ cells mL$^{-1}$. Every 12 hours, flasks were swirled and 10 µL were withdrawn for cell enumeration. Cell counts were done with a haemocytometer in conjunction with epifluorescence microscopy using a wide-green filter set (510-550 nm). Cultures were maintained in exponential growth while average growth rate, $\mu$, was determined from a linear regression of log cell number vs. time.

**Adsorption of virus to host**

Approximately $10^6$ cells from triplicate cultures of exponentially growing cells were combined with viruses to achieve a ratio of infectious virus to host (MOI) of 0.01 in 300 mL polystyrene tissue culture flasks (Falcon). A low MOI was used so that each infection was the result of only one cyanophage adsorbing to a host.

The high adsorption coefficients of cyanophages (Ackermann & DuBow, 1978) required samples to be taken frequently and made it impractical to use a most probable number (MPN) assay (Taylor, 1962; Suttle & Chan, 1993) to estimate viral adsorption kinetics. Instead, following virus addition, 2 mL samples were taken every 10 min, fixed with 2 % (final concentration) biological-grade gluteraldehyde, and centrifuged at 10,000x g for 2 min within 5 min of sample collection. Typically within 24 or 48 h, viruses in 0.8 mL of supernatent were filtered onto 0.02 µm pore-size Anodisc membranes (Whatman) and stained following the protocol of Noble & Fuhrman (1998). Filters were placed, sample side up, on 100 µL of 2.5 % SYBR Green I (Molecular Probes, formula proprietary) in plastic Petri dishes. Filters were incubated for 15 min and then blotted dry with a Kimwipe, before being mounted on a glass slide.
using a solution of 50 % glycerol and 50 % Phosphate Buffered Saline (0.05 M Na₂HPO₄, 0.85 % NaCl, pH 7.5) with 0.1 % p-phenylenediamine (Sigma Chem. Co., made fresh daily from frozen 10 % aqueous stock). The slides were stored at -20 °C until examined by epifluorescence microscopy within 7 days. Estimates of viral abundance on frozen SYBR-Green stained samples slides stored at -20 °C are stable for at least a year (unpublished data).

For each slide, 20 fields were selected randomly and a total of >200 viruses were counted at 1000 x magnification using an Olympus BX60 epifluorescence microscope with a wide-blue excitation (450-480 nm) filter. Viral abundance was calculated using the following equation: 
\[ N_v = P_f \times (A_s/A_f) \times (1/V_s) \]
where \( N_v \) = abundance (mL⁻¹), \( P_f \) = number of fluorescent particles per field, \( A_s \) = filtration area of filter (μm²), \( A_f \) = area of field (μm²), and \( V_s \) = volume of sample (mL) (Suttle & Chan, 1993; Cottrell & Suttle, 1995).

The adsorption coefficient (Ca) was calculated using the following equation: 
\[ C_d (mL \text{ min}^{-1}) = a / N \]
where \( N \) (cell mL⁻¹) is the abundance of host cells and \( a \) (min⁻¹) is the slope as determined by linear regression of the natural logarithm of the remaining fraction of free viruses plotted against sampling time and multiplied by 2.3 (Suttle & Chan, 1993).

**One-step growth experiments**

The length of the lytic cycle and burst size was determined by one-step growth experiments. Due to the interest in determining the number of infectious particles produced by lysis and the fact that the length of the lytic cycle of cyanophages is on the scale of hours (e.g. Ackerman & Dubow, 1978; Sherman & Brown, 1978; Padan & Shilo, 1973) the MPN assay was
chosen as the most appropriate method for cyanophage enumeration. Experiments were initiated when cell concentrations of exponentially growing *Synechococcus* spp. cultures were $\sim 10^6$ mL$^{-1}$.

In the experimental treatment, cyanophages were added to triplicate cultures (600 mL polystyrene Falcon tissue culture flasks) to achieve an MOI of 10 to ensure that all cells were infected. For the controls, the equivalent volume of ESAW was added to another set of triplicate cultures.

Following the addition of viruses to uninfected triplicate cultures, adsorption was allowed to occur for 1 h. Subsequently 200 µL of each infected culture was added to flasks containing 200 mL of SESAW. This 1,000-fold dilution decreased contact rates to greatly reduce adsorption of newly produced viruses to host cells. At predetermined time intervals, 5 mL were withdrawn from each flask to determine the number of infected host cells plus free infective viruses by MPN assay. Also, at approximately 1, 2, and 3 h after the start of the experiment, 5 mL were withdrawn, filtered through a Millipore 0.2 µm pore size filter, and used in the MPN assay for enumeration of free infectious viruses. Samples to be assayed were diluted with chilled (4 °C) culture medium (ESAW) using a series of ten-fold dilution steps. Subsamples (100 µl) of each dilution (or growth medium for the controls) were added to 16 wells in a 96-well microtiter plate (Corning Glass Works, No. 25860) containing 200 µL of exponentially growing host. The microtiter plates were incubated at $\sim 50$ µmol quanta m$^{-2}$ s$^{-1}$ and checked daily, for 7-9 days, for the visible occurrence of culture lysis. The number of culture wells in which lysis occurred was scored, and the abundance of infectious viruses determined using a modified version of Hurley & Roscoe’s (1983) BASIC program for Windows (Passmore *et al*., 2000).
The length of the latent period was determined as the time between viral adsorption and the beginning of the burst event. The length of the lytic cycle was calculated as the time between virus adsorption and the end of the burst event. Burst size was determined by the following equation: $B = (V_f - V_s)/H$, where $B =$ burst size, $V_f =$ abundance of free viruses at end of experiment, $V_s =$ abundance of free viruses at start of experiment, and $H =$ host cell abundance at start of experiment.
RESULTS

Growth rate data

The growth rate of *Synechococcus* DC2 as a function of irradiance is shown in Figure 1. The maximum growth rate (μmax) was 1.61 d⁻¹. Growth limitation began to occur at irradiances < 300 μmol quanta m⁻² s⁻¹. Irradiances at near μmax (250 μmol quanta m⁻² s⁻¹) and 10 % μmax (19 μmol quanta m⁻² s⁻¹) were chosen for experiments.

Figure 1. Growth rate of *Synechococcus* DC2 grown at varying light irradiances.

Adsorption Kinetics Data

The adsorption kinetics of cyanophages on their hosts were well described by a logarithmic decrease in the percentage of free virus with time (Figure 2). However, in the case
of S-PWM1 adsorbing to *Synechococcus* DC2 grown at $\mu_{\text{max}}$, it was decided to use only the first three points in the graph as this represented the adsorption of over 90% of the free virus (Figure 2A). The coefficients of adsorption ($C_d$) varied widely between *Synechococcus* grown at $\mu_{\text{max}}$ and 10 $\%\mu_{\text{max}}$ (299 and 19 $\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$, respectively) (Table 1). The $C_d$ for S-PWM1 adsorbing to *Synechococcus* DC2 at saturating irradiance was approximately 12-fold higher than for cells grown under light limitation. In contrast, $C_d$'s for S-PWM1 and S-PWM3 adsorbing to *Synechococcus* DC2 and S-PWM3 adsorbing to *Synechococcus* SNC1 both growing at $\mu_{\text{max}}$ were within a factor of 2 of each other. A one-way ANOVA showed the mean $C_d$ of the four experiments to be significantly different ($P < 0.001$). A Tukey Test (Tukey, 1949) showed that, except for S-PWM3 adsorbing to *Synechococcus* DC2 and SNC1 grown at $\mu_{\text{max}}$ ($P = 0.845$), all the $C_d$'s were significantly different (Table 2).

**One step-growth curve data**

Following infection, the abundance of infectious cyanophages increased in a one-step pattern (Adams, 1959) (Figure 3). A summary of the data on latent period, length of lytic cycle, and burst size estimated from these experiments is given in Table 3. An exception to the one-step pattern was the data for S-PWM1 infecting *Synechococcus* DC2 grown under high light (299 $\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$) (Figure 3A). The abundance of infectious cyanophages appeared to continue beyond 17 h even though culture lysis was complete (data not shown). Thus, for SPWM-1 infecting *Synechococcus* DC2 at near $\mu_{\text{max}}$ (1.1 d$^{-1}$), the length of lytic cycle and burst size were estimated to be 17 h and 328, respectively. Although phage abundance in Figure 3A did not saturate, the fact that complete culture lysis had occurred (data not shown) indicated that phage production was complete by 22 h. Under growth limiting irradiances of 19 $\mu\text{mol quanta}$
Figure 2. Adsorption kinetics for: A. S-PWM1 adsorbing to *Synechococcus* DC2 grown at 299 μmol quanta m⁻² s⁻¹, B. S-PWM1 adsorbing to *Synechococcus* DC2 grown at 19 μmol quanta m⁻² s⁻¹, C. S-PWM3 adsorbing to *Synechococcus* DC2 grown at 299 μmol quanta m⁻² s⁻¹, and D. S-PWM3 adsorbing to *Synechococcus* SNC1 grown at 299 μmol quanta m⁻² s⁻¹. Symbols represent individual replicates. Lines are linear regression through each of the triplicate experiments.

m⁻² s⁻¹ (μ = 0.44 d⁻¹) viral production began at about the same time but the calculated burst size was approximately half that in the high-light culture (151 viruses per lytic event) (Figure 3B).
Table 1. Summary of adsorption coefficients ($C_d$) using the data presented from Figure 2.

<table>
<thead>
<tr>
<th>Host</th>
<th>Irradiance (μmol quanta m$^{-2}$ s$^{-1}$)</th>
<th>Cyanophage</th>
<th>Adsorption coefficient (mL min$^{-1}$)</th>
<th>Std Dev</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus DC2</td>
<td>299</td>
<td>S-PWM1</td>
<td>$1.26 \times 10^{-8}$</td>
<td>$1.88 \times 10^{-9}$</td>
<td>3</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>16</td>
<td>S-PWM1</td>
<td>$1.03 \times 10^{-9}$</td>
<td>$3.75 \times 10^{-10}$</td>
<td>3</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>299</td>
<td>S-PWM3</td>
<td>$9.04 \times 10^{-9}$</td>
<td>$4.33 \times 10^{-10}$</td>
<td>3</td>
</tr>
<tr>
<td>Synechococcus SNC1</td>
<td>299</td>
<td>S-PWM3</td>
<td>$9.72 \times 10^{-9}$</td>
<td>$3.34 \times 10^{-10}$</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Summary of pairwise multiple comparisons (Tukey test) for adsorption coefficients.
Statistically significant differences (P<0.05) are marked with a *.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>S-PWM1 adsorbing to DC2 (low light)</th>
<th>S-PWM3 adsorbing to DC2 (high light)</th>
<th>S-PWM3 adsorbing to SNC1 (high light)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-PWM1 adsorbing to DC2 (high light)</td>
<td>&lt;0.001*</td>
<td>0.010*</td>
<td>0.030*</td>
</tr>
<tr>
<td>S-PWM1 adsorbing to DC2 (low light)</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>S-PWM3 adsorbing to DC2 (high light)</td>
<td></td>
<td></td>
<td>0.845</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of the lytic cycle of cyanophages grown on different hosts and under high and low irradiance.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Irradiance (μmol quanta m$^{-2}$ s$^{-1}$)</th>
<th>Cyanophage</th>
<th>Latent Period (h)</th>
<th>Lytic Cycle (h)</th>
<th>Burst Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus DC2</td>
<td>299</td>
<td>S-PWM1</td>
<td>7</td>
<td>17</td>
<td>328</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>19</td>
<td>S-PWM1</td>
<td>7</td>
<td>13</td>
<td>151</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>299</td>
<td>S-PWM3</td>
<td>5</td>
<td>13</td>
<td>135</td>
</tr>
<tr>
<td>Synechococcus SNC1</td>
<td>299</td>
<td>S-PWM3</td>
<td>7</td>
<td>17</td>
<td>8</td>
</tr>
</tbody>
</table>
Figure 3. One-step growth curves for: A. S-PWM1 infecting Synechococcus DC2 (μmax), B. S-PWM1 infecting Synechococcus DC2 (25% μmax), C. S-PWM3 infecting Synechococcus DC2 (μmax), D. S-PWM3 infecting Synechococcus SNC1 (μmax). Error bars represent standard deviation.
The lytic cycle of cyanophage SPWM-3 infecting *Synechococcus* DC2 grown under high light was very different to that for S-PWM1 with viral production beginning within 2 h of each other. For S-PWM-3, lysis began at 5 h post infection, the length of the lytic cycle was ~13 h, and the burst size was determined to be 135. The control culture grew at approximately 1.1 d$^{-1}$. For cyanophage SPWM-3 infecting *Synechococcus* SNC1 grown at 299 μmol quanta m$^{-2}$ s$^{-1}$, host-cell lysis began at 7 h post infection with the cells swelling slightly beforehand (Figure 4). Upon lysis many cells still fluoresced but had irregular shapes. The length of the lytic cycle was ~17 h and the burst size was ca. 8. The uninfected cultures grew at an average rate of 1.32 d$^{-1}$. 
**Figure 4.** Progression of uninfected (left) and infected (right) *Synechococcus* SNC1 cells with S-PWM3. Numbers on side represent time in hours since infection. Samples were unstained and observed at 1000 X using epifluorescence microscopy with a wide green filter set.
DISCUSSION

Despite the fact that unicellular cyanobacteria are major primary producers in the world's oceans, data on the interactions of marine cyanophages with their hosts are largely lacking. This thesis demonstrates that light plays a significant role in the adsorption of cyanophages to their hosts, the length of the lytic cycle, and the burst size. This research also demonstrates that different strains of cyanomyoviruses have different infection characteristics when infecting the same host grown at the same growth rate. Finally, there appears to be significant differences in the length of lytic cycle and the burst size for a given virus infecting strains of *Synechococcus* from marine clusters A and B. These findings are considered in detail, below.

**Adsorption kinetics**

The adsorption kinetics observed in this study fell within a very narrow range relative to the wide range of $6.3 \times 10^{-12}$ and $2.5 \times 10^{-5}$ ml/min$^{-1}$ reported for tailed bacteriophages (Ackermann & DuBow, 1987). However, there was a significant ca. 12-fold difference between the adsorption coefficients for S-PWM1 adsorbing to *Synechococcus* DC2 grown at high and low irradiances ($P < 0.001$) indicating host physiology can have a marked affect on the rate of adsorption of cyanophages to their hosts. These results imply that in DC2 the number of receptors are lower, the configuration of the receptors has changed, or a cofactor required for adsorption is reduced under light-limited growth.

While these results are the first to demonstrate that light limitation affects marine cyanophage adsorption, similar results have been shown for freshwater cyanophage-cyanobacterium systems. Cseke & Faras (1979) found that the adsorption rate of cyanophage
AS-1 to *Anacystis nidulans*, placed temporarily in the dark, was only about half that of cells in the light. The rate of adsorption increased again when cells were transferred again to the light. The coefficients for other marine cyanophages adsorbing to hosts grown at low irradiances are also relatively low. For S-BBS1 adsorbing to *Synechococcus* BBC1 (grown at 40-50 μmol quanta m$^{-2}$ s$^{-1}$) the adsorption coefficient was 3.94 x 10$^{-9}$ mL min$^{-1}$ (Suttle & Chan 1993) while for S-PM2 adsorbing to *Synechococcus* DC2 (grown at 5-36 μmol quanta m$^{-2}$ s$^{-1}$) the adsorption coefficient was 6.06 x 10$^{-9}$ mL min$^{-1}$ (Wilson *et al.*, 1996).

The adsorption coefficients for S-PWM1 and S-PWM3 adsorbing to *Synechococcus* DC2 grown under high light were significantly different (1.26 x 10$^{-8}$ and 9.04 x 10$^{-9}$ mL min$^{-1}$ respectively, P = 0.01). While the number or configuration of receptors on *Synechococcus* DC2 may be similar, differences in host specificity of the two cyanomyoviruses (Suttle & Chan, 1993) suggest that there are differences in receptor recognition between the two viruses. While the adsorption coefficients for S-PWM1 adsorbing to DC2 and S-PWM3 adsorbing to SNC1 both grown at μmax are significantly different (P = 0.03), the difference is negligible compared to the differences between S-PWM1 adsorbing to DC2 grown under high and low light.

It is interesting that there was not a significant difference in adsorption coefficients for cyanophage S-PWM3 adsorbing to *Synechococcus* DC2 and SNC1 as these host organisms are different in size. Measurements with a light microscope showed that in this study, *Synechococcus* DC2 was ~1 μm in diameter while SNC1 had a diameter of about 0.5 μm. Based on increased surface area, one would expect a larger number of receptors to be displayed on *Synechococcus* DC2.
As reviewed by Suttle (2000b), the rate of cyanophage adsorption to their hosts plays an important part in the rate of cyanophage production. The fact that adsorption coefficients vary due to changes in light intensity and hence growth rate indicates that, in the marine environment, viral production may vary due to host growth rates. While it should be noted that differences in adsorption coefficients were obtained using cultures that had been acclimated to irradiances, the immediate effect of changing irradiances on adsorption rates observed by Cseke & Faras (1979) suggests that adsorption kinetics in natural communities will change on a diel basis.

One-step growth data

The values obtained for the length of the lytic cycle and the burst size in my experiments fall within those previously reported in the literature. Burst sizes range from 40-300 for cyanophages (Table 4), 5-610 (185 average) for marine bacteriophages (Børsheim, 1993), and 2-2000 (50-100 mean) for the tailed bacteriophages (Ackermann & Dubow, 1978). Typically the length of the lytic cycle for cyanophages is measured in hours (Table 4) as opposed to minutes for bacteriophages (Ackerman & DuBow, 1978).

A comparison of the infection characteristics of S-PWM1 infecting *Synechococcus* DC2 grown at 299 and 19 μmol quanta m$^{-2}$ s$^{-1}$ shows light limitation seems to affect the length of the lytic cycle and the burst size. These results do not agree with observations, for marine heterotrophic bacteria, that the length of the latent period is negatively correlated with the growth rate of the host (Proctor *et al.*, 1993; Guixa *et al.*, 1996).
Table 4. Summary of infection characteristics of cyanophages from this study and the literature.

<table>
<thead>
<tr>
<th>Host</th>
<th>Virus</th>
<th>Length of Lytic Cycle (h⁻¹)</th>
<th>Burst Size</th>
<th>Irradiance (μmol quanta m⁻² s⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus DC2</td>
<td>S-PWM1</td>
<td>17</td>
<td>328</td>
<td>299</td>
<td>Present Study</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>S-PWM1</td>
<td>13</td>
<td>151</td>
<td>19</td>
<td>Present Study</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>S-PWM3</td>
<td>13</td>
<td>135</td>
<td>299</td>
<td>Present Study</td>
</tr>
<tr>
<td>Synechococcus SNC1</td>
<td>S-PWM3</td>
<td>17</td>
<td>8</td>
<td>299</td>
<td>Present Study</td>
</tr>
<tr>
<td>Synechococcus BBC1</td>
<td>S-BBS1</td>
<td>9</td>
<td>250</td>
<td>40-50</td>
<td>Suttle &amp; Chan, 1993</td>
</tr>
<tr>
<td>Synechococcus DC2</td>
<td>S-PM2</td>
<td>9</td>
<td>45</td>
<td>5-36</td>
<td>Wilson et al., 1996</td>
</tr>
<tr>
<td>Synechococcus cedrorum</td>
<td>AS-1M</td>
<td>12</td>
<td>40</td>
<td>NA</td>
<td>Sherman et al., 1976</td>
</tr>
<tr>
<td>Synechococcus cedrorum</td>
<td>AS-1</td>
<td>15</td>
<td>50</td>
<td>NA</td>
<td>Safferman et al., 1972</td>
</tr>
<tr>
<td>Anacystis nidulans</td>
<td>AS-1</td>
<td>16</td>
<td>75</td>
<td>NA</td>
<td>Pearson et al., 1975</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>N-1</td>
<td>14</td>
<td>100-300</td>
<td>NA</td>
<td>Adolph &amp; Haselkorn, 1971</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>N-1</td>
<td>17</td>
<td>100-200</td>
<td>60</td>
<td>Amla et al., 1987</td>
</tr>
</tbody>
</table>

Other studies have also shown that light affects viral replication. Unfortunately most studies use incomparable units of irradiance (units in lux or foot-candles and wavelength is not provided) and predominantly focus on differences in viral replication between hosts exposed to light or in complete darkness. With cyanophage AS-1M, MacKenzie & Haselkorn (1972c) found viral synthesis to be completely inhibited by darkness. Similarly, with cyanophage AS-1 infecting Anacystis nidulans, the rate of phage production decreased at lower light intensities (173 vs 2700 lux) and no lysis occurred under very low light (108 lux) (Allen & Hutchison, 1976). In the filamentous cyanobacteria Plectonema boryanum, the length of the lytic cycle increased and the burst size decreased in darkness (Padan et al., 1970; Sherman & Haselkorn,
Similarly, with cyanophage AS-1 infecting *Anacystis nidulans*, dark incubation of infected cells for 5 h increased the latent period by 1-2 h, the eclipse period by 1 h, and reduced the burst size to 2% of that observed in the light (Allen & Hutchison, 1976). It is interesting to note that the reported burst size and length of the lytic cycle for cyanophage S-BBS1 infecting *Synechococcus* BBC1 represent a host potentially grown under light limitation (36 μmol quanta m⁻² s⁻¹) and thus might be different if the host was grown at μmax. Based on the results of my research, the length of the lytic cycle and burst size for S-BBS1 could have been larger if the host had been grown at a higher irradiance. My results demonstrate the need to consider the physiological status of the host cells when attempting to model mortality due to viruses.

Effects of light intensity on viral replication have also been seen with viruses infecting eukaryotic phytoplankton. Viral replication in the prasinophyte *Micromonas pusilla* was significantly reduced or prevented entirely in darkness (Waters and Chan, 1982; Cottrell and Suttle, unpublished data). In the haptophyte *Phaeocystis poucheti*, viral replication was found to proceed in darkness, however the burst size was reduced from a mean of 370 to 100 viruses per infected cell (Bratbak et al., 1998).

I emphasize that in the experiments described in this thesis, the cultures were adapted to low-light conditions for at least 8 doublings prior to experiments. While this ensures that the cells were in steady-state, the growth conditions were not analogous to those experienced in the marine environment. It should also be noted that while growth irradiance of the host seems to be one of the most important factors in cyanophage replication (reviewed in Suttle, 2000b) nutrient limitation can also affect cyanophage production. For example, the burst size of S-
WHM1 was reduced, whereas that of S-PM2 was not, when these cyanophages were amplified by infection and lysis of *Synechococcus* DC2 grown under nitrate limitation (Wilson *et al.*, 1996). In the same study, titers of cyanophages S-PM2, S-WHM1, and S-BM1 amplified on phosphate-depleted *Synechococcus* DC2 ranged from 59 to 91% of nutrient-replete controls. Detailed examination of the amplification of S-PM2 showed that the lytic cycle remained the same and that the decreased burst size was due not only to decreased number of viral progeny released per cell but was also because only 55% of infected cells produced progeny.

It is interesting that the length of lytic cycle and burst size for cyanophage S-PWM3 infecting DC2 grown under high light are closer to those of S-PWM1 infecting DC2 under light limitation. These results stress that even under the same physiological conditions, infection characteristics of different cyanophages capable of infecting the same host can vary markedly. Differences in infection characteristics by different cyanophages infecting the same host have been observed in other studies. In cyanophage AS-1M (Sherman *et al.*, 1976) and AS-1 (Safferman *et al.*, 1972) infecting *Synechococcus cedrorum*, the length of the lytic cycle was found to be 12 h for AS-1M and 15 h for AS-1 and the burst size for AS-1 was 1.25-fold larger. Another example of different cyanophages infecting the same host are cyanophages SM-1 and SM-2. Both infect *Synechococcus* sp. (PCC 6911), which in the past has been mistakenly identified as *Microcystis aeruginosa* (NRC-1 = PCC 7941) (Stanier *et al.*, 1971; MacKenzie & Haselkorn, 1972a; Suttle, 2000b). Cyanophage SM-1 has a lytic cycle of 48 h and a burst size of 100 (MacKenzie & Haselkorn, 1972b), while the lytic cycle of SM-2 was simply described as being 30-40 h long (Fox *et al.*, 1976). Given the importance of host physiology, it should be noted that none of the freshwater cyanophage work adequately describes the host growth rates or
conditions. Based on the data obtained in my research, it is important to provide the growth rate before in-depth comparisons of different cyanobacteria-cyanophage systems are made.

In my study, the length of lytic cycle and burst size for cyanophage S-PWM3 was shown to be different depending on whether the virus was replicating in *Synechococcus* DC2 or SNC1. These are the first infection characteristics reported for a single virus that infects *Synechococcus* spp. from marine clusters A and B. These two taxonomically different groups have different accessory pigments and occupy different habitats (Waterbury *et al.*, 1986). Also, SNC1 is considerably smaller than DC2. In the case of S-PWM3 infecting *Synechococcus* SNC1 this is also the smallest burst size reported for a marine cyanophage. The effect of the same strain of cyanophage infecting taxonomically different hosts has also been studied in freshwater cyanobacteria. Safferman *et al.* (1972) isolated cyanophage AS-1, which infects *Synechococcus cedrorum* and *Anacystis nidulans*. Cyanophage AS-1 had a 15 h lytic cycle with an average burst size of 50 when infecting *Synechococcus cedrorum* (Safferman *et al.*, 1972) and a 16 h lytic cycle with an average burst size of 70 when replicating on *Anacystis nidulans* (Pearson *et al.*, 1975).

The MOI used for the one-step growth curve experiments in my studies was 10. Other studies with freshwater cyanophages have shown that varying the MOI has little effect on cyanophage replication. Sherman *et al.* (1976) found an MOI of greater than ten caused the burst size to drop by 10-15 particles per cell while the length of the lytic cycle remained the same; no changes in the intracellular growth pattern of the virus were detected. With cyanophage LPP-1 infecting *Plectonema boryanum*, no difference in burst size or latent period was observed when
varying MOI from 1 to 50. Allen & Hutchison (1976) also found no difference in the one-step growth curve though varying the MOI. However, in the haptophyte *Phaeocystis pouchetii*, varying the MOI was shown to affect burst size (Bratbak *et al.*, 1998).

Cyanobacterial mortality due to cyanophage infection can be calculated as: $R = V \times P \times (B)^{-1}$ where $R =$ the number of host cells lysed (No. mL$^{-1}$ d$^{-1}$), $V =$ viral abundance (No. mL$^{-1}$), $P =$ viral production rate (d$^{-1}$), and $B =$ burst size. The fact that the burst sizes observed in this study differed by up to an order of magnitude demonstrates the potential for a wide range of estimates of cyanobacterial mortality due to the possible range in burst sizes for cyanophages. It is important to make sure that the data obtained in the laboratory are comparable to the marine environment. The host cyanobacteria and viral isolates used in this study were isolated from the marine environment. Cyanomyoviruses are the most frequently isolated cyanophage in the marine environment (Suttle & Chan, 1993; Suttle *et al.*, 1993; Waterbury & Valois, 1993; Wilson *et al.*, 1993; Lu *et al.*, 2001) and *Synechococcus* spp. exist in high abundances in much of the world's oceans (e.g. Waterbury *et al.*, 1979; Johnson & Seiburth, 1979). The fact that burst size in this study ranged by about 8-fold due to host physiology and 40-fold due to host differences implies that researchers should be careful when using a burst size from a single cyanophage-cyanobacteria system to calculate cyanophage production. The results from my work indicate that there are profound differences in cyanophage replication characteristics that are affected by host strain type and physiological status. Consequently, inferences on the effect of cyanophages on host dynamics in nature are fraught with uncertainty.
CONCLUSION

The results of this thesis show that cyanophage replication in the same host varies under different growth conditions, between different viruses infecting the same hosts, and for the same strain of virus infecting the different hosts. With regards to adsorption kinetics, light limitation decreased the adsorption coefficient by an order of magnitude. By comparison, adsorption coefficients for different combinations of viruses and hosts differed by less than a factor of 2. One-step growth experiments demonstrated that light limitation caused a 50% decrease in burst size. In contrast, the burst size and latent period for different cyanophages infecting the same host differed by 50% and 30%, respectively. When the same cyanophage infected different strains of the same genus the burst size differed by a factor of 16 and the length of the latent period and lytic cycle were also very different. In conclusion, although there were differences in all of the infection characteristics measured, burst size was the factor that varied over the widest range.

These findings improve upon sparse data regarding the infection characteristics of marine cyanophages. While these data were collected in the laboratory, the ultimate aim is assess the impact of cyanophage mortality on cyanobacterial populations in the marine environment. The irradiance-induced changes in adsorption kinetics, burst size, and length of lytic cycle indicate that host physiology is an important factor affecting cyanophage replication and hence cyanobacterial mortality resulting from phage infection. Finally the fact that the adsorption kinetics, length of the lytic cycle, and burst size varied between host and cyanophage isolates indicates that host mortality may vary markedly depending on which cells and viruses predominate a given area.
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