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Viruses are abundant in aquatic environments and are known to have an important role in aquatic microbial food webs as well as in structuring the architecture of aquatic microbial communities. The magnitude of viral induced microbial mortality is comparable to the mortality caused by bacterivorous protists. Viral lysis of host cells affects the carbon flow in the microbial food web as well the organic N and P and Fe turnover. Viral lysis of the cells releases cell wall components and other cell constituents (in addition to virus particles) into the water column, which then become components of dissolved organic material. Cell lysis caused by viruses will produce nutrient elements in organic complexes, differing in nature from the inorganic nutrients released by grazers in these systems. On the whole, viral infection is thought to shift more production and respiration toward the bacteria, effectively creating a short circuit of carbon between the bacteria, viruses and components of dissolved organic material (DOM). Recycling of material also leads to better retention of nutrients in small non-sinking particles at the euphotic zone of the water column, which is especially important for nutrients that limit primary production in some areas. The potential role that viruses play in nutrient regeneration can be determined from turnover rates of viruses.

In this study viral production and turnover rates were determined in the North Atlantic and in the equatorial Pacific Ocean using a novel dilution method. New viruses were produced at a rate of 0.7×10^6 viruses $\text{ml}^{-1} \text{h}^{-1}$ in the Sargasso Sea and at a rate of $0.23 - 213 \times 10^6$ viruses $\text{ml}^{-1} \text{h}^{-1}$ in the Pacific. Viral population turnover times measured by this method were ca. 40 day^{-1} in the Sargasso Sea and $6 - 225 \text{ day}^{-1}$ in the Pacific. These rates suggest more rapid turnover rate of viruses than has been measured in earlier studies by different methods.

The effects of viral infection on cellular carbon production and protein production of a bacterial community were studied by enriching natural seawater incubations with viral concentrate (VC) and with control additions of heat-treated VC (htVC) or ultrafiltered seawater. Observations of the effects were made through direct counts of bacteria and viruses by epifluorescence microscopy using SYBR Green I nucleic acid stain, and by monitoring heterotrophic bacterial carbon production by ^3H -Leu incorporation over time. Both additions of active, infectious VC and htVC caused an increase in bacterial abundance and bacterial production over control levels. Addition of VC caused the greatest net increases in bacterial abundances, while addition of htVC resulted in the largest net increases in bacterial production. Addition of DOM by htVC enrichments directly stimulated bacterial biomass production and metabolism. Viral lysis of bacterial subpopulations in VC enriched cultures effectively generated nutrients and other energy rich material that supported the bacterial production and cell production of noninfected populations. Our experiments have shown that viruses are an active component of pelagic microbial communities. Viral infection causes lysis of a significant proportion of a bacterial community on a daily basis. Upon viral lysis, organic carbon and other nutrient elements are released in the water column. The released nutrients support the growth of the noninfected bacterial population.

PREFACE

This study was based upon research which was done under the supervision of Dr Steven Wilhelm at the University of Tennessee at Knoxville, USA and on research cruises at the North Atlantic and Pacific Ocean. The research was done during 1999 and 2000. Dr Wilhelm was the principal advisor and Dr Pertti Martikainen of the University of Kuopio, Finland, was the second advisor of this thesis.

I would like to warmly thank Dr Wilhelm for providing an opportunity for me to get familiar with this fascinating field of study and his help and encouragement in the course of this work. Working in the “Wilhelmlab” was an excellent and memorable experience both educationally and personally.

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Johanna Rinta-Kanto

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1 INTRODUCTION

1.1 VIRUS RESEARCH IN AQUATIC MICROBIAL ECOLOGY

Water column microbes represent a significant proportion of the total living biomass in marine environments. During the last two decades of the 20th century, our knowledge about the role of water column microbes and their role in aquatic food webs has increased rapidly. New knowledge has also changed the conventional view of considering viruses as solely pathogenic entities for plants or animals. The presence of viruses in aquatic environments was documented already at the beginning of the 20th century (d'Herelle, 1917); by mid-century, Spencer had given a detailed description of a marine bacteriophage (Spencer, 1955). Until 1989, when Bergh et al. (1989) reported high abundances of viruses found in aquatic environments, the viral populations were still an unknown part of aquatic ecology and protozoan grazers were assumed to be the primary cause of bacterioplankton mortality. Active investigation of the role of viruses in aquatic food webs started at the beginning of the 1990's when Proctor and Fuhrman (1990) suggested that viral infection could be a significant cause of marine bacterioplankton mortality and that viruses can also create a new significant pathway for carbon and nitrogen cycling in the marine food webs.

Currently, over ten years after the initial "awakening", viral ecology has become an important discipline in the research field of aquatic microbial ecology. Rapid development of study methods and volume of published data are strong indications of powerful research in the field.

1.2 FEATURES OF WATER COLUMN VIRUSES

Most of the free virus particles in marine systems appear to be pathogens of prokaryotes and small planktonic eukaryotes. Direct evidence that viruses infect native bacteria and cyanobacteria came from transmission electron microscopy (TEM) studies of thin-sectioned plankton, in which the assembled viruses were observed in about 0.8-4.3 % of the cells from a variety of marine habitats: high nutrient coastal waters, oligotrophic open ocean, and sinking material collected in offshore sediment traps (Proctor and Fuhrman, 1990). Information obtained from several studies (reviewed by Wommack and Colwell, 2000) indicates that most aquatic viruses are bacteriophages. Key

indicators include: viruses in marine systems have shown a lack of significant correlation with algal biomass (chlorophyll *a* concentration); changes in bacterial abundance allow for the prediction of changes in viral abundance; there is a greater abundance of bacteria over that of other planktonic hosts; and there is a predominance of viruses within virioplankton having bacteriophage-sized genomes. Typically, viruses found in transmission electron microscopy micrographs from natural water samples are small; primarily bacteriophages with tails of various length and capsid diameters varying between 20 – 250 nm (e.g. Fuhrman and Suttle, 1993).

Marine viruses are host-specific: a given type of virus usually has a restricted range of hosts, often a single species, although some viruses demonstrate potential for cross-infection of a limited number of hosts of the same genus that are related at the species level (Fuhrman, 1999; Wilhelm and Suttle, 1999). Host specificity of viruses has also an important ecological implication that will be discussed later in this section.

Microbial ecologists believe that only a small proportion (<1 %) of naturally occurring heterotrophic bacteria can be cultured in laboratory conditions (Ferguson et al., 1984; Lee and Fuhrman, 1991). This represents only a very narrow host range for viruses and thus limits our ability to interpret culture-based studies. However, culture-based studies provide direct measures of viral and bacterial activities that may provide models when looking at the whole pelagic microbial community.

1.2.1 VIRAL INFECTION AND PRODUCTION OF NEW VIRUS PARTICLES

Viruses encounter hosts in the water column through random diffusion. A successful contact will lead to the attachment of a phage to its host, determined by the receptors in the cell wall. The receptors for phages are exposed structures of the cell wall; they can be part of the lipoprotein component or the receptors can be contained on the lipopolysaccharide layer of the cell, e.g. transporter molecules can act as an entry point to a host cell (Fuhrman, 2000).

Three kinds of viral life cycles have been defined for marine bacteriophages: lytic, lysogenic and chronic infections (Figure 1). Viruses that cause lytic infection are called virulent and viruses causing lysogeny are called temperate viruses. In the lytic cycle the genome of a virulent phage replicates immediately after infection in the host cell, which leads to a bursting of the host, releasing numerous progeny viruses into the water column. In a lysogenic infection, the prophage (i.e. the genome of the infecting virus) is

carried by the host cell from generation to generation in a noninfectious form. The cell enters the lytic cycle when the prophage is spontaneously activated or induced by mutagenic agents, such as ultraviolet (UV) radiation, temperature shock, or some other stress factors (Bratbak et al., 1994; Cochran et al. 1998). Vigorous growth of the host may also induce the lytic cycle. Among both prokaryotes and eukaryotes, “chronic infections” can also occur, where viral progeny are produced and shed from the host via budding or extrusion of filaments, while host metabolism and reproduction proceed relatively unaltered (Fuhrman and Suttle, 1993).

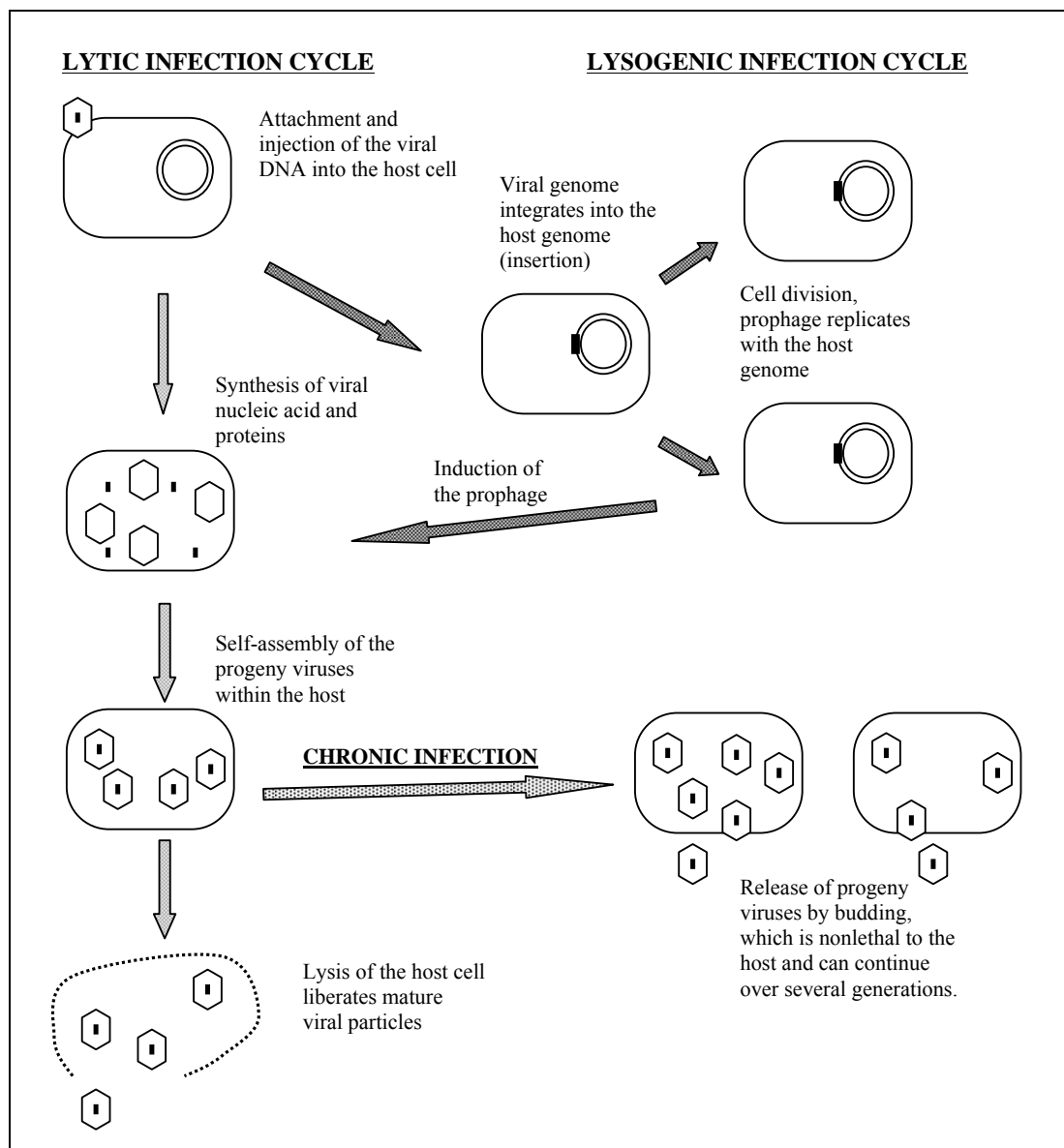


Figure 1. Viral life cycles (modified from Fuhrman, 1993).

A less well-defined type of virus-host interaction is pseudolysogeny, in which the viral nucleic acid may remain within a host cell for some time before lysis or cell destruction occurs. Pseudolysogeny may be related to host starvation, in which the virus adopts an inactive state, unable to initiate viral gene expression owing to the low energy state of the cell. Normal viral activity returns when the cell gains nutrients (Ripp et al., 1997).

It has been suggested that more than 90 % of all known phages are temperate, i.e. cause lysogeny in their hosts (Bratbak et al., 1994; Wilcox and Fuhrman 1994).

Lysogeny may be advantageous to the host since, once it has been infected, the host is immune to multiple infections by the same strain of phage. Also, lysogeny might be a survival strategy for phages to persist when the host abundance is low (Weinbauer and Suttle, 1996).

1.3 ENUMERATION OF THE NATURAL VIRAL COMMUNITY

Enumeration of virus particles is one of the basic methods in studying marine viruses. Counting has been, and still is, a central part of many studies on aquatic viral communities.

Compared to microscopy-based methods, culture-dependent methods to determine viral abundance may provide great underestimates because of constraints in culturing native marine bacteria in laboratory conditions. Development of culture-independent direct-count methods, including TEM and epifluorescence microscopy, has enabled us to assess the true size of the natural viral community more quantitatively.

TEM provides a method for studying viral morphology and the viral infection cycle in natural samples. This feature has allowed scientists to use TEM to detect visibly infected cells, i.e. bacteria that contain mature viral particles. Sample preparation for TEM requires either ultracentrifugation or ultrafiltration, which are rather time-consuming steps, require expensive equipment and include a series of steps where viruses in samples could be lost; thus TEM is not a suitable method for use e.g. on board a ship. Moreover, given the constraints of TEM, only relatively small sample volumes can be examined.

Epifluorescence microscopy combined with the use of nucleic acid binding fluorochrome stains has become an alternative to elaborate TEM counts. Even though viral particle size is beyond the theoretical limit of resolution of light microscopy, viral particles with DNA content as small as 4 to 6 kilobases can be seen by epifluorescence

microscopy (Proctor and Fuhrman, 1992). Bright-fluorescing fluorochrome binds to tightly packed viral DNA, rendering the virus visible as a brightly colored pinpoint against a dark background. The first viral counts through epifluorescence microscopy were done with Acridine Orange and 4,6-diamidino-2-phenylindole (DAPI) stains, but these stains are still not optimal for that purpose and resulted in underestimations in viral abundance. DAPI-stained viral particles do not fluoresce brightly enough under epifluorescence microscope for all particles to be enumerated.

Other techniques have been devised to overcome the problems that arise with DAPI-based methods. Hennes and Suttle (1995) developed a similar method for enumerating viruses and bacteria in water samples using a cyanine-based stain (Yo-Pro-1). Noble and Fuhrman (1998) developed a method for use of SYBR Green I for even faster, inexpensive routine counts of viruses and bacteria. This method has become widely used because it is accurate and allows for fast processing of samples. As well, the SYBR Green I stain allows for an analysis of samples that have been preserved with glutaraldehyde or formalin. In a SYBR Green I stained water sample viruses and bacteria are distinguished according to their size; against a dark background, viruses appear as bright fluorescent green, round shaped pinpricks and bacteria are usually distinctive because of their brightness and relative size. At low viral densities ($>0.5 \times 10^7$ virus ml^{-1}) SYBR Green I counts appeared to give results similar to TEM counts from the same sample. At higher viral densities ($1 \times 10^7 - 2 \times 10^7$ virus ml^{-1}), however, TEM counts give lower numbers than SYBR Green I counts (Noble and Fuhrman, 1998). Combined with better stains and staining techniques, the development of epifluorescence microscopes has allowed more accurate bacterial and viral counts.

1.3.1 ABUNDANCE AND DISTRIBUTION OF VIRUSES AND BACTERIA IN THE WATER COLUMN

1.3.1.1 Viruses

Viral abundances typically reach 1×10^{10} particles L^{-1} in coastal marine environments and $1 \times 10^7 - 1 \times 10^{11}$ L^{-1} across other marine habitats (Wilhelm and Suttle, 1999). Wilhelm et al. (unpublished) have collected viral and bacterial abundance data from numerous independent studies performed at different times and different depths, across a variety of aquatic habitats. A correlation chart (Appendix 1) based on this data indicates strong correlation between viral and bacterial numbers, despite significant

variation in environmental conditions. This strong correlation further supports the previously presented views about correlation of viral and bacterial abundances also on a larger scale. The wide variation that can be seen in estimates of viral abundance suggests that viruses are indeed an active component of aquatic microbial communities. The great variability might also reflect the variation in host abundance or diversity, since every virus is dependent on its specific host for reproduction. Viral abundance typically parallels that of bacteria and it is assumed to be 5-25 times the bacterial abundance (Fuhrman, 1999). The highest viral abundances are usually observed in rich coastal surface waters. Viral abundances decrease, with increasing depth, from nearshore to more oligotrophic offshore waters (Hara et al., 1996). Seasonal variations in viral abundance have been reported, with higher abundances during the productive part of the year and lower abundances during the unproductive part of the year (Bergh et al., 1989; Bratbak et al., 1994).

1.3.1.2 Bacteria

Heterotrophic prokaryotes are abundant and represent a significant proportion (up to 70 %) of the living biomass in the euphotic zone (the upper 200 m of the water column) and an even larger proportion in deeper waters (Fuhrman, 1999). For the continental shelf and the upper 200 m of the water column, the cellular density is about 1×10^8 cells ml^{-1} , while for the deep oceanic waters it is about 5×10^7 cells ml^{-1} , on average. From global estimates of volume the upper 200 m of the ocean contains a total of 3.6×10^{28} cells, of which 2.9×10^{27} cells are autotrophs; the water below 200 m contains some 6.5×10^{28} cells (Whitman, 1998). This shows that, although heterotrophic prokaryotes form the majority of microbes in the water column, photosynthetic prokaryotes (cyanobacteria and prochlorophytes) are also an abundant group of microbes in the euphotic zone. The term “bacteria” used in the majority of studies refers to prokaryotes, because most studies do not distinguish between Bacteria and Archaea (Fuhrman, 1999).

1.4 PRODUCTION AND DESTRUCTION OF VIRAL PARTICLES

1.4.1 VIRAL PRODUCTION

The abundance of viruses in natural waters depends on the rate of production and release of new viral particles and on the decay (destruction) of free viral particles. Stability is an important property of virulent viruses, especially in waters where the host population density is low and where the time between lysis and infection might be long. With stable particles, a high abundance may be maintained with low production rates (Bratbak et al., 1994).

Burst size is a commonly used term for the number of progeny viruses released upon lysis of a host cell. Burst size can be used for calculations to estimate bacterial mortality caused by viruses. Several attempts have been made to estimate the burst size (e.g. Børsheim, 1993, Heldal and Bratbak, 1991; Weinbauer and Suttle, 1996) and different studies have used different estimates of burst sizes, ranging from 10 up to 300 viruses released per ruptured bacterium. In a steady-state situation, it is estimated that only one virus from every burst survives to infect another host successfully (Fuhrman et al. 1993). Otherwise the viral abundance would not stay stable, which seems to be the case in pelagic environments.

1.4.2 VIRAL DECAY

Viral decay can mean two things: decay or loss of infectivity of a virus, or loss of structural integrity of the intact viral particle. Loss of infectivity is not necessarily coupled to the process of degradation, because viral infectivity may be affected by factors other than viral particle disappearance (Wilhelm et al., 1998b). Measurements of viral decay have produced estimates of viral turnover time. Viral turnover time is the time it takes to replace decaying viral particles of a standing stock, with new viral particles (e.g. Fuhrman, 1999).

It has been suggested in the past that biochemical degradation of virus particles in natural systems occurs at similar rates for many different taxa, but that the loss of infectivity of specific taxa can be different because of the susceptibility of certain types of viruses to various damaging agents (Noble and Fuhrman, 1999). Sunlight, especially ultraviolet-B (UV-B) radiation (280 – 320 nm), can cause considerable damage to

cellular and viral DNA. The importance of sunlight as a decay factor decreases in deeper waters because of light attenuation with depth. Certain components of sea water, such as DOM, particulate organic matter (POM, comprising bacteria, protists, aggregates), exoenzymes, proteases, nucleases and other bioactive molecules, act as contributors to loss of viral infectivity and are also important agents of viral degradation acting synergistically with sunlight (Noble and Fuhrman, 1997). Potential other mechanisms causing disappearance of viruses are aggregation with sinking particles and grazing by protists. Viruses native to regions receiving much sunlight exhibit more resistance to solar-induced damage, which may represent an adaptation to this type of an environment. Typical overall decay rates reported for marine viruses are 3-10 % per hour, implying a turnover time of about 0.5-1.5 days (Fuhrman, 1999). I will discuss viral turnover in more detail in the next section.

Wilhelm et al. (1998a) have suggested that, in a system where the processes of production and loss of viral particles keep the viral abundance relatively constant, infectivity could not be lost at a higher rate than the viral particles are degraded. This model would lead to an increase in the total abundance of viral particles in the system. Therefore, the difference between the rates at which infectivity and viral particles are lost must be balanced by the restoration of infectivity to noninfectious viruses by repair mechanisms. Sunlight-induced damage is the primary causative agent contributing to the decay of viral particles in the ocean's surface layer (Fuhrman, 1999; Weinbauer et al. 1997). However, sunlight inactivated viruses may also be photoreactivated in coastal marine environments by host-cell-mediated DNA-repair mechanisms (Weinbauer et al., 1997; Wilhelm et al. 1998b). Moreover, phage-encoded light-independent repair mechanisms exist, which in functioning together with host-dependent photorepair may maintain high concentrations of viruses infecting photoautotrophic hosts (Wommack and Colwell, 2000). *In situ* measurements have demonstrated that infectivity is restored to 39 – 78 % of the viruses in which infectivity was lost, and that light-dependent repair mechanisms are essential for the maintenance of high virus concentrations in surface waters (Wilhelm et al., 1998b).

1.4.3 METHODS OF ESTIMATING VIRAL PRODUCTION AND TURNOVER

One approach to investigate viral activity quantitatively is to study the rate at which new viruses are produced. Viral production rates can also be inferred from decay rates

of viruses, i.e. disappearance of directly countable virus particles over time (e.g. Heldal and Bratbak, 1991; Wilhelm et al. 1998a; Noble and Fuhrman, 2000). Rates of viral decay and production of new viral particles must be equal in order to maintain viral abundances in surface waters (Wilhelm et al. 1998a). In culture-based studies viral decay is usually measured as the decrease over time in the abundance of infectious viruses. In whole community studies decay rate is determined as disappearance of countable viral particles. These methods are, however, controversial in determining viral decay, since the loss of infectivity and decay of the structure of a viral particle may be uncoupled and, for example, by direct-count methods it is impossible to make a distinction between infectious and noninfectious particles. Thus, measuring viral production and removal has been suffering from methodological constraints, until recently developed new techniques. In the next section I will summarize methods that have been used to estimate viral production and decay.

The earliest approach for determining viral production in a natural microbial community was developed by Heldal and Bratbak (1991). In their approach, the viral production rate in a natural sample was compared to the viral community decay rate in a natural sample where production of virus particles was stopped with cyanide treatment. These experiments were implying viral turnover times of 1-2 days. However there seemed to be uncertainties in interpreting results obtained through this method (Fuhrman, 1999). Another method, developed by Steward et al. (1992a, b) involves incorporation of radiolabeled thymidine (^3H) or phosphate (^{32}P) into viral nucleic acid. A conversion factor is used to estimate the number of viral particles per mole of radiolabel incorporated. Comparison of results obtained using different methods to assess viral production has implied that this method is not sensitive enough to detect low levels of viral production (Noble and Fuhrman, 2000).

Recently, Noble and Fuhrman (2000) determined rates of virus production and removal simultaneously using fluorescently labeled viruses (FLV) as tracers. Viruses are concentrated from seawater, then labeled with fluorescent stain and added back to seawater samples. With this method, it is possible to determine rates of virus production and removal simultaneously. Using this method, the virus production rates ranged from 10^9 to 10^{10} viruses liter $^{-1}$ day $^{-1}$. Estimated turnover times of virus populations were from 1 to 2 days. These results are within the same range reported by Steward et al. (1992b).

1.5 VIRUS-INDUCED BACTERIAL AND PHYTOPLANKTON MORTALITY

The role of viruses as contributors to phytoplankton death in the sea has been a widely studied subject area for the past decade. Results from numerous studies have shown that viruses in some environments can cause up to 50% of the bacterial mortality (Fuhrman 1999). These findings have changed radically the earlier idea of bacterivory being the most important bacterial loss factor. Generally, viruses are estimated to cause about 10 – 50 % of the total bacterial mortality in marine surface waters. The effect of viruses on bacterial mortality has shown variation between different bacterial populations and different habitats. Some groups of organisms and some habitats display relatively little virus-induced mortality, and the effect of grazer-induced mortality seems to be higher. This effect may be seasonal, local, or sporadic.

1.5.1 VIRAL CONTROL OF THE BACTERIAL POPULATION SIZE AND DIVERSITY

Viruses are a species-specific cause of bacterial mortality, so viruses regulate the abundances of specific bacterial populations and thereby they control the structure of the microbial community (Hennes and Simon, 1995). Thingstad (2000) developed a mathematical model to assess the biogeochemical role of bacteria in the food web by analyzing mechanisms that control viral abundance and partitioning between loss of bacterial production and protozoan predation. The key principle of the model is that viruses are acting as a balancing factor among bacterial species that have different growth rates but similar loss by predation in the steady state. The equilibrium of diversity is maintained by mechanisms that selectively kill the dominant species. According to this proposed “killing the winner”-theory, host specific viruses balance the competition between bacterial species and prevent any particular species from building up a high biomass and becoming dominant in the population, thus enabling the coexistence of bacteria. Wommack and Colwell (2000) suggested, based on several studies, that selective events could lead to a monospecific phytoplankton bloom, though there is also strong evidence for involvement of viruses in phytoplankton bloom dynamics and for the role of viruses in ending blooms. Overall the highest viral concentrations have been shown to occur when bacteria and phytoplankton are most abundant (Hennes and Simon, 1995). Also, by lysing bacteria and releasing nutrients, viruses prevent the bacterial population from immobilizing all available mineral

nutrients in the water column and in that way viruses enable the coexistence of nutrient-limited phytoplankton and bacteria.

1.6 THE MICROBIAL LOOP

1.6.1 RESOURCES FOR BACTERIAL BIOMASS PRODUCTION

Carbon is often considered a general tracer of energy flow through biological systems. About 50% of organic carbon enters the biological pool of the oceans via primary producers, which convert inorganic carbon into organic form (Azam et al. 1983). The biggest external sources of organic carbon are river and aeolian input. All organic matter, including dissolved organic carbon, present in the water column is divided into operational classes of dissolved organic matter (DOM) and particulate organic matter (POM). Material that passes 0.45 μm pore size filter is considered DOM and the retained material is POM. In addition to deriving directly from phytoplankton production, DOM is also produced through zooplankton sloppy feeding and excretion, particle solubilization, and viral lysis of bacterial and algal cells (Carlson et al, 1998). Resulting from the operational division of organic material into classes of DOM and POM, DOM contains also material which does not meet the chemical definition of “dissolved”, such as viruses, cell debris, or material resulting from sloppy feeding of planktonic grazers.

DOM and its constituents differ in bioavailability. Microorganisms take up low molecular weight DOM (500-1000 Da) in its original form and larger molecules after enzymatic hydrolysis or chemical or photochemical modifications; processes are needed to convert the less labile matter before it can be taken up as nutrients (Ducklow, 2000; Wilhelm and Suttle, 1999).

The composition of the plankton community may be an important factor controlling the DOM production, however, oceanic regions can be different in production, biolability and accumulation of DOM, as well as in the flux of carbon through the microbial food web (Carlson et al., 1998). The pool of DOM is estimated to be one of the major carbon reservoirs in the whole biosphere and the largest exchangeable pool of organic carbon on earth (Williams, 2000).

1.6.2 MEASURING OF BACTERIAL PRODUCTION

Bacterial production is commonly determined indirectly by using DNA or protein precursors labeled with radioisotope. Incorporation of tracer over time yields incorporation rate, which can be converted to production rate by using empirical conversion factors. The most common approaches are measurement of [³H]-thymidine (TdR) incorporation into bacterial DNA (Fuhrman and Azam, 1982) and measurement of [³H]-leucine incorporation into bacterial protein (Kirchman et al., 1985). TdR-method is specific for estimating bacterial production of cells in active growing phase, [³H]-leucine method measures bacterial protein synthesis. Bacterial biomass production can be calculated from rates of protein synthesis because protein comprises a large, fairly constant fraction of bacterial biomass (Kirchman, 1993).

1.6.3 DOM AND THE MICROBIAL LOOP

Azam et al. (1983) presented a hypothesis of a “microbial loop”, which provides a model for microbial carbon recycling in the aquatic food webs. Heterotrophic bacteria efficiently use DOM as an energy source and this way part of the organic carbon produced by phytoplankton is diverted from the grazing food chain and recycled numerous times by bacteria as it passes through the entire food web. In this process heterotrophic bacteria also have a role as a source of POM through their rapid assimilation of dissolved DOM and their efficient conversion of DOM into bacterial biomass (Maranger, 1995). Significant amounts of POM, including bacteria and other members of the planktonic community, are transferred to higher trophic levels by planktonic grazers (Wilhelm and Suttle, 1999). Altogether, this could be interpreted as an indication that primary production directly or indirectly governs the supply of DOM, which in turn determines the rate of bacterial production (Kirchman and Rich, 1997). Bacterial production itself is the basis of DOM flux through the microbial loop; production estimates establish the importance of the loop and of bacterivory initiated food webs in marine ecosystems (Ducklow, 2000).

1.7 EFFECTS OF VIRAL INFECTION ON THE MICROBIAL LOOP

The original model of the microbial loop did not include viruses in its account of carbon recycling. Since viruses are now known to be abundant throughout the aquatic

planktonic community, their role in carbon and nutrient cycling has become a subject of numerous studies. Viruses divert carbon and nutrients from secondary consumers of the food web by destroying daily up to 50 % of their host marine heterotrophic bacteria, cyanobacteria and eukaryotic plankton (Fuhrman and Suttle, 1993). Viruses exert both positive and negative controls over microbial communities. Viral infection increases the mortality of the specific host populations, and the growth of the noninfected populations, which efficiently take up cell lysis products. Viral infection is also enhancing nutrient recycling within the bacterioplankton (see the next section).

On the whole, viruses are thought to shunt carbon flow for production and respiration from higher trophic levels towards bacteria. Repeated cycling of organic matter from bacteria to viruses to DOM to bacteria, causes the bacteria to be efficient sinks for carbon and regenerators of nitrogen and phosphorous (Fuhrman and Suttle 1993).

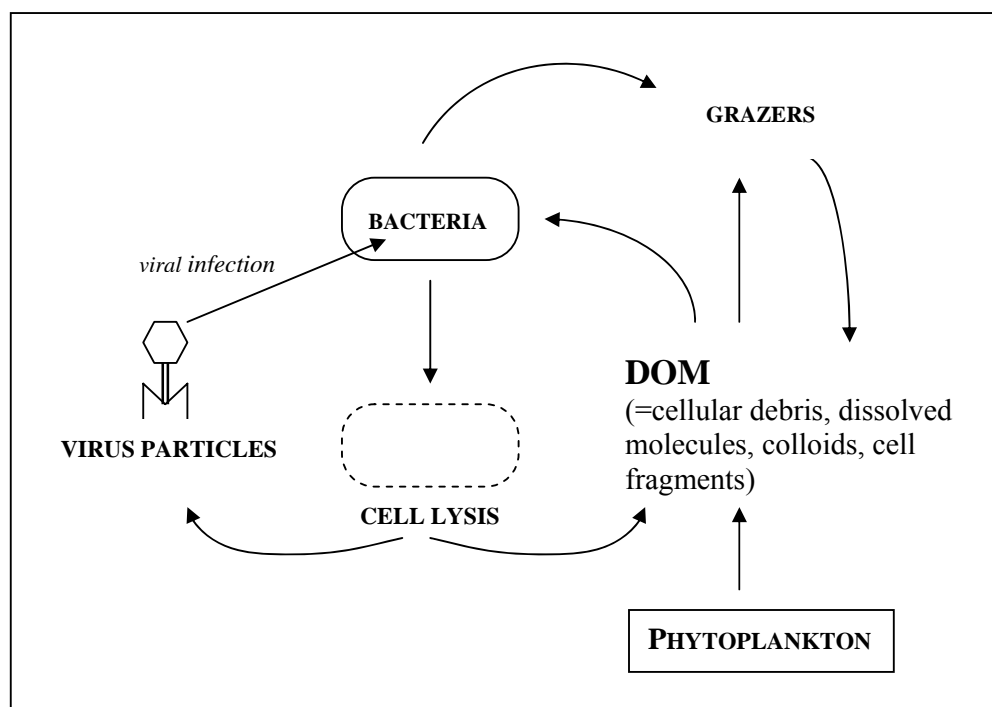


Figure 2. Viruses in planktonic microbial food web. In this diagram viruses and DOM are separated, but operationally they belong to the same class (modified from Fuhrman 1999).

Wilhelm and Suttle (1999) suggested that proportionately from 6 to 26% of photosynthetically fixed carbon is recycled back to the pool of dissolved organic

material through the “viral shunt” (Figure 2). In contrast to viruses, heterotrophic nanoflagellates and other bacterivores recycle a maximum of 9% of the primary production.

1.7.1 EFFECT OF VIRUSES ON NUTRIENT RECYCLING

Lysis of the host cell leads to release of dissolved organic compounds, such as viral progeny, structural and cytoplasmic materials, dissolved molecules and carbohydrates. This material is readily available as nutrients to noninfected bacteria (Middelboe et al., 1996; Noble et al., 1999). The cell lysis material is rich in nutrient elements, particularly organic nitrogen, phosphorous and iron, which all play a different geochemical role in aquatic systems. Release of these nutrient elements has a considerable effect on carbon transfer through the entire food web, especially in areas where the low concentration of a particular element in the water is limiting primary production. Lysis of infected hosts will release the nutrient elements in a labile, organically complexed form, which is different from the form in which it is released by grazers. Some of the labile material will be immediately available to noninfected bacterioplankton, other components may require photochemical modification or actions by exogenous enzymes or grazers before they can be utilized and thus this material would be biologically available over a longer time scale (Wilhelm and Suttle, 2000). Previous studies have shown that uptake of cell lysis material is especially efficient under phosphorous-limited conditions. In investigating fates of radiolabeled cell lysis products in a microbial assemblage, Noble and Fuhrman (1999) demonstrated that microbial uptake of ³³P-labeled virus material was rapid in nutrient limited waters, where the regeneration of phosphorous is vital to microbial growth. Also Middelboe demonstrated (1996) that under P-limitation, the uptake of dissolved organic carbon increased 72 % after adding viruses to the culture.

Considering the large biomass of phytoplankton and heterotrophic plankton in the euphotic zone, viral activities can play a significant role in large-scale carbon and nutrient cycling processes in the world's oceans. Due to viral infection, nutrients will remain in small non-sinking forms in the euphotic zone. Viral lysis also facilitates nutrient recycling within the planktonic microbial community in nutrient poor areas. Reduced viral activity would result in carbon transfer to larger organisms, which either sink or, as detritus, transport carbon and nutrients from the euphotic zone to the deep sea (Fuhrman, 1999).

2. MATERIALS AND METHODS

2.1 STUDY SITES

Studies in the North Atlantic were performed during a research cruise on R/V *Cape Henlopen* from 21 May to 16 June, 2000; and in equatorial Pacific Ocean during a research cruise on R/V *Melville* from 21 August to 28 September, 2000. Exact sampling sites are marked on the maps (Figures 3 and 4) and the date at each location corresponds with the date reported on the summary table with the results of each experiment (Tables 1a and 2).

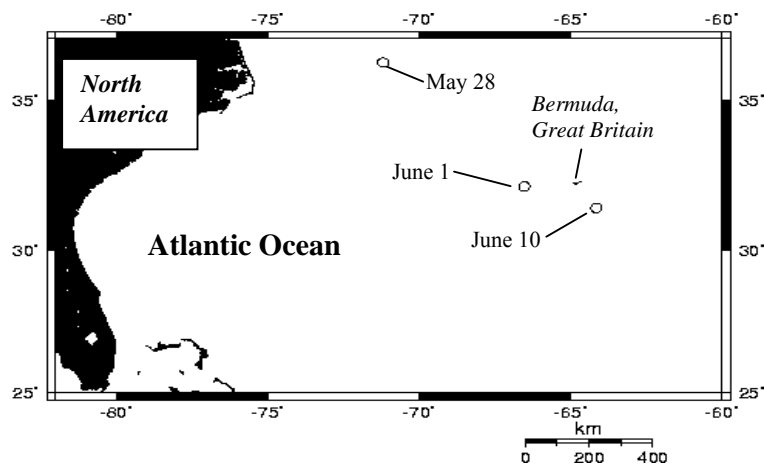


Figure 3. Sampling locations at the North Atlantic. Dates marked on locations correspond with dates marked with each experiment on summary tables.

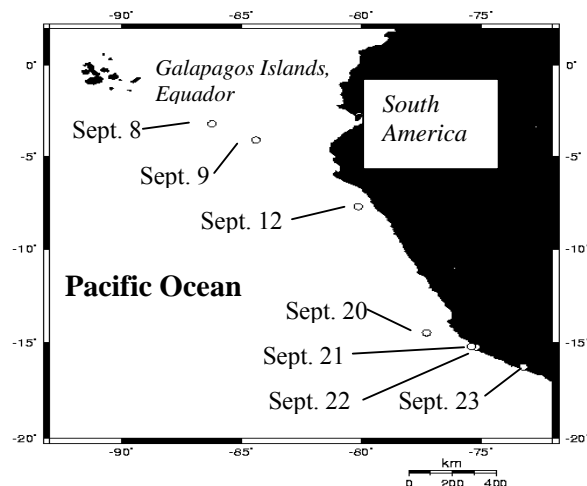


Figure 4. Sampling locations at the eastern equatorial Pacific Ocean.

2.2 GENERAL WATER SAMPLING PROCEDURES

Water for experiments was collected into acid washed polyethylene carboys, through the ship's clean surface water system or from 10 l Niskin-bottles attached to the ship's rosette-sampler. The carboy was protected from direct sunlight by covering it with a black plastic bag. Collected water was taken to the laboratory and processed immediately.

2.3 ON-DECK INCUBATIONS

All incubations (if not otherwise mentioned) were carried out in incubators placed on the deck of the ship. Incubators were made out of transparent Plexiglas®. Light and temperature conditions in the incubators were similar to *in situ* conditions in the sea just below the surface. *In situ* temperature was maintained with constant flow of surface water through the incubator. Excess sunlight was screened with tinted Plexiglas® so that the light inside the incubator corresponded to *in situ* light levels.

2.4 ENUMERATION OF VIRUSES AND BACTERIA USING EPIFLUORESCENCE MICROSCOPY

The method of Noble and Fuhrman (1998) was modified to determine the total abundance of viruses and bacteria in the seawater samples using SYBR Green I staining and epifluorescence microscopy. All seawater samples collected for enumeration of viruses and bacteria by direct epifluorescence microscopy were preserved in 2.5 % glutaraldehyde (final concentration) and stored in the refrigerator at +4°C until processed in the laboratory.

The formula of SYBR Green I is proprietary and the molecular weight and concentration are not reported by the manufacturer (Molecular Probes Inc., Eugene, Oregon, USA). SYBR Green I is light-sensitive; thus to prevent the stain from fading, all work steps that involved handling the stain, or stained samples, were conducted in reduced light. For staining solution, the SYBR Green I stain used for this study was diluted from the manufacturer's stock 1:10 with sterile, 0.02 µm filtered deionised water. For the working solution, 10 % dilution of SYBR Green I was further diluted with sterile, 0.02 µm filtered deionised water so that the final dilution became 2.5×10^{-3} .

800 µl of sample was pipetted directly onto the 0.02 µm pore size aluminium oxide filter (Anodisc 25, Whatman), which was backed with a 0.45 µm pore size HAWP (mixed cellulose acetate and cellulose nitrate) filter (Millipore) and filtered (<10 kPa) on a 10-place filtration manifold (Hoeffler Scientific). To obtain optimal density of particles for counting, samples were diluted appropriately for counting with autoclaved, ultrafiltered seawater so that each field of view contained approximately 20-200 particles. To stain bacteria and viruses collected on the filter, a 100 µl drop of SYBR Green I working solution was pipetted on the bottom of a clean plastic Petri-dish. A 25-mm, 0.02 µm nominal pore-size Anodisc (Whatman) filter was placed on the drop with the sample side up for 10 minutes. During staining the dish was kept in the dark. After the staining period, excess stain was wiped off from the back of the filter and the dry filter was placed on a glass slide. A drop of sterile mounting solution was pipetted on top of the Anodisc filter to prevent fading of the stain. The mounting solution (50% glycerol, 50% phosphate buffered saline (PBS [0.05 M Na₂HPO₄, 0.85 % NaCl, pH 7.5] with 0.1 % of *p*-phenylenediamine (Sigma chemicals)) was made daily using 10 % frozen aqueous stock of *p*-phenylenediamine. A glass cover slip (25mm x 25mm) was placed on top of the Anodisc filter and the mounting solution. A drop of low-fluorescence immersion oil was added on the coverslip before observation with the epifluorescence microscope. Virus and bacterial numbers were determined immediately after preparing the slides or they were stored frozen at -20°C until processed.

A Leica DMRXA epifluorescence microscope equipped with a standard acridine orange filter set ($ex_{\lambda} = 450-490$ nm; $em_{\lambda} = 510$ nm; suppression filter $\lambda = 510$ nm) and ocular grid was used for microscopy. For each sample 20 fields of view, or a total of >200 bacteria and >200 viruses, were counted. Grid size was calibrated with a stage micrometer and used to develop a calibration factor.

2.5 TOTAL CHLOROPHYLL A MEASUREMENTS

Chlorophyll *a* concentration was determined fluorometrically according to protocol of Welschmeyer (1994). A known volume of sample was filtered through 0.22 µm pore size Isopore polycarbonate filter (Millipore). Chlorophyll *a* was extracted from the filters in 90 % acetone for 24 h at +4°C in the dark. After 24 h, samples were warmed to room temperature and the extract transferred into 5 ml borosilicate tubes (Fisher). The

chlorophyll *a* concentration of the sample was measured with a Turner Designs fluorometer. Chlorophyll *a* concentration in the original sample was calculated by formula:

$$\text{Chl } a \text{ (}\mu\text{g/L)} = \frac{V_A}{V_S} \times \text{fluorometer reading (}\mu\text{g/L)}$$

V_A = volume of 90 % acetone added (in litres); V_S = volume of the filtered sample (in litres).

2.6 VIRAL PRODUCTION EXPERIMENTS

2.6.1 EXPERIMENTAL SETTING

Viral production was measured according to Wilhelm et al. (2001). Seawater (300 ml) was filtered under gentle vacuum through 0.22 μm pore size polycarbonate filter (Millipore) using an acid washed and rinsed Millipore polycarbonate filter funnel. During filtration, the volume was maintained at >50 ml through addition of virus-free ultrafiltrated (<30 000 kDa) seawater. The final volume of the retained cell suspension was 300 ml. The bacteria were prevented from settling on the filter by drawing in and injecting the cell suspension with a transfer pipette during filtration. This way the concentration of naturally occurring viruses was reduced 3.5 - 43 % of the initial concentration. 100 ml aliquots of the retained cell suspension were transferred into acid-clean 250 ml polycarbonate flasks, which were incubated in the dark at *in situ* temperature. Subsamples (5 ml) were taken at t_0 from whole water (before filtration) and from the cell suspension after filtration, and then every 3 hours from each replicate bottle. Water samples were preserved in 2.5 % glutaraldehyde (final concentration) and stored in a refrigerator until processed. Abundances of viruses and bacteria were determined in the laboratory by SYBR Green I- staining and epifluorescence microscopy.

2.6.2 CALCULATIONS

To determine the *in situ* rate of viral production using the viral production rate in the sample, a correction for bacterial abundance was calculated by dividing bacterial

abundance in natural seawater by bacterial abundance in retained cell suspension. Correction was needed because some infected cells were lost during dilution. Viral turnover time was calculated by using *in situ* viral production rate and *in situ* viral abundance. The abundance of bacteria destroyed by viruses per day was calculated for each experiment. Estimated burst size was 100 viruses per lytic event (Fuhrman and Suttle 1993).

$$VP_{is} = \frac{BA_{nw}}{BA_{ret}} \times VP$$

VP_{is} = *in situ* rate of production of viruses ($ml^{-1} min^{-1}$); BA_{nw} = bacterial abundance in natural seawater sample (ml^{-1}); BA_{ret} = bacterial abundance in retained cell suspension (ml^{-1}); VP = viral production in the sample ($ml^{-1} h^{-1}$).

$$VT = \frac{VP_{is}}{VA_{nw}}$$

VT = viral turnover (min^{-1}); VP_{is} = *in situ* rate of production of viruses ($ml^{-1} h^{-1}$); VA_{nw} = viral abundance in natural seawater sample (ml^{-1}).

$$D_b = \frac{VP_d}{100}$$

D_b = bacteria destructed (d^{-1}), VP_d = viral production (d^{-1}), calculated directly from VP_{is} .

2.6.3 STATISTICAL ANALYSIS

In viral production experiments, viral and bacterial abundances were calculated as the mean value of the independent replicates in each experiment. For each replicate, the viral production rate was determined from a slope of first-order regression of viral abundance versus time. Mean values and standard deviations for viral production rate were determined for independent replicates.

2.7 VIRAL CONCENTRATE ADDITION EXPERIMENTS

2.7.1 PREPARING VIRAL CONCENTRATE BY ULTRAFILTRATION

Water for the incubations was collected from the same location and at the same time as the water to be used to make the concentrate. Prefiltration and ultrafiltration system is illustrated in Figure 5.

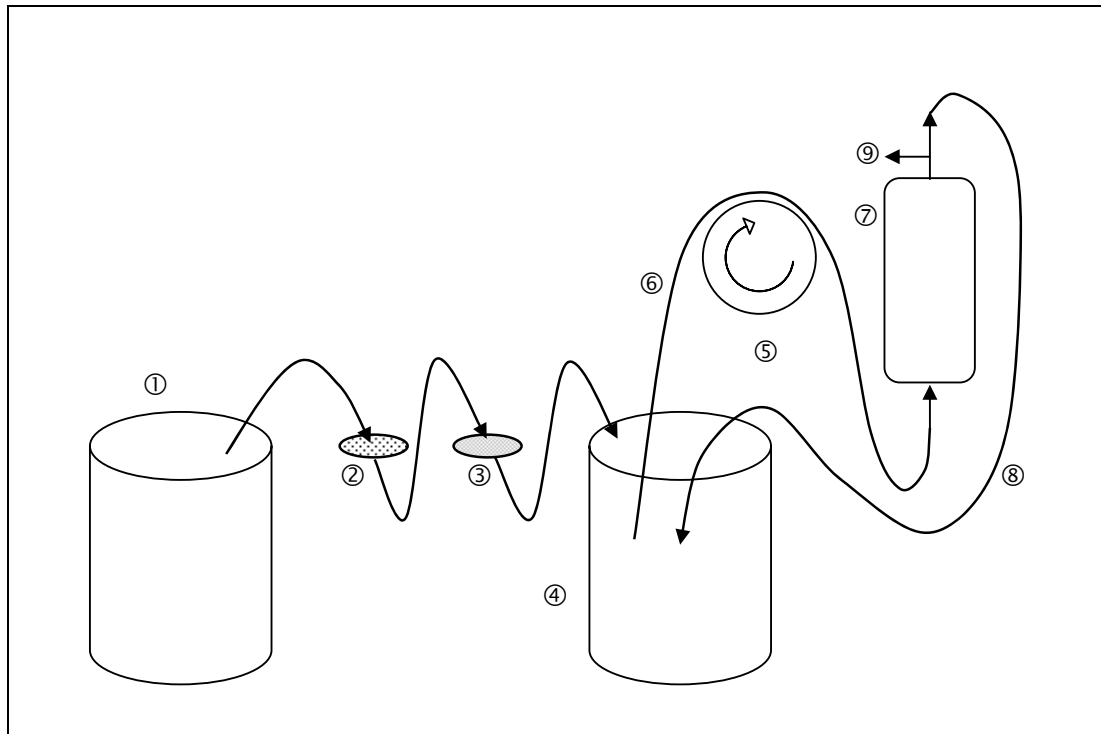


Figure 5. Prefiltration and the ultrafiltration system: 1. Whole seawater; 2. Glass fiber filter; 3. 0.45 μm membrane filter; 4. Prefiltered seawater; 5. Peristaltic pump; 6. Feed; 7. Ultrafiltration cartridge; 8. Retentate (concentrate); 9. Permeate (ultrafiltrate).

For preparation of a VC, 50–100 l of seawater was pressure filtered (<15 kPa) through 142 mm diameter GF/F glass fiber filter and 142 mm diameter low protein-binding Durapore (Millipore) 0.45 μm nominal pore-size membrane filter. Filters were held in place by Millipore stainless-steel filter holders. To concentrate the 30 000 kDa - 0.45 μm size fraction, an M12 Tangential Flow Filtration System (Millipore) was used with 30 000 kDa cut-off spiral wound ultrafiltration cartridge (S10Y30 Amicon Inc.). After

each use the cartridge was washed with 2 L of 0.1 M NaOH and stored filled with 0.01 M NaOH in a refrigerator according to the manufacturer's instructions. The cartridge was flushed with 10 L of deionized water before preparation of each concentrate. The final volume of the concentrate was 450 – 800 ml. The ultrafiltration system is illustrated in Figure 5. After concentration, the concentrate was filtered through 47 mm diameter Durapore (Millipore) 0.45 µm pore size membrane filter to remove any remaining larger particles and bacteria from the solution.

Samples (5 ml) were taken and preserved in 2.5 % glutaraldehyde (final concentration) from both the whole water before prefiltration and concentration, and from the concentrate, for enumeration of bacteria and viruses in the laboratory and to determine the concentration efficiency.

2.7.2 EXPERIMENTAL DESIGN

The general experimental design involved sea water incubations in 9 bottles per experiment, which included 3 replicates of each treatment: VC addition, heat-treated VC addition and ultrafiltrate (UF) addition as a control. Ultrafiltrate (i.e. permeate) was collected from the ultrafiltration system.

One litre of seawater to be used for the incubations was dispensed into nine acid washed and rinsed 1-liter Nalgene polycarbonate bottles. An equal volume of VC, heat-treated VC or ultrafiltrate was dispensed into replicate bottles. Heat treated VC was prepared in a microwave oven by bringing VC to near-boiling temperature 3-5 times. Heating was stopped when the temperature of VC reached the boiling point; the VC was then let to cool down 5-10 minutes, after which it was reheated until the boiling temperature was reached again. This was repeated as long as a total of 10 minutes of microwaving had elapsed. The heat treated VC was let to cool back down to room temperature before adding it to incubation containers. Time zero (t_0) samples were taken from the bottles and subsamples collected for total bacteria and virus counts, samples for [^3H]-leucine incorporation. After this, bottles were incubated in on-deck incubators at *in situ* temperature in ambient sunlight and subsampled at known intervals. The sampling intervals varied between the experiments 1-3; samples from experiments 1 and 2 were taken every 12 hours and from experiment 3 every 36 hours. Experiments 4-6 were all sampled every 24 h.

The increase in viral abundance due to VC addition was calculated by dividing viral abundance in the VC enriched culture with viral abundance in UF controls at t_0 . In experiments 1 and 2 only UF control sample at t_0 was available; therefore in these experiments the factor was calculated indirectly. The factor was estimated by multiplying the viral abundance of VC by the volume of VC added into the culture. This gave the number of viruses added into the culture with known volume and known viral and bacterial abundance. Using this information, the new viral concentration of the culture was calculated and the enrichment factor was determined as in other experiments.

2.7.3 BACTERIAL PRODUCTION MEASUREMENTS

[^3H]-leucine incorporation into bacterial protein over time was used to measure bacterial production rates. In the first two experiments performed in the North Atlantic, we used modified protocol from the works of Kirchmann et al. (1985) and Wicks and Robarts (1988). Ten ml samples and killed controls, which were killed with ice-cold trichloroacetic acid (TCA, 5 % final concentration), were inoculated with [^3H]-leucine (final concentration 20 nM). Samples were incubated 60 min in the dark at ambient seawater temperature. After that, samples were filtered on 0.22 μm nominal-pore size mixed cellulose acetate and cellulose nitrate filter (Millipore) using a 10-place filtering manifold (Hoeffler Scientific) and cold steel filtration towers. Samples were rinsed twice with 3 ml of ice cold 5 % TCA and twice with 3 ml of ice cold 80 % ethyl alcohol. Dry filters were placed in 10 ml polyethylene scintillation vials and were frozen until processed further in the laboratory. In the laboratory, filters were dissolved with ethyl acetate and radioassayed with liquid scintillation counter.

For the three experiments performed in the Pacific Ocean, we used the microcentrifuge protocol by Smith and Azam (1992) as modified by Kirchman and Cottrell (personal communication). 1.5 ml of sample was pipetted in a polyethylene screw-cap microcentrifuge tube (Fisher). In control samples TCA was added to final concentration of 5 %. Samples were inoculated with [^3H]-leucine (10 nM leucine + 10 nM [^3H]-leucine, 20 nM total; specific activity 75 $\mu\text{Ci/nmol}$). Tubes were capped, mixed well and incubated 30 minutes in the dark at ambient seawater temperature. After incubation the tubes were placed on ice bath for 5 minutes and ice-cold TCA was added to live samples to terminate incubation (final concentration 5 %). Samples were

centrifuged in a refrigerated microcentrifuge (Eppendorf) for 10 min at 13 000 rpm. After centrifugation the tubes were placed on the ice bath and supernatants were aspirated. 1 ml of ice cold 5 % TCA was added to the tubes and centrifugation was repeated as on the previous step. The TCA was removed and 1 ml of ice-cold 80 % ethanol was added into the tubes. The centrifuge step was repeated as before. After the final centrifugation step, ethanol was aspirated from the tubes and the tubes were left uncapped in a fume hood to allow the remaining ethanol to evaporate from the samples. The next day, 1 ml of scintillation cocktail (Ultima-Gold, Packard) was added into the tubes, the tubes were placed in 10 ml plastic scintillation vials, and the samples were assayed after 24 h with liquid scintillation counter.

Biomass production ($\text{g C l}^{-1} \text{ h}^{-1}$) was calculated from rate of leucine incorporation using conversion factor $3.1 \text{ kg C mol}^{-1}$ (Kirchman, 1993). Bacterial carbon production was converted to cell production assuming $23.3 \times 10^{-15} \text{ g C cell}^{-1}$ (Lee and Fuhrman, 1987). The final results are calculated as mean values of three independent replicates.

2.7.4 STATISTICAL ANALYSIS

In VC addition experiments each treatment had three independent replicates, except experiment 2, which had 2 replicates for each treatment. Viral and bacterial abundances were calculated as the mean value of the replicates for each time point. Student t-test was used to test differences for significance between VC-enrichments, inactivated VC-enrichments and UF control. Differences were compared at single time points and considered significant if $p < 0.05$.

3. RESULTS

3.1 WATER SAMPLE CHARACTERISTICS

In the North Atlantic the *in situ* bacterial abundance ranged from $0.2 - 0.4 \times 10^6 \text{ ml}^{-1}$, while *in situ* viral abundances ranged from $0.2 - 1.0 \times 10^6 \text{ ml}^{-1}$. In the eastern equatorial Pacific Ocean the corresponding abundances of bacteria and viruses ranged from $0.4 - 18.0 \times 10^6 \text{ ml}^{-1}$ and from $2.0 - 60.0 \times 10^6 \text{ ml}^{-1}$, respectively.

Total chlorophyll a concentrations *in situ* in the the North Atlantic ranged from $0.1 - 0.6 \mu\text{g l}^{-1}$ and in the eastern equatorial Pacific Ocean from $0.9 - 1.3 \mu\text{g l}^{-1}$.

3.2 VIRAL PRODUCTION EXPERIMENTS

Sampling locations, dates and results of viral production experiments are summarised in Tables 1a, 1b and 1c.

Table 1a, Sampling locations, depths and sea water characteristics for viral production experiments. Dates correspond to locations and dates presented in Figures 3 and 4.

Experiment	Date	Location	Temp. (°C)	Depth (m)
1	June 1, 2000	North Atlantic Lon. 66°54' W Lat. 32°18' N	23.5	90
2	Sept. 9, 2000	Pacific Ocean Lon. 84°40.3' W Lat. 4°10.7' S	20.1	Surface
3	Sept. 12, 2000	Pacific Ocean Lon 80°13.1' W Lat. 7°7.6' S	17.0	Surface
4	Sept. 21, 2000	Pacific Ocean Lon 75°21.1' W Lat. 15°23.4' S	16.8	Surface
5	Sept. 23, 2000	Pacific Ocean Lon 73° 24.9' W Lat 16° 27.3' S	15.0	Surface

3.2.1 THE NORTH ATLANTIC

One viral production experiment was performed at the North Atlantic. In this experiment the ambient bacterial abundance was reduced to ca. 83 % and the viral abundance to ca. 44 % of ambient abundances by filtration and dilution with virus free water (Table 1b). Viruses were produced in the sample at a rate 0.6×10^6 viruses $\text{ml}^{-1} \text{h}^{-1}$. The *in situ* rate of production was 0.7×10^6 viruses $\text{ml}^{-1} \text{h}^{-1}$, which was determined using a correction for bacterial hosts (see Materials and methods). Viral turnover rate was calculated to be 40 day^{-1} . Using an assumed burst size of 100 viruses released per lysed bacterium, we could assume 74% of the bacterial population being destroyed by viral lysis daily (Table 1c). During the experiment, there was a slight net increase in bacterial numbers in the incubations (Figure 6).

3.2.2 PACIFIC

In these four experiments, the ambient bacterial abundance was reduced to ca. 4-29 % and the viral abundance ca. 4-40 % of *in situ* abundance by filtering and adding virus-free water to the sample. Viral production rates in incubations ranged from $0.1 - 16.0 \times 10^6$ viruses $\text{ml}^{-1} \text{h}^{-1}$. These production rates were converted to *in situ* rates of viral production, which ranged from $0.23 - 213 \times 10^6$ viruses $\text{ml}^{-1} \text{h}^{-1}$. Viral turnover was determined in three of the four experiments, ranging from 6 up to 255 day^{-1} . The assumed burst size of a bacterium was 100; using this, we could assume viruses causing bacterial mortality of 5 – 36 % of the bacterial population per day (Table 1c). While the viral abundance in the diluted samples increased through out the experiment, the net bacterial abundance showed only small changes during incubations (Figure 6).

Table 1b. Comparison of bacterial and viral abundance at t_0 *in situ* and after dilution.

Expt.	N	t_0 bacterial abundance ($\times 10^6 \text{ ml}^{-1}$)		% cells remaining	t_0 viral abundance ($\times 10^6 \text{ ml}^{-1}$)		% viruses remaining
		<i>In situ</i>	Diluted		<i>In situ</i>	Diluted	
1	3	0.18	0.15	83	0.41	0.18	44
2	3	9.78	1.45	15	27.8	6.8	24
3	2	18.2	5.3	29	60.7	24.4	40
4	2	ND	0.36	ND	ND	1.62	ND
5	3	5.39	0.20	3.7	20.0	0.7	3.5

ND: not determined

Table 1c. Viral production rates in diluted samples and *in situ*, viral turnover rates and % of bacterial population destroyed by viral lysis in five viral production experiments.

Expt.	Viral production rate in diluted samples (x 10 ⁶ viruses ml ⁻¹ h ⁻¹)	<i>In situ</i> viral production rate (x 10 ⁶ viruses ml ⁻¹ h ⁻¹)	Viral turnover rate (d ⁻¹)	% of bacteria lysed (d ⁻¹)
1	0.56 (±0.17)	0.68 (±0.21)	40	74
2	4.67 (±0.67)	10.4 (±1.7)	29	11
3	0.07 (±0.08)	0.23 (±0.28)	6	5
4	16.0 (±3.4)	ND (±ND)	ND	ND
5	8.03 (±2.95)	213 (±78)	255	36

ND: not determined

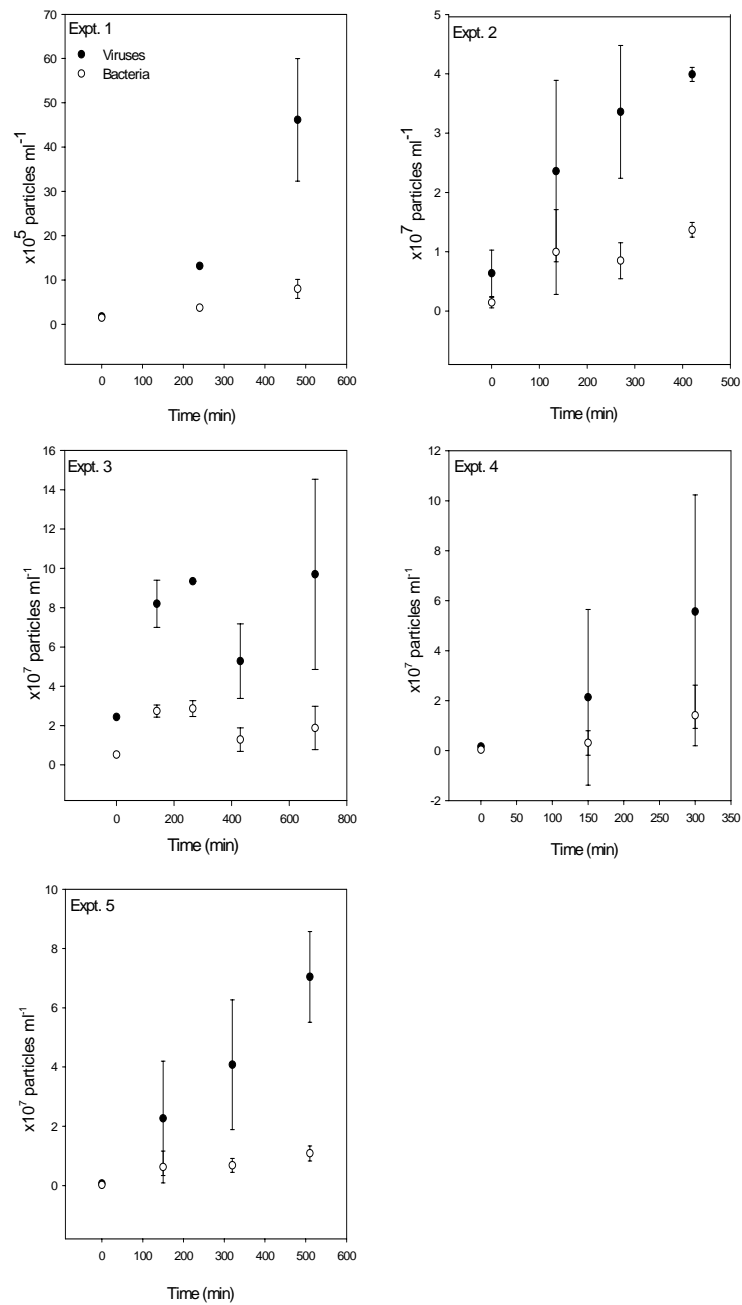


Figure 6. Viral and bacterial abundances over time in viral production experiments. Error bars represent SD, calculated from triplicate incubations. Where no error bars are shown, SD is smaller than the symbol.

3.3 VIRAL CONCENTRATE ADDITION EXPERIMENTS

3.3.1 INITIAL PARAMETERS

Sampling locations, dates, seawater characteristics and viral enrichment factors for VC amended incubations in each VC addition experiment are summarised in Table 2.

Table 2. Sampling locations, sampling depths, seawater characteristics and number of times viral abundance was increased in viral concentrate (VC) enriched incubations compared to ambient viral abundance in six VC addition experiments.

Experiment	Location	Date	Temp. (°C)	Depth (m)	Total chl a ($\mu\text{g l}^{-1}$)	Times virus concentration increased by adding VC	N
1	North Atlantic, Lat. 36°23'N; Lon. 71°20'W	28 May 2000	22	Surface	0.01	72	3
2	North Atlantic, Lat. 32°18'N; Lon. 66°54'W	1 June 2000	24	5	0.03	94	3
3	North Atlantic, Lat. 31°46'N; Lon. 64°16'W	10 June 2000	25	92	0.50	77	2
4	Pacific Ocean Lat. 3°2'S; Lon. 86°23'W	8 Sept. 2000	20	Surface	1.3 (± 0.01)	4	3
5	Pacific Ocean Lat. 14°47'S; Lon. 77°27'W	20 Sept. 2000	16	Surface	0.93 (± 0.03)	23	3
6	Pacific Ocean Lat. 15°20'S Lon. 75°40'W	22 Sept. 2000	16	Surface	1.2 (± 0.04)	5	3

3.3.2 VIRAL CONCENTRATES

Ultrafiltration resulted in a viral concentrate. When examined by epifluorescence microscopy the concentrate appeared to be full of uniformly stained virus-like particles. However, it appeared that some bacteria had passed the prefiltration into the concentrate. Viral abundances in concentrates ranged from 16 to 1030 fold from *in situ* abundances. The addition of heat-treated viral concentrate also increased the amount of virus-like particles in samples taken immediately after addition of htVC in each experiment. Some of the small-size fluorescing material visible under microscope may

have originated from cells that got lysed during heat-treatment. However we assumed that due to the heat-treatment, the intact virus-like particles observed in heat-treated viral concentrate were noninfective. Thus, viral abundance of the UF controls at t_0 was also used as the t_0 viral abundance for heat-treated VC-enriched incubations. Except in experiments 1 and 2, viral abundances in UF controls were used as t_0 for all treatments.

3.3.3 VIRAL ENRICHMENT

3.3.3.1 The North Atlantic

The addition of viral concentrate increased the viral abundance in incubations 72-94 fold (Table 2). Viral abundances at t_0 and at t_{end} are summarised in Table 3a. In experiments 1 and 3 viral abundances were higher in VC-amended incubations after 12 h, than in htVC-enriched incubations or in UF controls. As well, viral abundances in htVC incubations in experiments 1 and 3 were slightly above UF controls, but significantly lower than in VC-amended incubations. In all three experiments viral abundances in UF controls remained constant throughout the experiment. In experiment 2, no clear differences in viral abundance between treatments could be seen during the first 40 hrs.

In experiments 1 and 3, viral abundances in VC-enriched incubations declined towards the end point of the experiment after the second time point. In experiments 1 and 2 also htVC-incubations had a peak in viral abundances at the 20 h time point.

3.3.3.2 Pacific

Addition of viral concentrate in incubations increased the viral abundance 5-22 fold (Table 2). Viral abundances at t_0 and at t_{end} are summarised in Table 3b. In experiments 4 and 5, VC-enrichments kept the viral abundances at high levels from the start to the end of the experiment, despite that the viral abundances started to decrease after t_0 in all three experiments. In experiment 5 the viral abundance in VC-enriched incubations decreased steadily towards the end of the experiment, unlike in experiments 4 and 6, where viral abundances decreased from 0 to 24 h, but began to increase after 24 h. In all experiments, VC-enriched incubations demonstrated a net increase in viral abundances between the start and end points of the experiments.

In experiment 6, viral abundance data for htVC-treatment were available only for the start and end points of the experiment. In this experiment viral abundances in UF-controls and in htVC-enriched incubations demonstrated a significant net increase, unlike in other experiments.

3.3.4 BACTERIAL COUNTS

Bacteria that passed the prefiltration caused interference in t_0 bacteria counts in VC-amended incubations. Because the viability of bacteria in concentrate may be altered during the concentration process, the bacteria were not therefore expected to behave like the natural community in the incubations. Thus, the t_0 bacterial numbers of the UF control were used as t_0 values for all incubations. Bacterial abundances were determined at all time points of all six experiments (Figures 7 and 8). Bacterial abundances at t_0 and at t_{end} are summarised in Table 3a for experiments 1-3 and in Table 3b for experiments 4-6.

3.3.4.1 The North Atlantic

The highest bacterial abundances (compared to controls) were found in virus-enriched incubations in two of the three experiments performed in the North Atlantic (Figure 7). In experiment 3, the bacterial abundance in VC enriched incubation was significantly ($p < 0.05$) greater at the end of the experiment than in htVC-enriched bottles or in UF controls. In experiments 1 and 2, bacterial abundances had a peak value at 20 h; after this bacterial abundances declined towards the end of the experiment. In all three experiments, bacterial abundance in UF controls did not show a significant increase.

The first four time points of experiment 2 did not demonstrate differences in bacterial abundances between the treatments. The highest bacterial abundance in htVC-amended incubation was reached at the last time point. Notably, in this experiment had low bacterial abundance in all incubations until 40 h.

Generally, bacterial abundances followed similar trends as viral abundances and the highest bacterial abundances were observed at the same time points as peaks in viral abundance were recorded.

3.3.4.2 Pacific

In experiments 4, 5 and 6 the VC-enriched incubations demonstrated a net increase in bacterial abundances (Figure 8). In experiments 1 and 2, the net change in bacterial abundance in VC-enriched bottles was greater than in htVC-incubations or in UF controls, and at 72 h the bacterial abundances in VC incubations were significantly ($p < 0.05$) greater than in UF controls or in htVC-enriched incubations. In incubations that received heat-treated VC, bacterial numbers peaked at 24 h, after which, in experiments 4 and 5, the numbers declined towards the end of the experiment. In experiments 4 and 5, the difference between the treatments could clearly be seen after 48 h. In experiment 6, VC-enriched incubation had significantly the greatest bacterial abundance at 48 h compared to htVC-incubations and UF controls. In experiments 4, 5, and 6, bacterial abundances in UF controls varied over the time course of the experiments.

Table 3a. Abundances (\pm SD) of viruses and bacteria measured in viral concentrate (VC) enriched, heat-treated (ht) VC enriched and ultrafiltrate (UF) control incubations in VC addition experiments 1, 2 and 3 from the North Atlantic.

Expt.	Treatment	t_0 bacterial abundance ($\times 10^5 \text{ ml}^{-1}$)	t_0 viral abundance ($\times 10^6 \text{ ml}^{-1}$)	t_{end} bacterial abundance ($\times 10^6 \text{ ml}^{-1}$)	t_{end} viral abundance ($\times 10^7 \text{ ml}^{-1}$)
1	UF control	1.77 (\pm ND)	0.36 (\pm ND)	0.15 (\pm 0.03)	0.65 (\pm 0.41)
	VC		ND	5.50 (\pm 2.17)	30.8 (\pm 0.9)
	ht VC		ND	2.00 (\pm 1.06)	1.37 (\pm 0.75)
2	UF control	1.51 (\pm 0.50)	0.26 (\pm 0.11)	3.10 (\pm 0.6)	0.63 (\pm 0.10)
	VC		ND	0.55 (\pm 0.24)	1.26 (\pm 0.12)
	ht VC		ND	7.37 (\pm 0.79)	2.63 (\pm 1.45)
3	UF control	3.13 (\pm 0.11)	0.58 (\pm 0.34)	0.58 (\pm 0.026)	0.098 (\pm 0.007)
	VC		44.7 (\pm 0.3)	17.3 (\pm 0.5)	4.88 (\pm 1.99)
	ht VC		0.58 (\pm 0.34)	1.44 (\pm 2.03)	0.31 (\pm 0.43)

Table 3b. Abundances (\pm SD) of viruses and bacteria measured in viral concentrate (VC) enriched, heat-treated (ht) VC enriched and ultrafiltrate (UF) control incubations in VC addition experiments 4, 5 and 6 from the Pacific.

Expt.	Treatment	t_0 Bacterial abundance ($\times 10^6 \text{ ml}^{-1}$)	t_0 Viral abundance ($\times 10^7 \text{ ml}^{-1}$)	t_{end} Bacterial abundance ($\times 10^6 \text{ ml}^{-1}$)	t_{end} Viral abundance ($\times 10^7 \text{ ml}^{-1}$)
4	UF control	6.81 (\pm 3.31)	3.38 (\pm 1.08)	12.8 (\pm 3.6)	4.54 (\pm 1.12)
	VC		13.9 (\pm 3.4)	23.6 (\pm 1.7)	107 (\pm 1)
	ht VC		3.38 (\pm 1.08)	7.11 (\pm 0.40)	4.32 (\pm 0.91)
5	UF control	0.443 (\pm 0.025)	1.20 (\pm 0.01)	2.90 (\pm 1.24)	1.46 (\pm 0.87)
	VC		27.5 (\pm 1.8)	8.79 (\pm 2.65)	4.02 (\pm 1.02)
	ht VC		1.20 (\pm 0.01)	4.11 (\pm 0.06)	2.27 (\pm 0.32)
6	UF control	2.91 (\pm 0.83)	1.73 (\pm 0.34)	11.3 (\pm ND)	6.97 (\pm ND)
	VC		8.15 (\pm 1.08)	10.6 (\pm 3.3)	6.78 (\pm 0.98)
	ht VC		1.73 (\pm 0.34)	9.73 (\pm 1.66)	6.03 (\pm 1.28)

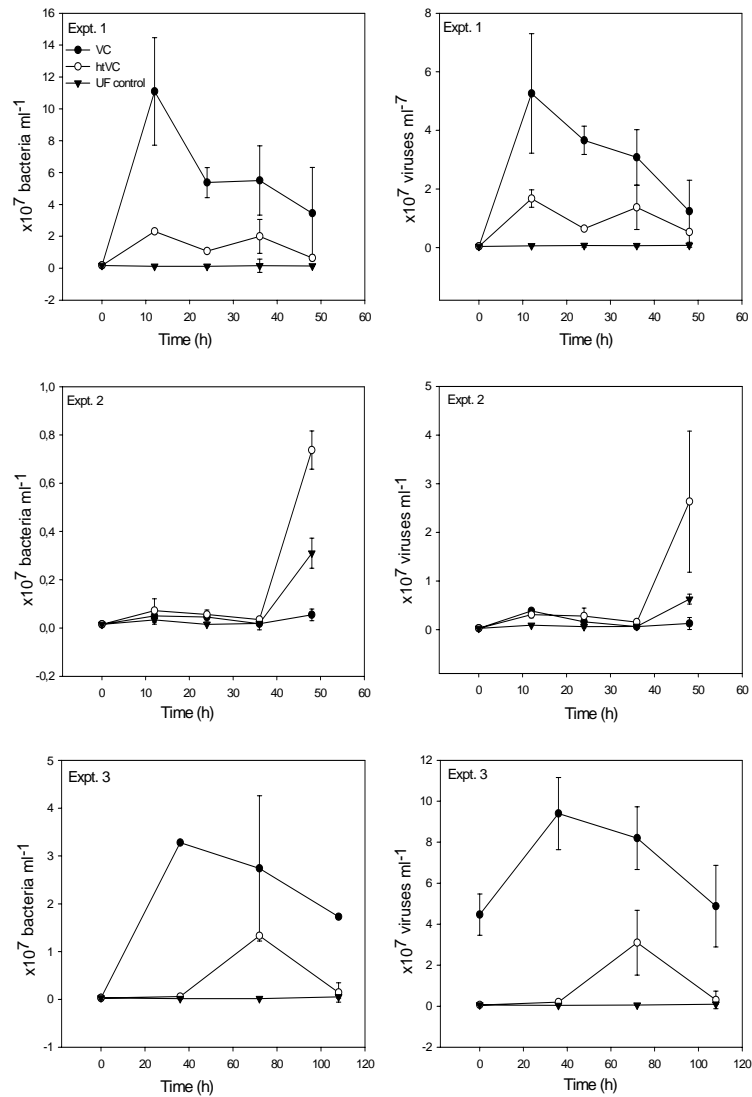


Figure 7. Bacterial and viral abundances over time in experiments 1, 2 and 3 from the North Atlantic. Error bars represent SD calculated from triplicate incubations. Where no error bars are shown, SD is smaller than the symbol.

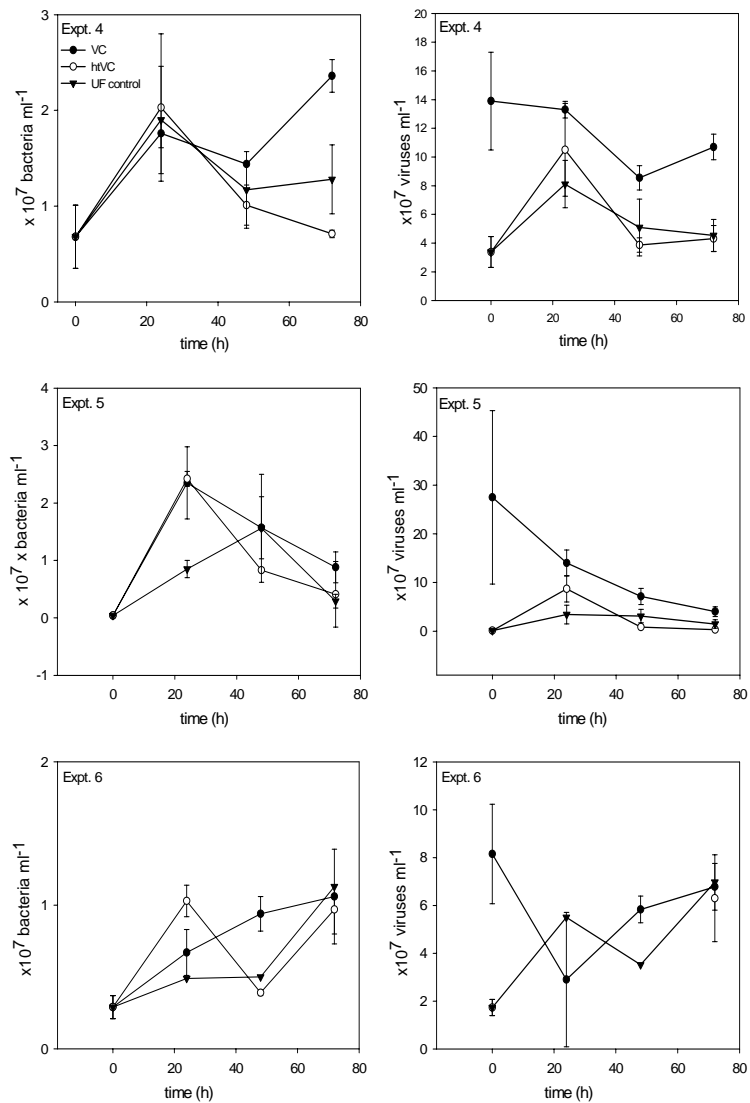


Figure 8. Bacterial and viral abundances in VC addition experiments 4, 5 and 6 from the Pacific Ocean. Error bars represent SD from triplicate incubations. Where no error bars are shown, SD is smaller than the symbol.

3.3.5 BACTERIAL PRODUCTION

Bacterial production ($[^3\text{H}]$ -leucine incorporation) rates over time were determined in two of the three experiments performed in the North Atlantic and in all three experiments performed at the eastern equatorial Pacific Ocean. Measurements suggest that both VC and htVC treatments induce bacterial production to exceed the control values (Figure 9).

3.3.5.1 The North Atlantic

Bacterial production rates in htVC-enriched incubations showed the greatest net increase over the time course of both two experiments. At the end points of experiments 1 and 2, bacterial production in VC enriched bottles was ca. 65 % and 40 % less, respectively, than in htVC-enriched incubations (Table 5, Figure 9).

In experiment 1, bacterial production in htVC and VC-enriched incubations demonstrated a small increase between t_0 and 12 h, but between 12 h and 24 h bacterial production in both incubations increased sharply. Over the time course of the experiment, bacterial production rates in htVC-incubations were significantly higher ($p < 0.05$) than in VC-enriched incubations at four of the six time points of the experiment. VC-enriched incubations demonstrated higher bacterial production rates than in UF control, but rates did not reach htVC-incubation production level. Bacterial productions in UF controls demonstrated a small net increase from $0.13 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$ to $2.4 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$ over the time course of the experiment.

In experiment 2, bacterial production rates in htVC-amended incubation were greater than in VC-enriched incubations at all time points, although the bacterial production rates in htVC and VC-enriched incubations did not demonstrate statistically significant ($p < 0.05$) difference from one another. Bacterial production in htVC-enriched samples increased from $0.21 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$ to $5.4 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$, while the increase in VC-enriched incubations was from $0.21 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$ to $3.4 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$. Both htVC and VC treatments demonstrated significantly ($p < 0.05$) greater values than in UF controls. Bacterial production in UF controls stayed visibly constant over the time course of the experiment and demonstrated only a slight increase, in the range of $0.21 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$ to $0.25 \times 10^{-10} \text{ gC l}^{-1} \text{ h}^{-1}$.

Table 4a. Bacterial production rates (\pm SD) ($\text{gC L}^{-1} \text{h}^{-1}$) at t_0 and at t_{end} in experiments 1 and 2 from the North Atlantic.

Expt.	Treatment	t_0 bacterial production ($\times 10^{-10} \text{ g C L}^{-1} \text{h}^{-1}$)	t_{end} bacterial production ($\times 10^{-10} \text{ g C L}^{-1} \text{h}^{-1}$)
1	UF control	0.12 (± 0.05)	2.37 (± 0.15)
	VC		6.48 (± 0.03)
	ht VC		14.0 (± 0.6)
2	UF control	0.21 (± 0.04)	3.97 (± 2.81)
	VC		7.13 (± 0.36)
	ht VC		14.5 (± 1.2)

3.3.5.2 Pacific

As in experiments performed in the North Atlantic, in the three experiments performed at the Pacific, the htVC-amended incubations demonstrated the greatest net increase in bacterial production rates and overall greater bacterial production through out the experiments than either VC enriched incubations or UF controls (Table 4b, Figure 9).

In experiment 4, htVC-amended incubations demonstrated significantly higher bacterial production at all time points after t_0 than VC-enriched incubations or UF controls. The addition of VC increased bacterial production significantly ($p < 0.05$) over UF control values, but the effect from adding active VC was significantly ($p < 0.05$) less than resulted from htVC-addition. In htVC-amended incubations bacterial carbon production increased from $0.49 \times 10^{-6} \text{ gC l}^{-1} \text{h}^{-1}$ to $5.2 \times 10^{-6} \text{ gC l}^{-1} \text{h}^{-1}$. VC-enriched incubations demonstrated a net increase in bacterial production from $0.49 \times 10^{-6} \text{ gC l}^{-1} \text{h}^{-1}$ to $3.7 \times 10^{-6} \text{ gC l}^{-1} \text{h}^{-1}$. In all experiments, bacterial production rates at the end points of the experiments (at 72 h) were ca. 52%-74% lower in VC-enriched incubations than in htVC-enriched incubations. Bacterial production rates in UF controls showed a slight increase in all experiments, in experiment 4, the net increase from $0.49 \times 10^{-6} \text{ gC l}^{-1} \text{h}^{-1}$ to $2.2 \times 10^{-6} \text{ gC l}^{-1} \text{h}^{-1}$ was observed.

In experiments 5 and 6, bacterial production in htVC-enriched bottles exceeded production in VC-enriched bottles after the 24 h time point. By t_{end} bacterial production in VC-enriched incubations had decreased to levels not significantly different from UF controls, while bacterial production in htVC-incubations was significantly higher than in VC-incubations or in UF controls.

Table 4b. Bacterial production rates ($\text{gC L}^{-1} \text{h}^{-1}$) at t_0 and at t_{end} in experiments 4, 5 and 6 from the Pacific.

Expt.	Treatment	t_0 bacterial production ($\times 10^{-7} \text{ g C L}^{-1} \text{h}^{-1}$)	t_{end} bacterial production ($\times 10^{-7} \text{ g C L}^{-1} \text{h}^{-1}$)
4	UF control	4.9 (± 0.2)	22.4 (± 8.4)
	VC		36.7 (± 3.6)
	ht VC		52.2 (± 6.1)
5	UF control	2.11 (± 0.32)	9.67 (± 2.94)
	VC		13.2 (± 1.3)
	ht VC		23.1 (± 1.2)
6	UF control	1.93 (± 0.16)	5.59 (± 0.53)
	VC		8.00 (± 1.94)
	ht VC		13.4 (± 1.9)

Table 5. Comparison of the effects of viruses and organic carbon on bacterial production at t_{end} of VC addition experiments. Experiments 1 and 2 were performed in the North Atlantic and experiments 4-6 in the Pacific Ocean.

Experiment	Increase in bacterial production (vs. control) at t_{end}		Bacterial production reduced due to viral enrichment (%) at t_{end}
	With htVC added (effect of DOM)	With VC added	
1	4.92	1.74	64.7
2	20.7	12.4	40.1
4	1.34	0.64	52.0
5	1.39	0.36	73.3
6	1.40	0.43	69.2

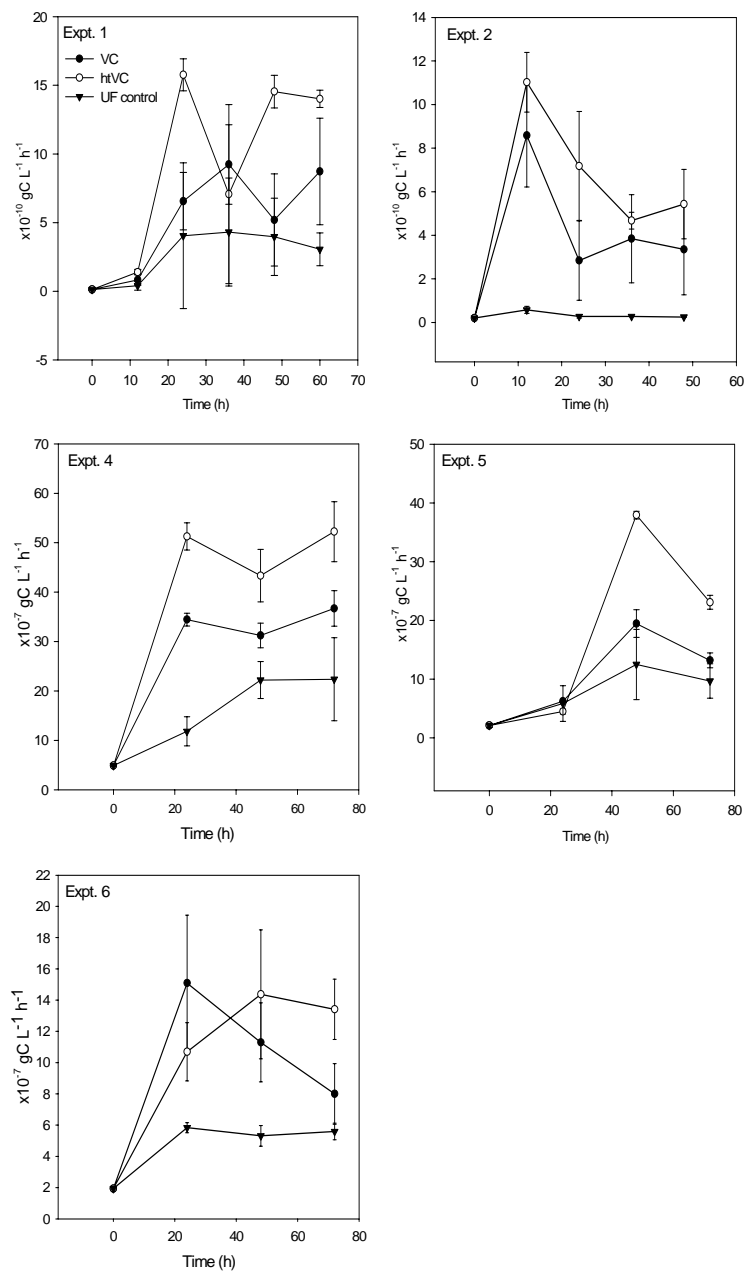


Figure 9. Bacterial productions $\text{gC L}^{-1} \text{h}^{-1}$ over time in VC addition experiments 1 and 2 from the North Atlantic, and 4, 5 and 6 from the Pacific Ocean. Error bars represent SD of triplicate incubations. Where no error bars are shown, SD is smaller than the symbol.

4. DISCUSSION

In this thesis I have focused on two topics: viral production in steady state conditions by a novel dilution method and the role of viruses in aquatic food webs by virus concentrate addition experiments. Detailed findings are listed below.

4.1 VIRAL AND BACTERIAL ABUNDANCE

The North Atlantic and eastern equatorial Pacific Ocean differed from each other by *in situ* measured parameters: the bacterial abundance, viral abundance and *in situ* total chlorophyll *a* (chl *a*). We did not observe great variations in bacterial and viral abundances in surface water samples between different offshore stations in the North Atlantic. In the eastern equatorial Pacific Ocean variations occurred in surface water viral and bacterial abundances and in chlorophyll *a* concentration along the transect.

Bacterial and viral abundance data is based on direct counts of SYBR Green I – stained viruses and cells using epifluorescence microscopy. Enumerating viruses in highly concentrated samples by this method may be a source of error, since samples have to be diluted as much as 20 fold before reaching a suitable abundance of particles to be counted. Also, when counting samples with a high density of particles, fading of the stain may lead to inaccurate results. We also ran into an unexpected problem when enumerating bacteria and viruses at t_0 in incubations of VC addition experiments. In samples taken immediately after addition of htVC, a high abundance of virus-like particles was observed as well as some fluorescent particles of bigger size-class than viral particles. We assumed that the total of 10 minutes microwaving was enough to render the viral particles non-infective, but as we found out, it was not enough to break the structure of all viral particles and therefore they were still visible in our counts. The large fluorescent particles may have formed during heating from aggregated organic material present in the viral concentrate. In the process of making a viral concentrate, some bacteria passed the prefiltration step and got into the concentrate. It is possible, however, that the viability of these bacteria had been affected during the ultrafiltration process.

4.2 VIRAL PRODUCTION EXPERIMENTS

By using the approach first described by Wilhelm et al. (2001) to assess viral production rates in two separate locations, the North Atlantic and eastern equatorial Pacific Ocean, we have demonstrated *in situ* viral production rates of ca. 0.68×10^6 viruses $\text{ml}^{-1} \text{h}^{-1}$ in the North Atlantic and a range from 0.23×10^6 to 213×10^6 viruses $\text{ml}^{-1} \text{h}^{-1}$ in the Pacific Ocean. The *in situ* viral production rate in the North Atlantic overlapped with the lower range of viral production rates observed in Pacific. A slower rate of production of viruses in the North Atlantic can be partly explained by overall lower host abundance and lower rate of encounters with viruses and suitable hosts in oligotrophic waters.

Our data on bacterial mortality rates implies that ca. 5-36 % of the bacterial population would be lysed due to viral infection on a daily basis in the equatorial Pacific and ca. 74 % of the bacterial population in the North Atlantic. Since only one viral production experiment was performed in the North Atlantic, we are not able to compare a range of viral induced bacterial mortality rates. In the Pacific, we observed that the fraction of bacteria lysed due to viral infection daily, increased along a transect from offshore to nearshore stations. This supports the idea presented by Noble and Fuhrman (2000) that viral infection may be a less significant factor causing bacterial mortality in offshore locations due to lower virus-host density and increased grazing pressure. Bacterial mortality rates from our study correspond with the range of bacterial mortality rates observed by Wilhelm et al. (2001) in tidally mixed waters of inlets and channels at the coast of British Columbia (Canada) and thus our results would support the view that bacterial mortality rates in a steady-state system are lower than in a non-steady-state system. Our data shows that virus induced mortality is an important loss factor within bacterial populations in variety of marine waters although mortality rates can vary significantly among different locations. If viruses are responsible for lysing indicated fractions of bacterial populations, significant amounts of organic carbon and other inorganic nutrients are released in the water column due to the lysis event. By applying the cell quota of each nutrient element ($23.3 \text{ fg C cell}^{-1}$; $5.6 \text{ fg N cell}^{-1}$; $0.5 \text{ fg P cell}^{-1}$; $1.1 \text{ ag Fe cell}^{-1}$ [Wilhelm and Suttle, 2000]), quantitative estimates of the amounts of nutrients released upon cell lysis can be made (Table 6).

Table 6. Estimates for release of carbon, nitrogen, phosphorous and iron due to viral-induced cell lysis in the north Atlantic (experiment 1) and in the Pacific Ocean (experiments 2-5). Estimates of the release of the elements are based on the estimates of cells lysed per day and the cellular quotas of each element.

Expt.	C ($\mu\text{g L}^{-1} \text{d}^{-1}$)	N ($\mu\text{g L}^{-1} \text{d}^{-1}$)	P ($\mu\text{g L}^{-1} \text{d}^{-1}$)	Fe ($\text{ng L}^{-1} \text{d}^{-1}$)
1	3,10	0,75	0,07	0,15
2	26,1	6,28	0,57	1,23
3	22,6	5,44	0,49	1,07
4	89,5	21,5	1,92	4,23
5	44,9	10,8	0,96	2,12

Viral turnover rates from this study ($6\text{-}255 \text{ day}^{-1}$) suggest overall more rapid turnover rates of the virus populations than has been reported previously in the literature (e.g. Heldal and Bratbak 1991; Steward et al., 1996; Noble and Fuhrman 2000), although our turnover rates corresponded with turnover rates determined with the same method by Wilhelm et al. (2001) in British Columbia, Canada.

As mentioned above in the Introduction section, previous studies of viral production and decay have been done through indirect methods, which have involved the use of tracers and various conversion factors (e.g. Heldal and Bratbak, 1991; Steward et al., 1996; Noble and Fuhrman, 2000). As an example, in a recent study, Noble and Fuhrman (2000) used fluorescently labeled viruses (FLVs) to measure viral decay and production. Viruses were concentrated from seawater, stained with SYBR Green I nucleic acid stain and added back to seawater. It is possible that decay of fluorescent tracer may have taken place already during the preparation steps of the FLV-material and over the time course of the experiment itself. Moreover, it cannot be overlooked that addition of tracers may have some unknown outcome in culture studies, which may affect directly or indirectly viral production or decay. Also, attempts to label viral nucleic acids with fluorescent tracer may turn out to be problematic due to the very low nucleic acid content of virus particles. Consequently, the nucleic acid of a single virus particle can bind a very small amount of tracer to itself, if compared for example to single a bacterium, and a detection signal from one single labeled virus particle is very weak. Viral particles may lose their label over the course of the experiment due to exposure to light, or the fluorescence may generally fade over time. This way an originally labeled virus becomes nondetectable and some accuracy of detection is lost. Some fluorescent nucleic acid stains have been shown to fade more rapidly than others,

SYBR Green I being one of the most unstable stains over time (Bettarel et al., 2000), which might set limitations on its use in long term experiments.

Comparatively rapid viral turnover rates from our study may reflect some methodological discrepancy between our study and the earlier studies. In our experiments, viral production was determined in a direct manner from natural microbial assemblages by monitoring reoccurrence of new viruses from previously infected cells. We did not expose bacteria or viruses to any treatments with foreign chemicals or tracers during the experiment. Staining of viruses and bacteria was done after returning to the laboratory just prior to the enumeration of stained particles with epifluorescence microscopy. By enumerating viruses and bacteria freshly stained with SYBR Green I, we were able to obtain very accurate counts of bacteria and viruses and this way potential errors resulting from fading of the stain were minimized. Our suggestion is that high accuracy in viral counts may have led us to detect higher viral production rates than has been reported in earlier studies that have used SYBR Green I-stain as a fluorescent tracer for viruses. In our study, viral production rates were used to calculate other parameters, therefore high viral production numbers affected our estimates of viral turnover and bacterial mortality rates, which were higher than reported previously.

In our experiments, the incubations were conducted in dark bottles protected from sunlight. Dark incubations may have led to overestimates of viral production, however, since sunlight mediated destruction of viral particles is an important loss factor *in situ* (Wilhelm et al, 1998a). Incubation in the dark also prevents primary production, which may have a negative effect on viral production through overall reduced microbial food web activity. The effect of bacterivorous protists was not examined in these experiments; therefore it is possible that the presence of bacterivores had an effect in our results. Generally it has been estimated that viruses and protists both cause 50 % of bacterial mortality, and it has been speculated previously that removal of protists will increase viral induced bacterial mortality (Noble and Fuhrman, 2000; Weinbauer and Peduzzi, 1995).

4.3 VIRAL CONCENTRATE ADDITION EXPERIMENTS

Two major conclusions can be drawn from our data from VC addition experiments: first, the data further support the idea that viruses are an active component of the bacterioplankton community and by lysing their hosts they enhance DOM and nutrient

recycling within the community. Second, it can be seen that without viruses being present, bacteria actively assimilate the labile DOM, and bacterial protein production increases significantly above the control levels as well as the bacterial production levels in VC-amended incubations.

VC addition experiments carried out for this study differed from the VC addition experiments done in the past, as we used size fraction 0.45 μm -30 000 kDa of seawater instead of 0.2 μm -30 000 kDa to make the concentrates. Because we used a larger size fraction of seawater components, we may have concentrated more non-virus particles in our concentrates. As well, in early studies of the effects of viral enrichments, heat treated VCs were not used as a control treatments, until the study by Noble et al. (1999). We used microwaving as the means for heat treatment instead of autoclaving simply because an autoclave was not available on board ship. Heat treatment in a microwave oven for 10 minutes is sufficient to denature active proteins and enzymes, and to kill most of the bacteria (Noble and Fuhrman, 2000; Karner and Rassoulzadegan, 1995).

All VC addition experiments demonstrate that both VC and heat-treated VC-enrichments resulted in an increase in bacterial abundances and enhanced the bacterial production in the incubations. Addition of VC stimulated bacterial production, as compared to the UF control values, but an even greater increase in bacterial production resulted from htVC-enrichment in all five experiments where bacterial productions were measured (Table 5). In experiments 1 and 2, bacterial production was overall approximately four orders of magnitude lower than in experiments 4, 5 and 6. This variation can be explained by the differences between conditions of the sampling sites. Although all our experiments were performed in separate locations and there were substantial differences between the initial conditions (bacterial and viral abundance, VC addition, temperature) among the experiments, the same general trend in bacterial abundance and bacterial production were observed. The results from experiments 1 and 2 did not conflict with observations from experiments 4, 5 and 6. In experiment 2, VC addition resulted in a 94-fold increase in viral abundance, and no significant response in bacterial and viral abundances could be seen during the first 40 hours. However, responses could be seen in bacterial production, which showed similar trends with other experiments.

The finding that bacterial production is increased in VC-enriched incubations is consistent with previous findings that enhanced viral infection of a bacterial population

will increase bacterial productivity and growth (e.g. Noble and Fuhrman, 1997; Noble and Fuhrman, 1999). In previous studies heat treated VC did not have any substantial effect on bacterial production levels or bacterial abundances (Noble et al., 1999).

It is possible that not only the viruses, but also the bacteria and other DOM, contained in the viral concentrates caused the rise in bacterial abundances in VC addition incubations. As well, htVC-addition increased the amount of bacterial and viral-size fluorescent matter and detritus in the samples. In this case, uncertainties about the origin of the fluorescent matter make it hard to interpret the actual effect of viral enrichment using bacterial counts. The elevation in bacterial production in htVC and VC enriched incubations may have been induced by the concentrated DOM contained in viral concentrates and in heat-treated viral concentrates. Active viruses that eliminated part of the bacterial community by viral infection and subsequent lysis repressed bacterial production in VC-enriched incubations compared to htVC-enriched bottles.

Another possible explanation for our findings is that the bacteria used the DOM provided in htVC as a source of energy and this caused an increase in bacterial cell size in the bacterial population but not an active division of bacterial cells. In this case the whole bacterial assemblage may have benefited from the addition of DOM contained in the htVC, and this would explain the greater bacterial production levels in htVC-enriched incubations. The growth of the bacterial population due to heightened viral impact may indicate that only some particular part of the population started dividing, while viral infection eliminated its competitor and also provided beneficial conditions for growth, increasing the concentration of nutrient-rich cell lysis products in the environment. If this was the case, the increased bacterial production could be caused by the active growth of this particular population, while other bacterial populations remained in a relatively unchanged state. It may also be possible that the noninfected cells growing at the expense of the infected cells are smaller (have a smaller carbon content), i.e. that biomass is not increasing as suggested in Middleboe (1996).

Kirchman and Rich (1997) have demonstrated previously the effect of added DOM on bacteria at the equatorial Pacific and their conclusion was that DOM is the main factor limiting bacterial production and growth rates in that area more probably than any nutrient. Carlson et al. (1998) investigated partitioning of organic carbon in the North Atlantic and concluded that the amount of DOC available to bacterial use may be a growth-limiting factor also in that area. Since bacterial production in two sampling

areas was increased after addition of heat-treated viral concentrate, this could be an indication that bacteria in our samples were under DOM-limitation.

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