The role of viruses in marine phytoplankton mortality
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Chapter 1

Introduction

Marine viruses, also referred to as virioplankton, are the most abundant and diverse biological entities in the ocean. The ecological implication of marine viruses goes beyond the mortality of their host. Viruses can substantially influence plankton community structure. The cell lysis of the infected host may furthermore affect processes of global significance, notably the cycling of nutrients. Viral pathogens are found to infect all the major classes of phytoplankton. Algal viruses appear to be involved in the disintegration of algal blooms, but only a limited number of studies has investigated their role in population dynamics of non-bloom forming phytoplankton. Therefore, the prevalence of virally mediated mortality in phytoplankton still remains elusive, and the significance of viral lysis related to other phytoplankton losses is essentially unknown. The purpose of this thesis is to address these issues in order to better understand the ecological significance of algal viruses, specifically examining the impact of these pathogens in marine environments with contrasting trophic status (eutrophic vs. oligotrophic).

This introductory chapter covers different aspects of viral ecology, with special emphasis on viruses infecting phytoplankton and the interaction with their host. The chapters that follow describe the results from field and laboratory studies investigating the ecological role of viral infection of phytoplankton as compared to other sources of phytoplankton losses (e.g. microzooplankton grazing) in different marine systems.

1. Viruses in marine ecosystems

In essence viruses are simple. They are defined as small particles composed of nucleic acids (either DNA or RNA) embedded in a protein shell (named caspid) that may be surrounded by an envelope. Viruses do not respire, move, or grow. They do not have inherent metabolism, therefore, they utilize the cellular machinery of a suitable host to replicate. A given virus infects a restricted range of hosts. Most viruses described to date are species-specific, i.e., they infect a single host species and sometimes even a single
strain within a species. As they do not move, viruses depend on passive diffusion to contact a suitable host. Consequently the encounter rate between a virus and a host is directly affected by their relative abundance.

1.1. Global abundance and diversity of marine viruses

In the years following the discovery of high virus abundance (typically $10^6$ to $10^8$ viruses mL$^{-1}$), field studies have emphasized that marine viruses are also a dynamic component of the planktonic community (Bergh et al. 1989). For example, virioplankton abundance varies with depth (Hara et al. 1996, Culley & Welschmeyer 2002), along trophic gradients (Weinbauer et al. 1993, Noble & Fuhrman 2000), and during the course of phytoplankton blooming events (Bratbak et al. 1990, Castberg et al. 2001, Brussaard et al. 2004b). It is now well accepted that viruses are present wherever life is found, and current estimates of $\approx 10^{30}$ viruses in the world’s ocean indicate that they are the most abundant marine biological entities (Suttle 2005).

The observation of natural virioplankton assemblages using transmission electron microscopy (TEM) has revealed that, beyond their high abundance, viruses vary in size and shape (Flint et al. 2000); most of them are polyhedral and range between 20 and 200 nm. The isolation and the characterization of viruses infecting specific microbial host cultures, including prokaryotic and eukaryotic microbes, consistently showed that at least one, and usually multiple, viruses can infect a single host species (see reviews by Brussaard 2004, Weinbauer 2004). It is, therefore, sensible to assume that the richness of viruses is at least as high as that of cellular life forms (Weinbauer 2004).

The development of culture-independent methodologies has considerably advanced our understanding of global virus diversity. The use of pulsed field gel electrophoresis (PGFE), that discriminates viruses based on their genome size, showed that natural viral assemblage typically comprise 7 to 35 distinct viral genome size classes ranging between 12 and 560 kilobases (Auguet et al. 2005 and reference therein). Using this approach, pronounced variations in virus community structure were detected in response to phytoplankton bloom formation (Castberg et al. 2001, Larsen et al. 2001), water column stratification (Wommack et al. 1999), or salinity gradient (Sandaa et al. 2003). Overall, PFGE results confirm that viral populations are spatially and temporally dynamic as previously predicted from changes in viral abundance.

The most striking evidence of high viral richness in the ocean was reported by metagenomic analyses of uncultured marine viral communities (Breitbart et al. 2002, Angly et al. 2006). The recent sequencing of shotgun libraries of 168 viral assemblages collected from four major oceanic regions revealed that several hundred thousand distinct viral species were dispersed in these waters (Angly et al. 2006). Most of these viral genotypes were not similar to previously reported sequences, indicating that much of the viral diversity is actually uncharacterized. An emerging view of viral diversity contends that the vast majority of viral species is widespread and that the local environmental conditions select for certain viral type through selective pressure.
1.2. Production vs. decay of marine viruses

In theory, all living marine organisms from ‘bacteria to whale’ are likely to be infected by at least one virus. However, the host-virus encounter is an abundance-dependent process; hence the majority of viruses probably infect the organism they most frequently encounter, i.e., the bacteria and phytoplankton.

Viruses are produced by their host through four types of replication. The lytic infection results in the release of virus progeny upon host lysis. The number of viruses produced per infected cells is called the burst size. The magnitude of viral burst size has important ecological implications as it directly influences the viral abundance, hence the propagation of viral infection.

Viruses can also interact with their host through lysogeny (or latency) where the viral genome is incorporated into the host cell genome (termed as prophage) and propagates along with host replication until an inducing event triggers the lytic pathway. The incidence of lytic or lysogenic replications may relate to ecological strategies. Lysogenic replication may prevail over lytic infection when successful host-virus encounter rate is too low to sustain lytic replication (Lenski 1988, Weinbauer 2004). The importance and mechanisms underlying lytic vs. lysogenic replication are unclear and therefore still need further investigation. So far lysogeny has only been reported for prokaryotic microbes.

Although the lytic and lysogenic infections are the most investigated forms of replication in marine environments, two other types of replication are described. These include the chronic infection where viruses are released by budding or extrusion without killing the host and the pseudolysogeny infection whereby the virus either enter a dormant intracellular phase or proceed with lytic infection. This type of infection resembles a true lysogenic infection except that the viral genome does not integrate into host genome (as cited in Williamson et al. 2001).

The term ‘viral decay’ includes the reduction in viral abundance and infectivity. Many different biotic and abiotic factors are involved in the loss of virus in the ocean. Among these, solar radiations, particularly ultraviolet B radiations (UV-B), are considered a major source of loss as they damage viral DNA (Suttle 2000, Wilhelm et al. 2002, Jacquet & Brathak 2003). The effect of damaging sunlight can still be significant at 10 m depth, as reported in the Gulf of Mexico offshore waters (Wilhelm et al. 2002). Viruses may also be inactivated, at least temporarily, by adsorption to host cells, high molecular weight dissolved organic matter, and transparent exopolymeric particles (Noble & Fuhrman 1997, Brussaard et al. 2005b, Ruardij et al. 2005). Viral losses have been observed due to grazing by nanoflagellates (Suttle & Chen 1992, Gonzalez & Suttle 1993) and adsorption of viruses to particles that sink out of the photic zone (Proctor & Fuhrman 1991). The latter observation is consistent with the report of high virus numbers in the sediments (Danovaro et al. 2001, Lawrence et al. 2001).
2. Ecological implications of marine viruses

2.1. The influence of viruses on the community composition

The majority of the marine viruses has a narrow host specificity, implying that only a small subset of the community is infected by a given virus. The current consensus is that virus will preferentially infect the most abundant host species (because of higher encounter rate) and by “killing the winner” viruses maintain the coexistence of competing species (Thingstad 2000). The incidence of changes in viral and microbial community structure following lysis events strongly support the role of viruses as a driving force for the interspecies competition and succession (Castberg et al. 2001, Larsen et al. 2001, Brussaard et al. 2005b). Lately, several field studies demonstrated that many marine viruses may even show intraspecies specificity, suggesting that viruses may also influence the clonal composition of the host species (Tarutani et al. 2000, Tomaru et al. 2004b, Mühling et al. 2005).

2.2. The effect of viruses on the biogeochemical cycles

Over the last two decades, it became evident that microbial processes largely drive the cycle of matter and energy in the ocean. Whilst phytoplankton constitute the base of the classical (grazing) food web, heterotrophic prokaryotes recycle dissolved organic matter (DOM) to inorganic nutrients and bacterial biomass through the microbial loop (Fig. 1). These microbes can be eaten by small predators, eventually leading back to the classical (grazing) food web. In the original hypothesis of the ‘microbial loop’, the primary source of DOM was assumed to derive from phytoplankton exudates and sloppy feeding by zooplankton (Azam et al. 1983). Through the lysis of their host, viruses also influence the cycling of DOM (Wilhelm & Suttle 1999). Prokaryotic and eukaryotic viruses efficiently convert the particulate organic matter (i.e., living biomass) to DOM that can be utilized by bacteria (Brussaard et al. 1996b, Middelboe et al. 2003). This “viral shunt” results in diverting the flow of matter and nutrient away from the higher trophic levels and, in turn, forces the food web towards a more regenerative system.

The incorporation of “viral module” in simple theoretical models systems demonstrated that viral lysis enhanced bacterial respiration and production (Fuhrman 1999, Wilhelm & Suttle 1999) and reduced protist production (Fuhrman 1999). The first mathematical ecosystem model based on empirical data confirmed that virally mediated mortality of the bloom-forming algal species Phaeocystis globosa was an essential regulating factor for the nutrient cycling (Ruardij et al. 2005). Experimental studies also supported these models’ predictions. Viral mediated release of DOM can constitute a significant supply of major nutrients (C, N, P) and trace nutrients (e.g. Fe) for other
photosynthetic and heterotrophic microorganisms (Middelboe et al. 1996, Göbler et al. 1997, Middelboe et al. 2003, Poorvin et al. 2004). Viral mediated nutrient cycling was furthermore shown to influence bacteria and phytoplankton species composition and succession (Göbler et al. 1997, Brussaard et al. 2005b).

Another possible effect of viruses on processes of global significance is to accelerate the production of dimethylsulfide (DMS). The DMS is a biogas that influences clouds formation, hence the global climate (Charlson et al. 1987). Many phytoplankton species produce dimethylsulfoniopropionate (DMSP) that may be cleaved into DMS and acrylic acid (AA) by the algal lyases and/or by the lyases of other organisms. Laboratory studies have demonstrated that viral lysis of *Micromonas pusilla*, *Phaeocystis pouchetii*, and *Emiliania huxleyi* was accompanied by a build up of DMSP in the media (Hill et al. 1998, Malin et al. 1998, Wilson et al. 2002). Therefore, viral lysis of phytoplankton may be an important source of DMSP in the environment.

Figure 1. Simplified diagram of the microbial loop. Through cell lysis of hosts, viruses divert living biomass away from the higher trophic levels of the food web (microzooplankton, heterotrophic nanoflagellates (HNF), larger grazers). Instead, living biomass is effectively converted to dissolved organic matter (DOM), available for heterotrophic bacteria, hence forcing the food web towards a more regenerative pathway. Black arrows indicate the flow of organic matter and grey arrows represent the flow of inorganic nutrients.
3. Viruses and phytoplankton hosts

Soon after the discovery of high abundance of marine viruses, Suttle et al. (1990, 1992) showed that adding virus concentrates to natural field samples could result in decreased primary production. Complementing these studies, the observation of viral infected algal cells suggested that viruses can account for significant phytoplankton losses, particularly during blooming events (Bratbak et al. 1993, Nagasaki et al. 1994, Brussaard et al. 1996b). With phytoplankton forming the base of the marine pelagic food web and the awareness of the ecological and socio-economical consequences of algal blooms (e.g., fisheries and tourism activities), the ecology of algal viruses and their contribution to phytoplankton mortality has gained considerable interest.

3.1 Taxonomy and phylogeny of algal viruses

Marine phytoplankton communities include a prokaryotic (cyanobacteria) and a eukaryotic component. Currently, host specific viruses are reported for both groups of phytoplankton. Viruses that infect cyanobacteria, referred to as cyanophages, were reported in unicellular (Proctor & Fuhrman 1990) and colonial cyanobacteria (Ohki 1999, Hewson et al. 2004). The ecologically important marine *Synechococcus* sp. and *Prochlorococcus* sp. are, by far, the most investigated cyanobacterial hosts (for reviews see Suttle 2000, Mann 2003). All known cyanophages have tails, present double stranded DNA, and belong to three families that also infect heterotrophic bacteria, the Myoviridae, the Siphoviridae, and the Podoviridae. Besides morphological differences, cyanophages belonging to these families also have variable “life styles”. For example, the Myoviridae are typically lytic and have a broader host range than the other tailed cyanophages. Conversely, the Podoviridae present the narrowest range of host. The replication of Siphoviridae differs from other tailed phages as they can interact with their host through lysogeny, and may thus propagate along with the host replication (see also section 1.3).

Viruses have been isolated for the major existing classes of eukaryotic phytoplankton. Unlike cyanophages, all known eukaryotic algal viruses propagate through a lytic pathway. Until recently, most of these viruses were consistently assigned to the family of large double stranded (ds)DNA viruses, the Phycodnaviridae (Wilson et al. 2005b). Molecular based analysis using the highly conserved DNA polymerase (pol) gene allowed distinguishing six genera within this family. With the increasing number of viruses characterized, it became evident that viruses infecting phytoplankton are extremely diverse with representatives in many more viral families than the Phycodnaviridae. For example, picorna-like positive sense single stranded (ss)RNA viruses were found to infect the diatom *Rhizosolenia setigera* (Nagasaki et al. 2004) and the two toxic harmful algal bloom (HAB) species *Heterocaspa circularisquama*
(Tomaru et al. 2004a) and *Heterosigma akashiwo* (Tai et al. 2003). The recently completed genomic sequence of the ssRNA *H. akashiwo* virus led to the creation of the new, distinct viral family, the Marnaviridae (Lang et al. 2004). Previously unknown nuclear inclusion viruses have also been reported to infect *H. akashiwo* (Lawrence et al. 2001) as well as the diatom *Chaetoceros cf. gracilis* (Bettarel et al. 2005). Another example of novel virus is the dsRNA virus that infects the cosmopolitan *Micromonas pusilla* assigned to the Reoviridae family (Brussaard et al. 2004a, Attaoui et al. 2006). These examples emphasize that many different viruses can infect the same algal species. We are only starting to uncover the diversity of marine algal viruses. Many more viruses need to be isolated and characterized in order to better evaluate algal virus richness.

### 3.2 Abundance and diversity of marine algal viruses

In the field, viral titer determination using the most probable number (MPN) culture based method has been proven very useful in the study of algal virus ecology. These studies indicated that infective algal viruses can be highly abundant (up to >10^5 mL^-1), dynamic, and exhibit a high level of diversity (Waterbury & Valois 1993, Suttle & Chan 1994, Cottrell & Suttle 1995a, Tomaru et al. 2004b).

The use of molecular tools allowed discriminating between different virus isolates infecting the same species. For example, Cottrel and Suttle (1995b) distinguished different *Micromonas pusilla* virus isolates using DNA hybridization. The diversity of cyanophages (namely, myocyanophages) was estimated by the sequence analysis of the gene encoding a structural protein g20 (Füller et al. 1998, Zhong et al. 2002, Mühling et al. 2005). Other genetic markers, such as the gene fragment of the putative major caspid protein of *Emiliania huxleyi* viruses also revealed a high molecular diversity among *E. huxleyi* viruses (Schroeder et al. 2002).

### 3.3 Algal viruses and phytoplankton mortality

The significance of algal viruses in terms of abundance, dynamics, and diversity indicates a significant role of viral lysis in phytoplankton ecology. However, to fully understand the role of viruses, it is essential to determine the extent of mortality they impose on their host. The studies that have addressed this issue were mainly conducted during conditions of high host cell abundance such as during phytoplankton blooms. In theory, when the host cell abundance is high, the probability of collision between a host and a virus increases, hence viruses may propagate rapidly through the host population. This may result in bloom collapse if the viral lysis rates exceed the specific host growth rates. This type of interactions is referred to as a control by “reduction” (Brussaard 2004). Several reports confirmed this theory and demonstrated that viruses are profoundly involved in the disintegration of algal blooms. For example, high proportions (10 - 50%) of algal cells were visibly (using TEM) infected at the end of a bloom of
Chapter 1  
Introduction

*Aureococcus anophagefferens* (Sieburth et al. 1988, Gastrich et al. 2004), *Heterosigma akashiwo* (Nagasaki et al. 1994), and *Emiliania huxleyi* (Bratbak et al. 1993, 1996, Brussaard et al. 1996b). Other approaches determining cell lysis from the number of putative algal viruses produced divided by an empirical viral burst size indicated that viruses accounted for substantial mortality (7 - 100%) during the bloom of *Phaeocystis globosa* (Brussaard et al. 2005a, Ruardij et al. 2005) and *E. huxleyi* (Jacquet et al. 2002).

While several studies have examined the role of viruses in controlling algal bloom dynamics, fewer studies have investigated the potential role of viruses in regulating non-blooming phytoplankton populations. Examinations of the picoeukaryote *Micromonas pusilla* and its specific viruses indicated a turnover of host standing stock between 2 and 25% d⁻¹ in various marine systems (Cottrell & Suttle 1995a, Evans et al. 2003). Studies conducted on the cyanobacterium *Synechococcus* reported that virally induced mortality daily removed <1 to 8% of the host population (Waterbury & Valois 1993, Suttle & Chan 1994, Garza & Suttle 1998). These results suggest a stable host-virus coexistence, where the viruses seem to maintain host population size to non-blooming level rather than causing a rapid decline in host abundance. This type of regulation is referred to as a “preventive” viral control (Brussaard 2004).

Overall, virally mediated mortality can occur at significant rates in phytoplankton populations. However, our understanding of the global significance of viral lysis is far from complete because non-blooming phytoplankton and more generally, phytoplankton in oligotrophic environments have been inadequately sampled as compared to bloom forming species, typically found in eutrophic (coastal) waters.

3.4. Potential factors regulating virally mediated mortality of phytoplankton

The above referred field studies indicate that viral lysis can be responsible for significant mortality in phytoplankton. Different factors can, however, regulate the dynamics of virally mediated mortality. These regulatory parameters include phytoplankton host abundance, morphology, physiology and their potential to develop defense mechanisms.

As viral infection is a stochastic process, the rate of encounter depends on the hosts and virus abundance and also on other morphological characteristics such as particle size and motility (Murray & Jackson 1992). At a given virus concentration, larger hosts will be more readily intercepted by a virus than the smaller counterparts. Host cell motility enhances transport rates which, in turn, increase the probability of collision with a given virus. Other host morphological characteristics can influence viral infection rate. For example, field and laboratory evidence suggested that non-coccolith-bearing *Emiliania huxleyi* are more readily infected than the lithed cells (Brussaard et al. 1996b, Jacquet et al. 2002). This is, however, not (yet) confirmed by controlled experiments. Furthermore, a mesocosm study showed that *Phaeocystis globosa* cells embedded in a colonial matrix tend to escape viral infection (Brussaard et al. 2005a,
Ruardij et al. 2005). Interestingly, this can be explained by the larger colonial size (Ruardij et al. 2005).

The most efficient defense of phytoplankton against viral infection is to be resistant. The incidence of resistant host strains has been reported for algal viruses in culture (Thyrhaug et al. 2003) as well as in the field (Waterbury & Valois 1993). Theory based on bacterial host-phages interactions suggests that resistance has a physiological cost for the host cells, resistant cells may have a competitive disadvantage against susceptible hosts (Levin et al. 1977). So far, the importance and the mechanisms underlying resistance of phytoplankton against viruses remain largely unclear.

A potential phytoplankton chemical defense against viruses was recently suggested by Evans et al. (2006). These authors related the negative effect of acrylic acid (AA) and dimethylsulfide (DMS) on the titers of *Emiliania huxleyi* virus to the inability to isolate viruses infecting *E. huxleyi* strains with high lyase activity (i.e., capable of efficient conversion of dimethylsulfoniopropionate (DMSP) into the AA and DMS). It was suggested that the cleavage of DMSP in DMS and AA during cell lysis of *E. huxleyi* may reduce the titers of *E. huxleyi* viruses, and therefore decrease the probability of infection of further cells. These observations led to argue that the DMSP system in phytoplankton may operate as a chemical defense against viral infection. This study supported the hypothesis that virucidal compounds can be produced alongside viruses during viral infection, and, in turn, can reduce infection rates of other algal cells (Thyrhaug et al. 2003). Another example of potential host defense strategy includes the enhanced sinking rates of *Heterosigma akashiwo* cells when infected by a virus (Lawrence & Suttle 2004). Viral infection may result in cells rapidly sink out of the euphotic zone, which, in turn, may prevent viral infection of conspecifics.

As obligate parasites, viruses depend on the host cellular machinery to propagate. Several studies have shown that the algal host growth stage may significantly influence the lytic viral growth cycle. Reduction in viral burst size (Van Etten et al. 1991, Bratbak et al. 1998) and even prevention of viral infection were observed (Nagasaki & Yamaguchi 1998) during the algal host stationary growth phase. The algal host cell cycle stage may also influence the production of algal viruses (Thyrhaug et al. 2002). Viral infection of *Pyramimonas orientalis* at the onset of the light period led to a higher viral production than when infected at the beginning of the dark period. Conversely, *Phaeocystis pouchetii* infection was independent of the host cell cycle.

Different environmental variables known to influence phytoplankton growth rates (i.e. light, nutrient, and temperature) may, furthermore, affect the viral growth cycle. For instance, darkness could prevent viral infection of different prokaryotic and eukaryotic algal hosts (MacKenzie & Haselkorn 1972, Allen & Hutchinson 1976, Waters & Chan 1982). Temperature may alter the susceptibility of host species to virus as shown for *H. akashiwo* (Nagasaki & Yamagushi 1998). Nutrient limitations were found to have variable effects; phosphate depletion resulted in a reduction of the burst size of *P. pouchetii* and *Emiliania huxleyi* viruses (Bratbak et al. 1993, 1998) or delayed the cell lysis in *Synechococcus* (Wilson et al. 1996). Under nitrogen depletion, the production of *E. huxleyi* viruses was delayed (Jacquet et al. 2002) and a reduction in the
viral burst size was observed for *P. pouchetii* (Bratbak et al. 1998).

In the ocean, phytoplankton cells experience strong fluctuations in natural resources (e.g. light, nutrient, temperature). For instance, light and nutrient levels can vary during phytoplankton bloom and across the water column of stratified systems (e.g. open ocean). These variations may thus control the impact of viruses on the host population. Furthermore, the contrasted nutrient conditions found in oligotrophic vs. eutrophic environments may underlie differential virally mediated mortality of phytoplankton in these respective environments.

4. Viral lysis and other sources of phytoplankton losses

4.1. Viral lysis and other sources of cell death by lysis

Algal cell lysis rates can be high and dynamic in marine environments (Brussaard et al. 1995, 1996a, Agusti et al. 1998). Estimates up to 0.3 d\(^{-1}\) have been reported not only during the termination of algal bloom (Brussaard et al. 1995, 1996a) but also in oligotrophic marine environments (Agusti 1998). Algal cell lysis has important implications on the trophic dynamics as the cell content released in surrounding waters upon lysis provides DOM, potentially available for heterotrophic bacteria. Several field studies indicated that DOM released upon algal cell lysis could be sufficient to fulfill most of the bacterial carbon demand (Brussaard et al. 1996b, 2005b).

Viruses are considered important agents killing phytoplankton. Although several studies have demonstrated that viruses can impose substantial mortality on their host (see review Brussaard 2004 and section 3.3), the quantitative significance of viral lysis in the ocean is not clear. One reason for this is that rates of virally mediated mortality are assessed using different approaches; therefore results from these studies are not necessarily comparable. More importantly, all known methodologies determining virally mediated mortality rely on differing assumptions and conversion factors (Table 1). Very few studies have, thus far, determined the contribution of viral lysis to total algal cell lysis. One recent mesocosm study has shown that viral lysis comprised 30 - 100% of the total lysis occurring during the bloom of *Phaeocystis globosa* (Brussaard et al. 2005a).

In addition to viruses, several other mechanisms responsible for cell lysis are currently described. For example, other pathogens (e.g., bacteria, fungi) are reported to kill phytoplankton (Fukami et al. 1992, Mayali & Azam 2004). Another example includes allelopathic interactions between phytoplankton species. In this type of interaction, the production of a metabolite by a phytoplankton species has an inhibitory effect on the growth or physiological function of another phytoplankton species that may result in cell lysis (Vardi et al. 2002, Legrand et al. 2003).
Table 1. Summary of the assumptions, advantages, and disadvantages of the methods used to determine virally mediated mortality of phytoplankton (adapted from Winget et al. 2005).

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Assumption</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>Frequency of infected cells using</td>
<td>- All cells containing viruses will lyse</td>
<td>- No incubation required</td>
<td>- Heavily dependent on theoretical conversion factors (eclipse time, relationship between latent period and host generation)</td>
<td>Sieburth et al. 1988</td>
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<tr>
<td>transmission electron microscopy</td>
<td>- The eclipse time (time from infection to mature virus appearance) is constant</td>
<td></td>
<td>- Host of interest may be difficult to discriminate in natural sample</td>
<td>Proctor et al. 1993</td>
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<td>(TEM)</td>
<td>- Latent period equals host generation</td>
<td></td>
<td>- Selective losses of infected cells may occur during sample preparation</td>
<td>Nagasaki et al. 1994</td>
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<td></td>
<td>- Host infection occurs continuously</td>
<td></td>
<td>- Time consuming</td>
<td>Bratbak et al. 1993, 1996</td>
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<td></td>
<td></td>
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<td>Brussaard et al. 1996b</td>
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<td>Binder 1999</td>
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<td></td>
<td>Gastrich et al. 2004</td>
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<tr>
<td>Contact rates</td>
<td>- All cells equally sensitive to viral infection</td>
<td>- Rapid, inexpensive</td>
<td>- Heavily dependent on theoretical conversion factors (diffusion, adsorption coefficient, cell size, burst size)</td>
<td>Suttle and Chan 1994</td>
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<td></td>
<td>- All virus-host contact result in infection</td>
<td></td>
<td>- MPN may underestimate virus abundance</td>
<td>Garza and Suttle 1998</td>
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<td></td>
<td>- All cell lyse after viral infection</td>
<td></td>
<td>- FCM cluster may include other viruses</td>
<td></td>
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<tr>
<td></td>
<td>- Diffusion, adsorption rate, burst size, and host cell size are constant</td>
<td></td>
<td>- Cross infection is not taken into account</td>
<td></td>
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<tr>
<td></td>
<td>- Virus and host of interest can be discriminated (Most Probable Number, MPN, flow cytometry, FCM)</td>
<td></td>
<td>- Lytic and lysogenic virus not distinguished</td>
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<tr>
<td>Viral decay</td>
<td>- Viral decay equals viral production</td>
<td>- Direct observation of changes in viral abundance/loss infectivity</td>
<td>- Use of theoretical burst size</td>
<td>Suttle and Chan 1994</td>
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<td></td>
<td>- Burst size is constant</td>
<td>- Rapid, inexpensive</td>
<td>- Relation between viral decay and production is disputable</td>
<td>Cottrel and Suttle 1995a</td>
</tr>
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<td></td>
<td>- Virus of interest need be discriminated (MPN, FCM)</td>
<td></td>
<td>- Lytic and lysogenic virus not distinguished</td>
<td>Garza and Suttle 1998</td>
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<td></td>
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<td>- MPN underestimates virus abundance</td>
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<td>- FCM cluster may include other virus</td>
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<td>Bongiorni et al. 2005</td>
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<tr>
<td>Viral production</td>
<td>- All virus produced from infected cells</td>
<td>- Direct changes in viral abundance</td>
<td>- Use of theoretical burst size</td>
<td>Bratbak et al. 1993</td>
</tr>
<tr>
<td></td>
<td>- Virus of interest can be discriminated from other virus (MPN, FCM)</td>
<td>- Rapid, inexpensive</td>
<td>- MPN underestimates virus abundance</td>
<td>Jacquet et al. 2002</td>
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<td></td>
<td>- Burst size constant or within a stated range</td>
<td></td>
<td>- FCM cluster may include other virus</td>
<td>Brussaard et al. 2005a</td>
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<td>Modified dilution method</td>
<td>- Algal growth rates unaffected by dilution level and diluent</td>
<td>- The only method excluding the use of conversion factors</td>
<td>- Only measures newly infected cells</td>
<td>Evans et al. 2003</td>
</tr>
<tr>
<td></td>
<td>- Phytoplankton cell lysis starts after 12 h</td>
<td>- Provides simultaneously viral lysis and grazing rates</td>
<td>- Initial phytoplankton concentration must be high enough to allow up to 5-fold dilution</td>
<td>This thesis</td>
</tr>
<tr>
<td></td>
<td>- Losses are proportional to the dilution effect of the loss agent (virus and grazers)</td>
<td>- Discriminates different algal groups when combined with FCM</td>
<td>- A 24h incubation required</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- No selective grazing on infected/ noninfected cells</td>
<td></td>
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</tbody>
</table>
Non-pathogenic forms of algal cell lysis are also reported. For example, the diatom *Ditylum brightwellii* was shown to experience a type of ‘intrinsic mortality’ under nitrogen controlled conditions using chemostat cultures (Brussaard et al. 1997). Recently, another form of autocatalyzed cell death was shown to share similarities with the programmed cell death (PCD) observed in higher plants and metazoans (Berges & Falkowski 1998, Vardi et al. 1999, Berman-Frank et al. 2004). The PCD, unlike “natural cell death” or “necrosis” refers to an active, genetically controlled degenerative process, which involves series of apoptotic features such as morphological changes (e.g. cell shrinkage, vacuolization) and complex biochemical events (e.g. activation of PCD markers like caspases). The PCD ultimately leads to cell lysis. Laboratory studies suggest that a wide range of phytoplankton is programmed to die in response to adverse environmental conditions (see review by Franklin et al. 2006). The PCD pathway in phytoplankton was found to operate under environmental stresses such as intense light (Berman-Frank et al. 2004), darkness (Berges & Falkowski 1998), nutrient depletion (Berman-Frank et al. 2004), CO₂ limitation and oxidative stress (Vardi et al. 1999). Some apoptotic features, possibly directing to PCD, have also been detected upon viral infection (Berges and Brussaard unpubl. data, Lawrence et al. 2001). The complete genome sequence of a virus infecting *Emiliania huxleyi* has recently revealed the presence of genes encoding the biosynthesis of ceramide, which is known to suppress cell growth and is an intracellular signal for apoptosis (Wilson et al. 2005a).

### 4.2. Viral lysis versus microzooplankton grazing

Prior to the recognition of algal cell lysis as an important loss factor, phytoplankton cells were essentially treated as immortal unless they were preyed upon by zooplankton or lost by sedimentation through the water column. Cell lysis, sedimentation, and predation by zooplankton may thus, separately or in concert, influence phytoplankton community structure.

Whether a phytoplankton cell sinks, is preyed upon, or undergoes lysis has more implications than the structuring of phytoplankton community. As nicely formulated by Kirchman (1999), “how phytoplankton die largely determines how other marine organisms live”. The phytoplankton biomass that sinks is lost from the surface to the benthic food web (Smetack 1985). In contrast, zooplankton grazing channels phytoplankton biomass to the higher trophic levels whereas the release of cell constituents mediated by lysis directly affects the standing stock of dissolved organic carbon, forcing the food web towards a more regenerative pathway (Wilhelm & Suttle 1999, Brussaard et al. 2005b, Fig. 1). The quantification of cell lysis in relation to sinking and grazing rates is, therefore, essential for an optimal understanding of the flow of matter and energy in marine systems.

There are differential controls of phytoplankton in oligotrophic vs. eutrophic environments. In oligotrophic waters (e.g. open ocean, surface coastal waters during summer), the import rate of the controlling nutrient is low and the regeneration of this limiting nutrient is critical to sustain high productivity. Small-sized picophytoplankton
dominate the autotrophic community due to their competitive growth characteristics. Considering their micrometer size range, picophytoplankton cells are not prone to sedimentation (Raven 1998). Instead, the rapidly growing small-sized predators (heterotrophic nanoflagellates and microzooplankton) are thought to largely control this phytoplankton biomass (Riegman et al. 1993, Kuipers & Witte 2000). As discussed above (section 3.3), there is also evidence of significant viral lysis of smaller-sized picophytoplankton. The relative importance of viral lysis as compared to grazing is, however, largely unknown in these environments.

In eutrophic waters the import rate of the controlling nutrient is higher and larger-sized phytoplankton can develop as they escape grazing by microzooplankton. The larger-sized phytoplankton biomass may not be immediately controlled by larger grazers as the development and the generation time of larger grazers is relatively long. Size-selective escape of grazing or non-edible phytoplankton may form algal blooms. Mass sedimentation can be involved in the disintegration of some of these blooms (Smetack 1985, Brussaard et al. 1995). Cell lysis was also found to be responsible for bloom termination (Brussaard et al. 1996a). As emphasized in this introductory chapter, one possible agent causing the cell lysis is viral infection. Episodes of light and/or nutrient limitations, typically occurring during algal blooms, may regulate the impact of virus on host abundance. However, the extent to which viral lysis varies and the underlying regulatory mechanisms remain poorly documented.

Summarizing the above, the understanding of the quantitative and qualitative importance of phytoplankton viral lysis in the oligotrophic vs. eutrophic marine waters is not clear. The present thesis aims to elucidate the role of algal viruses in these contrasting environments. Therefore, virally induced mortality rates of different phytoplankton groups were determined and related to microzooplankton grazing. In order to compare results from different field studies, a single method assessing viral lysis was used, namely an optimized version of the recently modified dilution method (Evans et al. 2003). In its original form, the dilution method is routinely used to estimate grazing on phytoplankton (Landry & Hassett 1982). The modified assay also includes virally mediated losses of phytoplankton. Although this dilution method has some restrictions, it has the benefits to exclude the use of conversion factors (i.e., it provides direct viral lysis rates), to minimize the handling of sample, and it can be applied to the different coexisting phytoplankton populations. In addition to these field studies, laboratory experiment aimed to characterize specific virus-host model systems and to study the virus-host interactions in relation to environmental relevant conditions.
5. This thesis

The aim of this thesis is to advance our knowledge on the ecological significance of algal viruses for marine phytoplankton mortality. More specifically, this research used a combination of field and laboratory approaches to explore three main issues:

1. The extent of virally induced lysis in phytoplankton mortality in marine environments with contrasting trophic status (oligotrophic vs. eutrophic)
2. The comparison of viral lysis rates with other algal loss factors (mainly microzooplankton grazing)
3. Possible factors regulating algal host-virus interactions.

Chapter 2 describes the significance of viruses during the course of an algal bloom that developed in the eutrophic southern North Sea. The prymnesiophyte *Phaeocystis globosa* is well known for its complex polymorphic life cycle (including colonies and single cells) and its potential to develop dense blooms in temperate coastal waters. The termination of *P. globosa* blooms is typically sudden. Earlier studies have demonstrated that cell lysis can account for up to 75% of the bloom decline (Brussaard et al. 1995). Recently, a mesocosm experiment showed that viruses could be a primary cause of cell lysis for *P. globosa* (Brussaard et al. 2005a, Ruardij et al. 2005). However, virally induced mortality of *P. globosa* has never been determined in the field. For completeness and to allow the study of inter-annual variability, the significance of viral lysis during 2 consecutive *P. globosa* blooms was investigated. To complement viral lysis estimates of *P. globosa*, we monitored the total abundance of putative *P. globosa* viruses (PgV) as well as the abundance of infective PgV.

Chapter 3 adds to the previous study by providing insight into the phenotypic diversity of PgV. An earlier phylogenetic analysis of 24 PgV isolated from the Southern North Sea revealed a close genetic relatedness among these isolates as they formed a tight monophyletic group within the family of the Phycodnaviridae (Brussaard et al. 2004b). In order to address biodiversity issues, it was thus very challenging to explore the phenotypic diversity among these isolates. Therefore, twelve of these isolates were further characterized. This study includes a morphological (particle size and shape) and molecular (genome size, major structural protein composition) characterization as well as the investigation of ecologically relevant characteristics such as the length of the lytic cycle, burst size, the range of host infected by these isolates, and their sensitivity to temperature.

Chapters 4 and 5 investigate the role of viruses as mortality agents for different picophytoplankton groups in oligotrophic waters. Chapter 4 describes a study conducted in the northeastern subtropical Atlantic Ocean with a permanent oligotrophic
status whereas the study described in Chapter 5 was executed in the North Sea under seasonal (summer) oligotrophic conditions. Sharp gradients of light, temperature, and nutrient level are typically encountered across the water column in these environments. At the bottom of the euphotic zone, an accumulation of phytoplankton, referred to as a deep chlorophyll maximum (DCM), marks the transition between the nutrient-depleted lit surface waters and the nutrient-enriched, light-depleted waters below the thermocline. Different algal virus communities were observed in the surface and DCM waters (Zhong et al. 2002). We investigated the role of algal viruses in the DCM (Chapter 4) and in the surface waters (Chapter 5) and related rates of viral lysis to microzooplankton grazing for 4 groups of picophytoplankton (including eukaryotes and prokaryotes).

Chapter 6 addresses the influence irradiance can have on virus-algal host interactions. Given the changes in light conditions that a phytoplankton cell may experience with depth or with time, investigating the effect of different irradiance on host-virus interactions was timely. Chapter 6 describes a laboratory experiment testing the effect of different light levels, including darkness, on two marine phytoplankton of ecological relevance, namely the bloom former *Phaeocystis globosa* thriving in eutrophic waters, and the picophytoplankter *Micromonas pusilla* ubiquitously distributed including in oligotrophic environments.

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Chapter 1

Introduction


