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Algal Bloom Viruses

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ABSTRACT

Although viral infections of vascular plants have been studied intensively for a long time already, it is only relatively recent that the ecological importance of algal viruses was recognized. The scientific field of phycovirology is exciting and rapidly changing as new viruses are isolated on a regular basis. The biology of algal viruses is diverse; including DNA and RNA viruses, double-stranded as well as single-stranded, ranging in genome size from a few to over 500 kb. The various sorts of algae play significant roles in aquatic ecology, with the so-called algal bloom forming species being able to occur in high abundance. Many of the algal viruses in culture infect bloom-forming algal hosts, probably as a result of the extra effort put into studying algal blooms for socio-political and economical reasons, and the high abundance of host cells. In the following review, a synopsis of the available information on viruses infecting bloom-forming algal species will be provided. It will describe the discovery, characteristics and molecular biology of the algal bloom viruses. The ecological impact of these algal bloom viruses on the host's population dynamics, ecosystem functioning and biogeochemical cycling will be outlined. Finally, challenges for the future of algal virology will be discussed.

Keywords: aquatic, cyanophage, phycoviruses, phytoplankton, virology

Abbreviations: **CDS**, coding sequence; **DGGE**, denaturating gradient gel electrophoresis; **DMS**, dimethylsulfide; **DMSP**, dimethylsulfoniopropionate; **DNA *pol***, DNA polymerase gene; **HAB**, harmful algal bloom; **NCLDV**, nucleocytoplasmic large DNA virus; **ORF**, open reading frame; **PFGE**, pulsed field gel electrophoresis; **RdRp**, RNA-dependent RNA polymerase; **RFLP**, restriction fragment length polymorphism; **TEM**, transmission electron microscopy; **VLP**, virus or virus-like particle

CONTENTS

GENERAL INTRODUCTION

Whilst there is a long history in vascular plant virology, the study of algal viruses is only a relatively recent field of research. Algal virology is expanding rapidly since the global ecological importance of these viruses is being revealed and new viruses are isolated and characterised on a regular basis. Algal virus is a functional term often used to indicate viruses that infect algae, an informal assemblage of chiefly aquatic, oxygen-evolving photosynthetic organisms. These are (1) the prokaryotic algal viruses, infecting cyanobacteria, and (2) the eukaryotic algal viruses, infecting single-celled forms to giant kelp. Algal hosts represent a diverse group of organisms, ranging from unicells to large multicellular thalli. Unicellular algae may be solitary or colonial, motile or nonmotile, free-living or attached. The cell size of unicellular algae may vary from smaller than 1 micrometer in diameter for the picoeukaryotes and cyanobacteria to hundreds of micrometers for e.g. diatoms.

The scope of this review is not to provide a detailed and complete overview of algal viruses, but is to bring algal viruses to the attention of a wider audience also working with photosynthetic host-virus model systems. In the following, general characteristics and the ecological relevance of algal viruses will be discussed. The focus will be on viruses infecting bloom forming eukaryotic algal species, which represent many of the algal virus-systems in culture.

History of algal virology

The earliest reports describing a virus or virus-like particle (VLP) in aquatic photosynthetic organisms consisted solely of single accounts of microscopic observations using electron microscopy (Torrella and Morita 1979; Bergh *et al.* 1989; Børsheim *et al.* 1990). Viruses infecting cyanobacteria (cyanophages) were first discovered in freshwater in the

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Invited Review

Table 1 Referenced characteristics of phycoviruses in culture at present.

Host algae	Type[a]	Size[b]	Location[c]	LP[d]	Virus code	References
Bacillariophyceae (diatoms)						
Rhizosolenia setigera[e]	ssRNA	11.2	cytoplasm	<24	RsRNAV	Nagasaki *et al.* 2004b
Chaetoceros cf. gracilise	ND	ND	nucleus	<24	CspNIV	Bettarel *et al.* 2005
Chaetoceros salsugineume	ssDNA	6	nucleus	12-24	CsNIV	Nagasaki *et al.* 2005b
Chaetoceros debilis[e]	ssDNA	ND	cytoplasm	12-24	CdebDNAV	Tomaru *et al.* 2008
Chlorophyceae (green algae)						
Chlorella-like green alga[i]	dsDNA	330-380	cytoplasm	6-8	PBCV	Dunigan *et al.* 2006
Chrysophyceae (golden algae)						
Aureococcus anophagefferens[e]	dsDNA	ND	cytoplasm	ND	AaV	Gastrich *et al.* 1998
Cyanophyceae (cyanobacteria)						
Synechococcus sp.	dsDNA	80-100	cytoplasm	6-10	S-XM/P/S[f]	Suttle and Chan 1993; Wilson *et al.* 1993
Microcystis aeruginosa[e, i]	dsDNA	160	cytoplasm	6-10	MaV	Yoshida *et al.* 2006
Nodularia spumigena[e]	dsDNA	ND	ND	ND	N-BM/S[f]	Jenkins and Hayes 2006
Phormidium persicinum[g]	dsDNA	50	nucleus	30	ND	Ohkiand Fujita 1996
Dinophyceae (dinoflagellates)						
Heterocapsa circularisquama[e]	dsDNA	350	cytoplasm	24	HcV	Tarutani *et al.* 2001; Nagaski *et al.* 2003
Heterocapsa circularisquama[e]	ssRNA	4.4	cytoplasm	ND	HcRNAV	Tomaru *et al.* 2004a
Prasinophyceae						
Micromonas pusilla	dsDNA	190-210	cytoplasm	7-14	MpV	Waters, Chan 1982; Suttle pers comm., January, 2008
Micromonas pusilla	dsDNA	190-220	cytoplasm	4-8, 8-12, 12-16	MpV	J Martínez Martínez unpubl data
Micromonas pusilla	dsRNA	25.6	cytoplasm	36	MpRV[h]	Brussaard *et al.* 2004a
Pyramimonas orientalis	dsDNA	560	cytoplasm	14-19	PoV	Sandaa *et al.* 2001
Phaeophyceae (brown algae)						
Ectocarpus siliculosus[j]	dsDNA	335	nucleus	lysogenic	EsV	Dunigan *et al.* 2006
Feldmannia simplex[j]	dsDNA	220	nucleus	lysogenic	FsV	Müller 1996
Feldmannia sp.[j]	dsDNA	170	nucleus	lysogenic	FsV	Lee *et al.* 1995
Hincksia hincksiae[j]	dsDNA	ND	nucleus	lysogenic	HincV	Wolf *et al.* 1998
Myriotrichia clavaeformisj	dsDNA	ND	nucleus	lysogenic	MclaV	Wolf *et al.* 2000
Pilayella littoralis[j]	dsDNA	280	nucleus	lysogenic	PlitV	Maier *et al.* 1998
Prymnesiophyceae						
Emiliania huxleyi[e]	dsDNA	415	cytoplasm	12-14	EhV	Castberg *et al.* 2002
Phaeocystis globosa[e]	dsDNA	177	cytoplasm	12 or16	PgV-Group II	Baudoux *et al.* 2005
Phaeocystis globosa[e]	dsDNA	466	cytoplasm	10	PgV-Group I	Baudoux *et al.* 2005
Phaeocystis globosa[e]	dsDNA	176	ND	ND	PgV	Wilson *et al.* 2006
Phaeocystis pouchetii[e]	dsDNA	485	cytoplasm	12-18	PpV	Jacobsen *et al.* 1996
Chrysochromulina brevifilum[e]	dsDNA	ND	cytoplasm	ND	CbV	Suttle and Chan 1995
Chrysochromulina ericina[e]	dsDNA	510	cytoplasm	14-19	CeV	Sandaa *et al.* 2001
Raphidophyceae						
Heterosigma akashiwo[e]	dsDNA	ND	cytoplasm	ND	HaV	Nagasaki *et al.* 1997
Heterosigma akashiwo[e]	dsDNA	180	cytoplasm	17	OIs1	Lawrence *et al.* 2006
Heterosigma akashiwo[e]	ssRNA	0.91	cytoplasm	35	HaRNAV	Tai *et al.* 2003
Heterosigma akashiwo[e]	ND	ND	nucleus	ND	HaNIV	Lawrence *et al.* 2001

[a] Genome type
[b] Genome size, kb
[c] Location of replication
[d] Latent period, h
[e] Viruses infecting bloom forming algae
[f] Nomenclature for aquatic cyanophage (Suttle 2000): Species-Origin and Virus family; so Algal species virus with origin X and belonging to either *Myoviridae* (M), *Podoviridae* (P) or *Siphoviridae* (S).
[g] Lysogenic, although Southern blot patterns suggested that some part of the lysogenized phage was in the lytic cycle under normal growth conditions (Ohki and Fujita 1996).
[h] Virus was originally designated *M. pusilla* RNA virus (MpRNAV), but renamed MpRV upon recognition as reovirus (Attoui *et al.* 2006).
[i] Freshwater algal species
[j] Multi-cellular algal species
ND = not determined

1960's and only about 20 years later were there the first reports on marine cyanophages (see for detailed review Suttle 2000b). First reports of phycoviruses, viruses that infect eukaryotic algae, date back to the early 1970's (for overview of older literature, see review by Van Etten *et al.* 1991). Most observations were with field-collected algae that were not available for further characterization. It was not until the 1980's that a new era of phycovirological research started with the discovery and detailed study of viruses that infect symbiotic *Chlorella* species (PBCV) by Meints and Van Etten (Van Etten *et al.* 1983 Meints *et al.* 1984; Van Etten *et al.* 1985). The unicellular *Chlorella*-like green algae could be easily cultured, the system could be assayed by plaque formation, and the viruses were produced in large quantities. It still needed another 10 years before the research field was generally recognized and in 1998 the first Algal Virus Workshop was organized in Bergen, Norway.

At present, viruses have been reported for many algal species and for most algal classes there are host-virus model systems in culture (**Table 1**). Many of the culture systems today originate from marine waters and infect bloom-forming alga. The latter is likely the result of several factors: (1) the relative easy access to the coastal environments where algal blooms occur, (2) the high abundance of the host cells that promotes an epidemic course of infection, and (3) the extra effort put into studying algal blooms for socio-political and economical reasons.

It was also in the last decade of the 20th century that interest in aquatic viruses increased with the realization that

free viruses were very abundant (for comprehensive review, see Weinbauer 2004). The most abundant hosts are bacteria, archaea and phytoplankton, important functional groups for ecosystem productivity. In recent years, it became increasingly clear that viruses are not only pathogens that cause disease in their host, but that they also play critical roles in biodiversity and cycling of nutrients and carbon (Suttle 2007). This recognition, together with the fact that phytoplankton form the basis of most aquatic food chains, added a new dimension to the research of algal viruses.

Detection and isolation of algal viruses

Most algal viruses in culture were isolated from single celled host organisms. In order to isolate lytic algal viruses generally a rather simple isolation protocol is used; adding a (prefiltered) natural water sample directly or concentrated to one or various strains of the algal species of interest and check for loss in chlorophyll autofluorescence. Natural samples may be concentrated by ultrafiltration, and/or prefiltered or centrifuged at low speed to remove cellular material. The loss of autofluorescence reflects the loss of cells as compared to the noninfected control (Suttle *et al.* 1990). Contrarily to the filamentous cyanobacteria, macroalgae and vascular plants, infection of a unicellular algal host with a lytic virus results directly in the death of the host. This is an important phenomenon, defining population dynamics, species succession, the continual arms race between virus and host, and cycling of organic matter.

Purified clonal cultures of infectious viruses are typically obtained using several cycles of a liquid extinction serial dilution procedure since most of the algal viruses in culture infect pelagic (living in open sea) hosts that are generally hard to grow on an agar plate. Plaque assays are, therefore, not an easy option to isolate and detect these algal viruses. *Chlorella*, cyanobacteria and *Emiliania huxleyi* are good exceptions, as they grow well in culture dishes (Suttle and Chan 1993; Wilson *et al.* 1993; Schroeder *et al.* 2002). The isolation of the *Chlorella* viruses was, however, atypical as they were isolated from the zoochlorellae (or *Chlorella*-like algae) that live as hereditary endosymbionts within the coelenterate *Hydra viridis* and the protozoans *Paramecium bursaria* and *Acanthocystis turfacea* (Van Etten *et al.* 1983; Fitzgerald *et al.* 2007).

Most algal viruses reported are lytic, except for lysogenic cyanophages that were induced after exposure to UV or mytomicin C (see detailed review by Suttle 2000b), and a lysogenic phage in the life cycle of the phycoviruses infecting brown macroalgae (Van Etten 2000). Brown macroalgae are unique among the algae in developing into multicellular forms with functionally and structurally distinctive tissues and organs. They have vascular systems comparable to terrestrial plants. After viral infection of specifically the reproductive cells of the algal host, the viral genome associates with the algal host genome and is passed on to all progeny cells of the developing thallus by mitotic divisions (Müller and Frenzer 1993). Viral particles are exclusively produced in mature sporangia and gametogangia instead of reproductive cells. All vegetative cells are free of viral particles. The deformed infected reproductive structures of the host cell can be easily detected using light microscopy. Isolation of virus particles is typically done by disrupting the specific host cells (Müller *et al.* 1990).

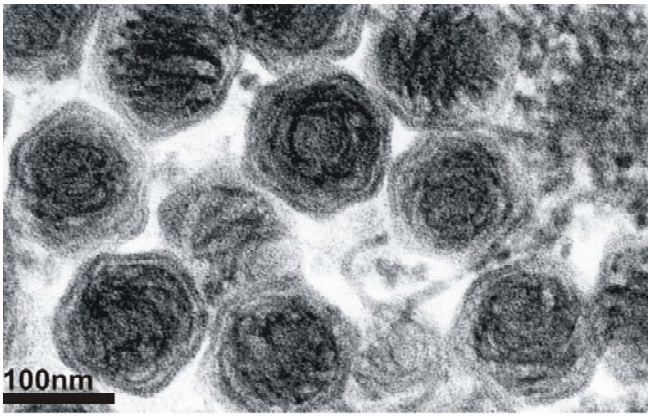

Fig. 1 Transmission electron micrograph of *Phaeocystis globosa*-virus particles (PgV-Group I).

Identification and discrimination of algal viruses

Algal viruses are detected by techniques that are routinely used in virology, such as transmission microscopy that has the advantage of providing information about morphology of the virus particles (**Fig. 1**). Some algal viruses can, however, also be discriminated using flow cytometry (Brussaard *et al.* 2000; Brussaard 2004a). Flow cytometry is a high throughput method in which many of the fluorescent stained algal viruses can be separated from bacteriophages. The sensitive nucleic acid-specific stains that are commercially available nowadays allow virus particles to be counted using epifluorescence microscopy and flow cytometry (Brussaard *et al.* 2000; Lawrence 2005). The latter has the advantage to allow discrimination of subpopulations, based on their fluorescence and scatter characteristics. As many of the algal viruses have large genomes (**Table 1**), staining of their nucleic acid may result in a distinct subpopulation (**Fig. 2**).

Molecular approaches have proven vital for the study of algal viruses. Pulsed field gel electrophoresis (PFGE) enables size fractionation and separation of viral genomes. PFGE has been proven very useful not only for determining the algal viral genome size, but also for detecting large genome-sized algal viruses in natural samples (Castberg *et al.* 2001; Larsen *et al.* 2001, 2004). Chen and Suttle (1996) showed the great potential of the highly conserved DNA polymerase (DNA *pol*) gene for phylogenetic analysis using specific algal virus primers to establish evolutionary relationships among many viruses. More specifically, primers have been developed to amplify DNA fragments coding for conserved regions of the viral capsid proteins from several cyanophages (g20 and g23; Fuller *et al.* 1998; Jenkins and Hayes 2006), the potential protein-coding regions (open reading frames, ORFs) of the red tide-causing *Heterosigma akashiwo* (Nagasaki *et al.* 2001), and the major capsid protein genes from the viruses infecting the globally important

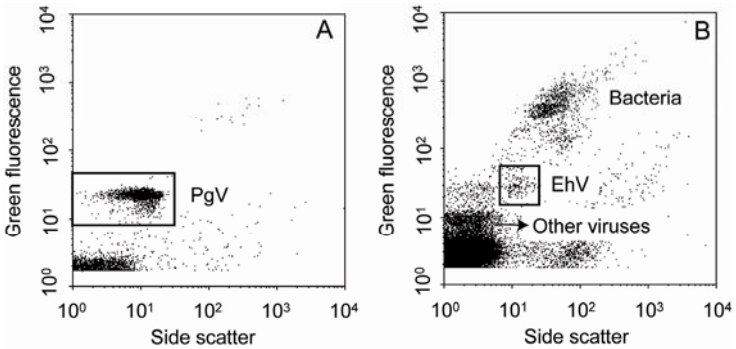

Fig. 2 Biparametric flow cytometry plots showing populations of algal bloom viruses. (**A**) *Phaeocystis globosa* virus (PgV) from a culture lysate; (**B**) *Emiliania huxleyi* viruses (EhV) from a natural non-filtered seawater sample. Viruses and heterotrophic bacteria were discriminated, after staining with the nucleic acid-specific dye SYBR Green I (excitation/emission maxima ~497/520 nm), on the basis of green nucleic acid-specific fluorescence versus side scatter signal. In flow cytometry, light scatter that bounces off in at small angles is called forward scatter and light that bounces off in other directions is called side scatter. A benchtop Becton-Dickinson FacsCalibur was used, equipped with a 15 mW 488 nm air-cooled argon-ion laser and a standard filter set.

calcifying microalga *Emiliania huxleyi* (Schroeder *et al.* 2002) and the bloom-former *Chrysochromulina ericina* (Larsen *et al.* 2007). The use of these algal virus primers in combination with sequence analysis, restriction fragment length polymorphism (RFLP) or denaturating gradient gel electrophoresis (DGGE) makes it is possible to obtain molecular fingerprints of specific algal viruses or even the natural algal virus community (Schroeder *et al.* 2003; Short and Suttle 2003; Mühling *et al.* 2005). Furthermore, using primers on DNA extracted from PFGE bands, it is possible to link bands of a certain genome size to a viral species (Sandaa and Larsen 2006). As a result of recent full viral genome sequence activities, microarrays have been subsequently developed and successfully employed to assess viral diversity (Allen *et al.* 2007). To our knowledge, there are no reports on using antibodies for the detection of algal viruses. Tests using antibodies for specific bloom-forming algal viruses showed that the results in natural systems with mixed populations were difficult to interpret and alternative molecular tools used gave better results (G. Bratbak pers. comm.).

Diversity in algal viruses

The number of algal virus isolates is still steadily growing, as is the number of different algal virus species. The majority of algal viruses that are characterized to date are dsDNA viruses, but recent studies show that other algal virus types, e.g. dsRNA, ssRNA and ssDNA algal viruses, are increasingly brought into culture (**Table 1**). The relatively large number of dsDNA viruses is opposite to what is reported for plant viruses. Plant viruses are dominated by ssRNA viruses, and only a few dsDNA viruses of small genome size (up to 8.2 kb) that are even not considered true dsDNA viruses as they employ a reverse-transcribing replication strategy through a positive-sense ssRNA intermediate (Knipe and Howley 2001).

Interestingly, the dsDNA phycoviruses can have large genome sizes that belong to the largest viral genomes reported (see reviews by Brussaard 2004b; Lawrence 2005). Viral genome sizes above 400 kb are no exception; 407 kb for *E. huxleyi*, 466 kb for *Phaeocystis globosa*, 485 kb for *P. pouchetii*, 510 kb for *Chrysochromulina ericina*, and even 560 kb for *Pyramimonas orientalis*. As a reference, the 560 kb virus of *P. orientalis* (PoV) is close to the genome size of the smallest living microorganism (Claverie *et al.* 2006). Not all the dsDNA algal viruses have a simple genome structure. The large dsDNA *Ectocarpus* virus (330 kb), for example, has a circular molecule with multiple single-stranded regions (Van Etten *et al.* 2002). Despite their differences in genome size and structure, many of the large genome-sized dsDNA phycoviruses are rather uniform in their morphology. The viruses have 5- or 6-sided polyhedral capsids with a multilaminate shell surrounding an electron dense core. The virions are 100-220 nm in size, lack an outer membrane, and belong to the family of Phycodnaviridae (Wilson *et al.* 2005b). Phylogenetic analysis of the DNA *pol* gene indicates that the phycodnaviruses are more closely related to each other than to other dsDNA viruses and they form a distinct monophyletic group. The viruses do, however, fall into different clades that correlate with their hosts, i.e. *Chlorovirus* (chlorella-like green alga viruses), *Prasinovirus* (*Micromonas pusilla* viruses), *Coccolithovirus* (*Emiliania huxleyi* viruses), *Prymnesiovirus* (*Chrysochromulina brevifilum* and *Phaeocystis globosa* viruses), *Phaeovirus* (brown algal viruses) and *Raphidovirus* (*Heterosigma akashiwo* viruses).

In contrast to these larger genome-sized phycodnaviruses, small viral genome sizes are reported for the ssRNA and ssDNA algal viruses, ranging in their genome size between 4 and 11 kb (Nagasaki and Brussaard 2008). For the positive-sense ssRNA viruses infecting the raphidophyte *Heterosigma akashiwo* the new family Marnaviridae was established, related to the picorna-like viruses (Lang *et al.* 2004). Phylogenetic analysis revealed that the other newly isolated ssRNA algal viruses infecting *Heterocapsa circularisquama* and *Rhizosolenia setigera* do not belong to the Marnaviridae. Further genomic comparison has to show whether these viruses form new virus groups.

Based on genomic comparison, the dsRNA virus MpRV (formerly coded MpRNAV), infecting the cosmopolitan picoprasinophyte *Micromonas pusilla*, was classified as a member of a novel genus Mimoreovirus within the family Reoviridae. RNA-dependent RNA polymerase (RdRp) phylogenetic analysis of this 11-segmented virus demonstrated that MpRV forms a separate cluster, separating the turreted from the non-turreted viruses (Attoui *et al.* 2006). This suggests that MpRV belongs to a third branch (although non-turreted), which is possibly ancestral. The reovirus MpRV is, furthermore, unique in that is the only virus in this family known to infect a protist (Brussaard *et al.* 2004a).

The dsDNA cyanophages form a separate group, belonging to the morphologically defined tail-bearing Myoviridae, Podoviridae, and the Siphoviridae that can have a lytic or temperate life cycle and also infect heterotrophic bacteria (Suttle 2000a). Representatives from each of these families infect unicellular and filamentous freshwater and marine cyanobacteria. In marine waters, Myoviridae, which have a contractile tail, are the most abundant cyanophages, whereas in freshwater ecosystems Siphoviridae and Podoviridae (with long and short noncontractile tail respectively) seem most common. Among the cyanophages, myoviruses tend to have a relatively broad host range (even across genera), whereas podoviruses and siphoviruses are very host-specific. Cyanobacteria can be found in almost any conceivable habitat and are evolutionary the principle primary producers. It is suggested that cyanophages originated more than 3 billion years ago, predating the divergence of cyanobacteria from other bacteria (Suttle 2000b). Cyanobacteria are found to be important agents of horizontal gene transfer (Lindell *et al.* 2007). A fascinating example of this is the transfer from host to cyanophage of key photosynthesis genes *psbA* and *psbD* (see review Clokie and Mann 2006). It has recently been shown that the cyanophage-encoded *psbA* gene is expressed during infection (Clokie and Mann 2006). Expression of these genes during infection is thought to increase cyanophage production through enhanced photosynthesis.

Cyanophages adsorb tail-first to the host outer cell surface and inject the DNA into the host cell (Hendrix and Casjens 2005). Similar to (cyano)bacteriophages, the *Chlorella* virus digests the cell wall at the point of attachment (Reisser and Kapaun 1991. In contrast, the brown algal phaeoviruses enter the cell by fusion with the plasmalemma of the host's spores or gametes that lack a cell wall (Van Etten *et al.* 2002). In the case of the wall-less prasinophytes, the virions also adhere to the host cell surface that is followed by fusion of adjacent host and viral particle surfaces and subsequent release of core contents. Less is known about the replication of the other algal viruses. There are no reports of viral transmission by vectors as commonly found for vascular plants that face special problems initiating an infection with their outer surface composed of protective wax and pectin layers and their cells surrounded by cellulose overlying the cytoplasmatic membrane. The intracellular location of the algal virions is typically in the cytoplasm, except for some of the ssRNA viruses and the ssDNA viruses that have been found in the nucleus of the algal host cell (**Table 1**). Although only one type of virus particle is usually present in the algal host cell, there are reports of coexistence of different virus types in a single cell (Brussaard *et al.* 1996b; Brussaard 2004b; Lawrence *et al.* 2006). The biology of such coexistence is, however, barely studied despite their potential significant ecological impact.

CHARACTERISTICS OF ALGAL BLOOM VIRUSES

Algal blooms are loosely defined phenomena, generally used to describe the accumulation of monospecific phytoplankton cell biomass through enhanced primary production (Legendre 1990; Cadée 1992). Traditionally only referring

to events of discolorations of the water due to the occurrence of algal concentrations in more eutrophic waters that are responses to favourable changing physical and chemical forcing, nowadays also used for substantial population increase of small-sized, low biomass picophytoplankton in nutrient-poor waters (e.g. *Synechococcus* sp. in the open ocean). What constitutes an algal bloom is still under debate, but it is not simply a biomass issue as illustrated by increasing harmful algal bloom (HAB) occurrences (Smayda 1997). Both HABs (whether as high or low biomass events) and the more traditional high biomass algal blooms are typically found in coastal regions. We exclude from the present section viruses infecting open ocean algae and focus on bloom-forming algal species in relatively eutrophic waters (e.g. coastal regions).

The brown macroalgae are worthy of special consideration. These large, bulky algae (commonly called kelp) are by far the largest of all algae, and can measure up to tens of meters and weigh hundreds of kilos. They inhabit nearshore sea coasts around the world, they can actually form dense underwater forests and can, therefore, in a way be regarded as algal blooms. However, algal bloom definitions generally insist on the temporal aspect of biomass accumulation, and kelp forest are clearly not examples of relatively short episodic events. Detailed discussion of the characteristics of viruses infecting kelp is beyond the scope of this review. We refer instead to the reviews on this topic by Van Etten *et al.* (2002) and Dunigan *et al.* (2006).

Virion properties and biological features

In almost all algal classes, bloom-forming species can be found, and for most we have representative host-virus model systems in culture. Three well-known bloom-forming algal genera with viruses in culture, *Emiliania*, *Phaeocystis* and *Chrysochromulina*, belong to the Prymnesiophyceae (**Table 1**). *Emiliania huxleyi* has gained tremendous interest over the years because of climate change issues. Coastal and midocean blooms of *E. huxleyi* in temperate regions (45-65°N) can be visualized by satellite imagery due to their reflective calcium carbonate coccoliths. The production of these coccoliths that are shed at the end of a bloom, and the production of dimethylsulfoniopropionate DMSP (precursor of cloud-forming dimethylsulfide, DMS) make it a key species with respect to sediment formation and climate (Archer *et al.* 2001). Several studies showed that during such blooms viruses infecting *E. huxleyi* (EhV) increase in abundance and are ultimately largely responsible for the demise of the bloom (Bratbak *et al.* 1993; Brussaard *et al.* 1996b; Castberg *et al.* 2001). Using transmission electron microscopy (TEM), up to 50% of the algal cells were visibly infected in the decaying phase of the blooms. *E. huxleyi* is one of the few algal species for which two different virus types were reported inside the same cell under natural conditions (Brussaard *et al.* 1996b). Both types were hexagonal in shape; the smallest VLP was 50-60 nm in diameter, whereas the larger one was 185-200 nm in diameter. Some of the EhV-84, isolated from the southern North Sea, show a small tail stub, indicating a potential attachment mechanism (Wilson *et al.* 2002). So far, only the relatively large EhVs has been brought into culture (160-190 nm in diameter; Castberg *et al.* 2002; Wilson *et al.* 2002). The dsDNA virus has a circular genome 407 kb in size and a nucleotide composition of 40.2% G+C (Wilson *et al.* 2005a). The latent period is around 12-14 h (Wilson *et al.* 2005a). The viral burst size (number of newly produced virus particles released from a single infected cell) under non-limiting growth conditions is between 400 and 1000 EhV host cell^{-1}, but strongly reduced under nutrient depletion (Bratbak *et al.* 1993; Brussaard *et al.* 1996b; Castberg *et al.* 2002; Jacquet *et al.* 2002). The number of virion structural proteins detected using SDS-PAGE ranged in size from 10 to 140 kDa; the major capsid protein was around 54 kDa (Castberg *et al.* 2002).

Viral burst sizes of viruses infecting *Phaeocystis pouchetii* (PpV) and *P. globosa* (PgV) are between 350 and 600 cell^{-1} under optimal growth conditions (Jacobsen *et al.* 1996; Baudoux and Brussaard 2005, 2008). The burst size becomes significantly reduced, however, when the algal host growth conditions are less favorable, i.e. late stationary growth phase and under nutrient depleted circumstances (Bratbak *et al.* 1998b; C Brussaard unpublished data). No effect was observed on latent period, lytic cycle length, or viral infectivity. Interestingly, in contrast to nutrient depletion (as found in batch cultures) no decreased burst size for PgV was found under nutrient limiting growth conditions (simulated under continuous culture conditions; C Brussaard unpublished data). Nutrient depletion can typically be observed at the end of the massive spring blooms, whereas nutrient limitation occurs during the summer season. The differences in response to nutrient depletion and limitation can, thus, be expected to affect the population dynamics of *Phaeocystis* significantly. *P. globosa* population dynamics is also influenced by the different virus species infecting the algal host. Based on genome size (466 and 177 kb), particle diameter (150 and 100 nm) and temperature sensitivity (above 35 and 25°C respectively), two major groups of PgVs were discriminated (Baudoux and Brussaard 2005). Within Group I with a 150 nm viral particle diameter, the latent period was 10 h and the host range for the different viral isolates was identical. Within Group II, however, host range was either 12 or 16 h and the specificity for different *P. globosa* strains varied largely, including colonial host strains. The structural proteins of PgV Group I ranged in weight between 41 and 257 kDa, with a main polypeptide of 52 kDa. The PgV virions in Group II consisted of polypeptides that ranged between 38 and 119 in weight, and with the major protein of 44 kDa (Baudoux and Brussaard 2005). One PgV of group I was used to infect *P. globosa* acclimated at different irradiance levels (25, 100, and 250 µmol photons·m^{-2}·s^{-1}) (Baudoux and Brussaard 2008). The low light-adapted as well as the high light-adapted cultures showed a 50% reduced burst size due to photolimitation and photoinhibition of the algal host. Furthermore, low light prolonged the latent period with 4 h. The fraction of infectious viruses was, however, unchanged under all conditions. The algal host's physiological status prior to infection also affected virus propagation under complete darkness. The viral yield decreased with the amount of light the host was adapted to prior to being placed in the dark, i.e. lowest viral yield for the cultures that were low light-adapted prior to darkness. Viral replication recovered when placed back into the light. Incubation of *P. pouchetii* in the dark also resulted in a reduced viral burst size of PpV01 (Bratbak *et al.* 1998b). This virus PpV01, which was isolated from Norwegian coastal waters, showed striking similarities in the capsid structure with a number of other large dsDNA viruses (Yan *et al.* 2005). Electron cryomicroscopy and 3D-image reconstruction methodology showed that the large PpV01 has a maximum capsid diameter of 220 nm (160-180 nm when using TEM, but this is likely due to shrinkage from the fixatives used). It is composed of 2,192 capsomers arranged with T=219 quasisymmetry. The virus contains a lipid membrane just below (inside) of the capsid. The presence of a lipid membrane in the *P. globosa* viruses was confirmed by chloroform treatment (C Brussaard unpublished data).

While dsDNA PgVs are tentative species in the genus *Prymnesiovirus* (family Phycodnaviridae), the dsDNA virus infecting *Chrysochromulina brevifilum* CbV-PW1 is the type species of this genus (Wilson *et al.* 2005b). CbV was isolated from the coastal waters of the Gulf of Mexico (Suttle and Chan 1995). From the other side of the Atlantic Ocean, the lytic virus CeV-01B that infects *C. ericina* was isolated from Norwegian coastal waters (Sandaa *et al.* 2001). The algal genus *Chrysochromulina* has a global distribution and includes the toxic species *C. polylepsis*, which is primarily implicated in fish kills. No virus infecting *C. polylepsis*, however, has been isolated yet. The virus particle size of the *Chrysochromulina* viruses in culture are

about 145 to 170 nm in diameter (Suttle and Chan 1995; Sandaa *et al.* 2001). From CeV we, furthermore, know that it has a latent period of 14-19 h and a burst size of 1,800 to 4,100 viruses cell^{-1}. Four polypeptides were recorded for CeV, of which the major polypeptide was approximately 73 kDa in weight (Sandaa *et al.* 2001). More interestingly, it has the second largest genome size of the algal viruses isolated to date (510 kb). Only the genome size of the virus infecting the non-bloom-forming species *Pyramimonas orientalis* is larger (560 kb; Sandaa *et al.* 2001).

Viruses that indeed infect HAB species do exist in culture and include the viruses infecting the brown tide chrysophyte *Aureococcus anophagefferens*, the shellfish-killing dinoflagellate *Heterocapsa circularisquama*, and the potential toxic raphidophyte *Heterosigma akashiwo*. The dense blooms of the picophytoplankter (2 μm diameter) *Aureococcus anophagefferens* are a recurring problem in regional coastal bays of New Jersey and New York, USA, negatively affecting the population of eelgrass (by reducing the light) and the recruitment of scallops (Garry *et al.* 1998). As for *E. huxleyi* and *Phaeocystis* spp. blooms, viruses seem to be a major source of mortality for the *A. anophagefferens* blooms (Gastrich *et al.* 1998). The *A. anophagefferens* virus AaV is approximately 140-160 nm in diameter and just as all other algal viruses discussed so far, AaV is formed within the cytoplasm of the algal host cell (Gastrich *et al.* 1998).

During blooms of the dinoflagellate *Heterocapsa circularisquama* along the coast of Japan, it became clear that two distinct virus species were present (Tomaru and Nagasaki 2004). Cells harbouring large (180-210 nm) and small (about 30 nm) viruses in their cytoplasm were detected, but co-infection was not observed. The larger-sized virus HcV has a dsDNA genome of approximately 350 kb, a latent period of around 24 h and a burst size of 1,800 to 2,400 infectious viruses cell^{-1} (Nagasaki *et al.* 2003; Wilson *et al.* 2005b). The smaller-sized virus HcRNAV has a ssRNA genome 4.4 kb in size (Tomaru *et al.* 2004a). Virus particles could be detected by TEM about 12 h after infection, and the burst size was estimated to be between 3,400 and 21,000 viruses cell^{-1} (Tomaru *et al.* 2004a). Protein analysis indicated only one major polypeptide, 38 kDa in size (Tomaru *et al.* 2004a). Based on intraspecies host-range tests of HcRNAV two ecotypes with complementary host specificity could be discriminated (Tomaru *et al.* 2004a).

There is also a high diversity among virus type, virus clone and host strains of *Heterosigma akashiwo*. Field studies clearly show that there is coexistence of many different virus clones and host strains, allowing a dynamic change of host clonal composition during the blooms (Tomaru *et al.* 2004b). At the same time, however, there is also significant diversity in virus species infecting this algal species. These findings are of special interest considering that *H. akashiwo* is a HAB species occurring in temperate and subarctic coastal waters of the world where it often causes mortality of caged fish such as salmon and yellowtail. Infecting the same strain of *H. akashiwo* (NEPCC522), small viruses of 25 to 30 nm in diameter have been observed in aggregates in the nucleoplasm (HaNIV; Lawrence *et al.* 2001), but also in the cytoplasm (HaRNAV; Tai *et al.* 2003). Furthermore, co-occurring isosahedral morphotypes of 30 and 80 nm in diameter, and 20 and 130 kb in genome size respectively (OIs1), have been observed for the same algal host strain (Lawrence *et al.* 2006). The 80 nm OIs1 is a dsDNA virus, but larger dsDNA virus particles (HaV) of 185 to 220 nm in diameter have also been detected for other strains of *H. akashiwo* (Nagasaki *et al.* 1994). The degree of characterization of these different *H. akashiwo* viruses differs largely. HaRNAV was the first ssRNA virus reported to infect a phytoplankton species and phenotypic characteristics suggest that HaRNAV is closely related to the picorna-like viruses (Tai *et al.* 2003). The virus has a genome size of 9,100 nucleotides, contains at least 5 structural proteins ranging in size from 24 to 34 kDa, and has a latent period of around 35 h. For the dsDNA OIs1 virus it takes about 17 h before virus progeny is released (Lawrence *et al.* 2006). In contrast to the earlier discussed *P. globosa* (Baudoux and Brussaard 2008), the lytic cycles of HaRNAV as well as OIs1 were of similar length in the light and in darkness and therefore not dependent on photophosphorylation (Juneau *et al.* 2003). For the other viruses we have more limited knowledge. First signs of HaNIV infection are detected 24 h after infection, and by 74 h nearly the entire culture has become visibly infected (Lawrence *et al.* 2001). For HaV we know that the onset of cell lysis of *H. akashiwo* is 24 h after infection (Nagasaki *et al.* 1999). Estimates of the viral burst size are as high as 21,000 for the ssRNA virus HaRNAV, and between 770 and 1,100 viruses cell^{-1} for the dsDNA viruses OIs1 and HaV (Nagasaki *et al.* 1999; Lawrence *et al.* 2006).

A group of algae for which only very recently the first viruses were brought into culture are the diatoms (Bacillariophyceae). Diatoms are considered to be the most widespread group of algae on earth, largely contributing to the world's net primary production. Currently, viruses have been isolated for four species of diatoms, *Rhizosolenia setigera*, *Chaetoceros salsugineum*, *Chaetoceros* cf. *gracilis*, and *Chaetoceros debilis*. *Rhizosolenia setigera* is a bloom-forming species that occurs widely throughout the world's oceans. *Chaetoceros* is the largest and most species-rich genus of the marine planktonic diatoms, with complex cell and colony morphology. Blooms of *Chaetoceros* typically occur in temperate and cold coastal waters, and can exist of many concurrent species that dominate the planktonic community for weeks at a time. The virus infecting *R. setigera* is a ssRNA virus (RsRNAV) 11.2 kb in size, was the first diatom virus to be brought into culture, and originates from the Ariake Sea, Japan (Nagasaki *et al.* 2004a). RsRNAV showed viral replication within the cytoplasm, has a particle size of 32 nm in diameter, owns 3 major structural proteins, and exhibits a latent period <24 h. The burst size varied with the physiological state of the host cell, about 3,100 viruses cell^{-1} in exponentially growing host cultures and around 1,000 viruses cell^{-1} for cultures in stationary growth phase. The *Chaetoceros* viruses are also small in particle size (25 to 32 nm in diameter), have a latent period between 12 and 24 h, and replicate either within the cytoplasm or the nucleus (Bettarel *et al.* 2005; Nagasaki *et al.* 2005c; Tomaru *et al.* 2008). Both have 2 major capsid proteins, ranging in size between 37.5 and 46 kDa. The viruses infecting *C. salsugineum* (CsNIV) and *C. debilis* (CdebDNAV) are the first ssDNA viruses known for algal viruses (Nagasaki *et al.* 2005c; Tomaru *et al.* 2008). CsNIV replicates within the nucleus of the host cell and has a burst size of 325 infectious units cell^{-1} (Nagasaki *et al.* 2005c). CdebDNAV seems to resemble CsNIV in many aspects, but it accumulates in the cytoplasm of the host (Tomaru *et al.* 2008).

Recent examples of cyanophage infecting dense bloom-forming and toxic cyanobacteria are Ma-LMM infecting *Microcystis aeruginosa* (colonial freshwater species; Yoshida *et al.* 2006) and cyanophages N-BM and N-BS infecting *Nodularia spumigena* (filamentous brackish and freshwater species; Jenkins and Hayes 2006). Blooms of these cyanobacteria can become quite striking if it floats or billowing near the surface. Both cyanobacteria produce potent hepatotoxins that may cause the death of livestock and wildlife after drinking significant quantities of the bloom waters. The *N. spumigena* cyanophage isolates belonged partly to the Siphoviridae (N-BS phage) with their long flexous non-contractile tails (188 up to 888 nm in length; Jenkins and Hayes 2006). The Ma-LMM and N-BM cyanophages belong to the Myoviridae based on their head size and contractile tail. Ma-LMM's head size was approximately 86 nm in diameter and the contractile tail 209 nm in length, which contracts to a length of 90 nm (Yoshida *et al.* 2006). N-BM myoviruses measured also approximately 210 nm in total particle length (including tail; Jenkins and Hayes 2006). The Ma-LMM phage genome consists of a linear dsDNA genome of 165 kb and the phage contains 11 polypeptides of which 4 are major (ranging in size between 26 and 84 kDa; Yoshida *et al.* 2006; Yoshida *et al.* 2008). The latent period is 6-12 h and the burst size between 50

and 120 infectious virions cell^{-1}. In contrast to the open-ocean cyanobacteria *Synechococcus* and *Prochlorococcus* myoviruses (Suttle 2000a), Ma-LMM has a narrow host range. This does not imply a distinct difference between marine and freshwater cyanophage, because Deng and Hayes (2008) isolated freshwater cyanophages with a very broad host range, able to infect not only *Microcystis* but also the dense HAB cyanobacteria *Anabaena* and *Planktothrix*. The morphotype of the majority of the cyanophages was alike the typical myo-, podo- or siphoviruses. A few of the isolates, however, were filamentous, a morphotype not previously reported for cyanophages. The PCR primer sets for g20, g23 and major capsid protein genes that have been shown to be conserved in other cyanophage, failed to give specific amplification products from most isolates. This suggests that these cyanophages were different from the ones previously characterized.

Genotypic characteristics

Three of the six genera that have been described within the Phycodnaviridae family are specific to bloom-forming phytoplankton species, i.e. *Coccolithovirus* (*Emiliania huxleyi* viruses), *Prymnesiovirus* (*Chrysochromulina brevifilum* and *Phaeocystis globosa* viruses) and *Raphidovirus* (*Heterosigma akashiwo* viruses). Furthermore, 4 other large dsDNA viruses infecting bloom-forming algae, i.e. *Aureococcus anophagefference*, *Heterocapsa circularisquama*, *Chrysochromulina ericina* and *Phaeocystis pouchetii* are tentative species still unassigned to the family (Wilson *et al.* 2005b). These viruses seem evolutionary related and genetic evidence furthermore suggests the existence of a common ancestor for Phycodnaviridae and other nucleocytoplasmic large DNA virus families (NCLDV), i.e. Poxviridae, Iridoviridae, Asfarviridae and Mimiviridae (Raoult *et al.* 2004; Wilson *et al.* 2005b; Monier *et al.* 2008). It should be noted, however, that the use of the DNA polymerase gene as phylogenetic marker has its limitations. Despite the earlier proven potential of the DNA polymerase gene, the designed degenerate set of primers gave negative results for some dsDNA algal viruses including the virus infecting the bloom-forming *C. ericina* (Sandaa *et al.* 2001). Very recently, Monier *et al*. (2008) showed that, based on the type B DNA polymerase sequences, the large dsDNA algal bloom viruses CeV-01 and PpV-01 are very closely related to their homolog in mimivirus and do not branch directly with other Phycodnaviruses. Furthermore, the degenerate set of primers did not work for HaV probably due to the presence of an intein insertion in the highly conserved motif I of the polymerase domain and a mismatch of the primers (Nagasaki *et al*. 2005). Their phenotypic characteristics suggest that they may indeed belong to the family Phycodnaviridae. Yet, the lack of phylogenetic information on their DNA *pol* genes hinders taxonomic assignment. Furthermore, recent studies with *P. globosa*-specific viruses showed that DNA *pol* does not have sufficient resolution to look for relationships within virus genera (Brussaard *et al.* 2004b; Baudoux and Brussaard 2005; Wilson *et al.* 2006). Phylogenetic analysis of DNA *pol* fragments placed PgV Group I and II in a monophyletic group, despite their morphologically distinct nature (Baudoux and Brussaard 2005). Surprisingly, another PgV with morphological and genome characteristics so similar to PgV Group II and that was even isolated from the same geographical region, clustered in another group (Wilson *et al.* 2006). This PgV-102P contained a 13 amino acid insertion that was similar to *C. brevifilum*-specific viruses (CbV). A different very recent study presented phylogenetic evidence for a close relationship between DNA polymerase sequences of the three algal viruses CeV, PpV and PoV (of which the first two infect bloom-forming phytoplankton) and mimivirus, and for the segregation of these from homologs from other known viruses (Monier *et al.* 2008). As to whether the phylogenetic classification of this virus family needs revision in the future, awaits further molecular analysis.

A diverse assortment of genotypic characteristics is found for the algal bloom phycodnaviruses. The range of genome sizes, between 176 and 510 kb for the algal bloom viruses (**Table 1**), indicates already towards an incredible genetic diversity. As we speak, there are about half a dozen dsDNA algal bloom viruses in the process of full genome sequencing and analysis (G Bratbak pers. comm.; C Brussaard unpublished data), but only one dsDNA algal bloom virus, i.e. Coccolithovirus EhV-86, has been published so far (Wilson *et al.* 2005a). Compared to the other sequenced algal phycodnaviruses that belong to the Chloroviruses and the Phaeoviruses, they only share <20 core genes from a total combined pool of about 1,000 genes. EhV-86 contains 25 of the core set of 40-50 conserved virus genes for NCLDVs (Wilson *et al.* 2005a). Sequence analysis of EhV-86 revealed a total of 472 predicted genes (coding sequences, CDSs) and three distinct families of repeats (designated A, B, C) throughout the genome (Wilson *et al.* 2005a). These repeat regions were suggested to be of crucial importance for virus propagation being involved with transcriptional control (Family A), virus adsorption/release (Family B) and DNA replication (Family C) (Wilson *et al.* 2005a; Allen *et al.* 2006a).

Out of the 472 CDSs, with an average length of 786 bp, only 66 have been annotated with functional product predictions. Among those were several unanticipated genes for algal viruses, such as RNA polymerase subunits, genes involved in biosynthesis of a sphingolipid (ceramide) known to induce apoptosis, or programmed cell death, and eight proteases (Wilson *et al.* 2005a). Microarray analysis that included 90% of the genes predicted in the genome of EhV-86 indicated that many of the unknown CDSs (65%) were transcriptionally active during infection and therefore likely to be functional (Wilson *et al.* 2005a). Using the microarray approach to test the genetic diversity and relationships between 11 EhV isolates showed that out of 425 genes examined 71 CDSs were absent or highly divergent in one or more strains (Allen *et al.* 2007). The other 354 CDSs were present in all virus strains.

The presence of the RNA polymerase genes in EhV-86 suggests a unique replication cycle as it implies the potential ability of this virus to transcribe its own genes from within the cytoplasm during host infection. The presence of genes involved in biosynthesis of ceramide led Wilson *et al.* (2005a) to hypothesise that EhV-86 encodes a mechanism for inducing apoptosis to kill the host cell and disseminate progeny virions. The viral RNA polymerases may be linked to the ceramide biosynthesis pathway. If host DNA is degraded during infection, then the host would not be able to encode new RNA polymerase. Manipulation of ceramide biosynthesis could result in delaying cell death and extending the infection time. The active prolonged production of virus-encoded RNA polymerases during infection would then prove very important for the replication strategy of the virus (Allen *et al.* 2006c).

The sequencing of approximately 80% of the genome of another EhV (EhV-163) and its comparison to EhV-86 have revealed a number of point mutations and insertions/deletions (Allen *et al.* 2006b). One notable gene deletion in EhV-163 is a putative phosphate permease (PPP) found in EhV-86 and all the other English Channel isolates (Allen *et al.* 2006b). Interestingly, EhVs isolated in the same year share identical PPP gene sequence at the nucleotide level (Martínez Martínez 2006). This finding is intriguing because the algal host species may regularly experience growth limitation due to inorganic phosphorus depletion. Further study is needed to determine its ecological relevance.

Besides the phycodnaviruses, there are several other small genome-sized algal bloom viruses have been fully sequenced, e.g. the ssRNA viruses infecting the dinoflagellate *Heterocapsa circularisqua* (HcRNAV; Nagasaki *et al.* 2005a), the flagellate *Heterosigma akashiwo* (HaRNAV; Lang *et al.* 2004) and the diatom *Rhizosolenia setigera* (RsRNAV; Shirai *et al.* 2006). Furthermore, also the ssDNA virus infecting the diatom *Chaetoceros salsugineum* (CsNIV;

Nagasaki *et al.* 2005c) is sequenced. Nagasaki and co-workers (2005a) sequenced the two ecotypes of HcRNAV that have intraspecies host specificities that are complementary to each other. They found that the nucleotide sequences of HcRNAV34 (4,375 nt) and HcRNAV109 (4,391 nt) were 97% similar. Each of the viruses lacks a poly(A) tail and has two open reading frames (ORFs), ORF-1 coding for a putative polyprotein having protease and RNA-dependent RNA polymerase (RdRp) domains and ORF-2 encoding the single major capsid protein. The most noticeable difference between the two ecotypes was 4 regions in ORF-2 that resulted in high frequencies of amino acid substitutions. Using an RT-nested PCR system for the variable regions, Nagasaki *et al.* (2005a) were able to show that the other HcRNAV strains tested could be grouped according to their ecotype. The tertiary structure of the capsid proteins predicted using computer modelling implied that the intraspecies host specificity of HcRNAV is determined by capsid alterations because many of the amino acid substitutions were located in regions on the outside of the viral capsid proteins (Nagasaki *et al.* 2005a). The use of particle bombardment in combination with highly sensitive-sense strand-specific Northern blot analysis confirmed that the intraspecies host specificity of HcRNAV is determined at the entry process of virus infection (Mizumoto *et al.* 2007).

Phylogenetic analysis of deduced amino acid sequences in the RdRp domain from positive-sense ssRNA viruses showed that HcRNAV (the 2 ecotypes showed identical amino acid sequences) is included in a group that formed a sister group with Picornaviridae, Caliciviridae, Dicistroviridae, Iflavirus, Comoviridae, Sequiviridae, and two marine algal bloom viruses (Nagasaki *et al.* 2005a). HcRNAV was, however, deeply branched and apparently distinct from the cluster containing the Tetraviridae, Barnaviridae and Luteoviridae at a bootstrap value of 97%, suggesting that HcRNAV belongs to a new unrecognized positive-sense ssRNA virus group.

Contrary to HcRNAV (see paragraph above), HaRNAV infecting *Heterosigma akashiwo* and RsRNAV infecting *Rhizosolenia setigera* have a poly(A) tail and a putative RNA helicase-coding region in their genome (Lang *et al.* 2004; Shirai *et al.* 2006). Furthermore, HaRNAV genome has, however, only one ORF (Lang *et al.* 2004). Sequence analyses of the HaRNAV genome revealed that this virus does relate to viruses from the picorna-like superfamily of viruses (Picornaviridae, Caliciviridae, Dicistroviridae, Comoviridae, Potyviridae). Like picorna-like viruses the genome has a protein attached to the 5' end and no overlapping ORFs, all are RNAs translated into a polyprotein before processing and they have a conserved RdRp protein. The 8,587 nt genome contains the RdRp domain, a conserved RNA helicase domain, and conserved picorna-like virus capsid protein domains (Lang *et al.* 2004). The HaRNAV protein sequence shows a mosaic pattern of relationships to picorna-like virus sequences. HaRNAV does, however, not belong to any of these defined picorna-like virus families. Based on overall structure of the genome and phylogenetic analysis of concatenated (putative) helicase / RdRp / VP3-like protein sequences, HaRNAV classified as type virus for a new virus family, the Marnaviridae (Lang *et al.* 2004). On the basis of analysis of RdRp sequences amplified from marine virus environments, Culley and colleagues (2003, 2007) found that a diverse array of picorna-like viruses exist in the ocean.

T he genome of RsRNAV (8,877 nt) has a 3′ poly(A) tail, uncapped 5′-termini, and two large ORFs (Shirai *et al.* 2006). ORF-1 has a length of 4,818 nt is a polyprotein gene coding for replicases, e.g. RdRp and RNA helicase, and ORF-2 (2,883 nt) encodes structural proteins. The deduced amino acid sequences for these ORFs showed considerable similarities to the non-structural and structural proteins of HaRNAV. The AU-richness of the RsRNAV genome is, however, much higher than HaRNAV and HcRNAV (63.7, 53.1, and 44.9% respectively), but comparable to dicistroviruses and other insect-infecting picorna-like viruses. Results of phylogenetic analyses (based on concatenated amino acid sequences of RNA helicase and RdRp domain) showed that RsRNAV is not a member of the Dicistroviridae, the monophyl of which was supported with a high bootstrap value (98%; Shirai *et al.* 2006). Interestingly, the RdRp phylogenetic tree showed that RsRNAV and HaRNAV, which infect Stramenopiles, and HcRNAV, which infects an Alveolata, fell into phylogenetically distant clades. This may suggest coevolution of host and virus. More ssRNA algal viruses need to be isolated and characterized to confirm this.

Another remarkable example of the immense potential of undiscovered algal viruses is the diatom virus CsNIV that replicates within the nucleus of *Chaetoceros salsugineum* (Nagasaki *et al.* 2005c). It has a genome structure unlike that of other defined viruses. The viral genome consists of a single strand of circular DNA 6,000 nt in size that is partly double-stranded (997 nt) and covalently closed. One of the ORFs showed low but noticeable similarity (E-value <2.5E-2) to replication-associated proteins of circoviruses. The secondary structure of the CsNIV genome disclosed that the dsDNA region is located between significant loop structures, which seem related to its specific structure. The partially sequenced genome of the ssDNA virus CdebDNAV that infects *C. debilis*, indicates that there is high similarity (E-value = 1E-56) between the putative replicase gene of CdebDNAV and CsNIV (Tomaru *et al.* 2008), despite the different location of replication in the host cell (cytoplasm versus nucleus).

ECOLOGICAL ROLE OF ALGAL BLOOM VIRUSES

Impact on algal host population dynamics

Viral infection of algal bloom-forming species has an instant impact on the population dynamics of the host. Contrary to vascular plants for which viral infection is scarcely strong enough to kill the entire plant, phytoplankton are unicellular and thus infection by a lytic virus will consequently result in rapid cell death of the organism (usually within a day; Brussaard 2004b). Yet, a high degree of specificity prevents mortality of the total population.

Typically, cell lysis results in the release of progeny viruses ready to infect new host cells. In comparison, vascular plant viruses do not use specific cellular receptors to attach to cells and thus are posed with special problems in recruiting new cells to infect upon replication in the initial cell. Although algal bloom viruses do not face this particular problem, they will become diluted strongly in the surrounding water in short time. Viral burst size and the total number of infectious particles is thus of great importance for viral proliferation in aquatic ecosystems. Viral infection depends on random encounter of host and infectious virus; hence bloom situations with high cell abundance provide perfect circumstances for a rapid spread of infection (epidemic). *Phaeocystis* and *Emiliania* blooms present good examples of such epidemic situations.

The abundance of specific algal bloom viruses can increase rapidly over the course of the bloom (Castberg *et al.* 2001; Tomaru *et al.* 2004b; Brussaard *et al.* 2005a; Baudoux *et al.* 2006). Up to 50% of the *E. huxleyi* cells were visibly infected (using TEM) during the decaying phase of a natural bloom, which translates back to the entire population being virally infected at that stage (Brussaard *et al.* 1996b). A similar record was reported for *Aureococcus anophagefferens*, 38% of the cells contained virus-like particles at the termination of the bloom (Gobler *et al.* 2004). At the peak of a bloom of *Heterocapsa circularisquama* even 88% of the cells contained small virus-like particles (Nagasaki *et al.* 2004b). As a potential alternative to the laborious TEM analyses live-dead viability assays in combination with flow cytometry can be used to rapidly determine when and at what rate unicellular algal host cells die (Brussaard *et al.* 2001; Lawrence *et al.* 2006). Actual rates of viral lysis for specific phytoplankton in the field were hard to obtain due

to methodological complications. Recent developments show that viral lysis rates are comparable to grazing rates, for example viral lysis of *P. globosa* cells was up to 0.35 d^{-1} during a bloom situation while microzooplankton grazed at maximum rates of 0.4 d^{-1} (Baudoux *et al.* 2006).

The fact that algal blooms do occur indicates that viral infection cannot actually prevent bloom formation. The availability of infectious viruses at the start of the blooming period of the host alga underlies the degree of viral control (Brussaard 2004b). For most bloom-forming algae there is only a restricted period per year when environmental conditions (light, nutrients etc) favour bloom formation. The remaining time, the alga is present in relatively low cell numbers and concomitantly the associated viruses are found in reduced abundance. The preservation of viral infectivity during this period is of course an essential factor for the success of infection at the start of the bloom period the next year. Temperature, decay by solar UV radiation, grazing, adsorption to inorganic colloids and organic matter all seem processes affecting the infectivity and even existence of the viral particles (Kapuscinski and Mitchell 1980; Suttle and Chen 1992; Noble and Fuhrman 1997; Wilhelm *et al.* 1998; Mari *et al.* 2005). Recently, Tomaru and colleagues (2007) found that the concentration of the ssRNA virus infecting *Heterocapsa circularisquama* in the sediment prior to the host's blooming season was an important factor in determining the magnitude and length of the summer blooms in the shallow (<15 m) bays in western Japan. The sediment had a protective effect on the viruses and retained them stable for a considerable period until brought back into the water column by hydrographical diffusion (e.g. by typhoons).

The response of the host cell to viral infection also affects the number of viruses available for infection. The virally infected *Heterosigma akashiwo* cells lost not only their motility within 24 h of infection (Nagasaki *et al.* 1999), but also showed enhanced sinking rates (Lawrence and Suttle 2004). Sinking rates were also enhanced when incubated in darkness. The impact of viral infection on sinking rates of the algal host was independent of the type of virus (ssRNA or dsDNA). Thus, the propagation of infection through a bloom depends also on the fate of the infected host. Under natural conditions enhanced sinking rates in shallow environments may even result in deposition of infected cells on the bottom. The same author (Lawrence *et al.* 2002) did indeed find viruses in the sediment as deep as 40 cm below the sediment-water interface that still could infect *H. akashiwo*. A similar outcome of reduced infectious viruses available to infect bloom-forming algae can be established when the algal host cells undergo apoptosis upon infection. Apoptosis as a response to nutrient limitation or other physiological stress factors rather than virus infection has been previously reported in phytoplankton (Brussaard *et al.* 1997; Berges and Falkowski 1998; Bidle and Falkowski 2004). Recently, apoptotic features have been reported for virally infected *H. akashiwo* (Lawrence *et al.* 2001) and *P. globosa* (C Brussaard and J Berges unpublished data), and genes encoding for apoptosis have been found in viruses infecting *E. huxleyi* (Wilson *et al.* 2005a). Very recently Bidle and colleagues (2007) documented factually an interaction between autocatalytic programmed cell death and lytic viral infection of *E. huxleyi*. Viral infected *E. huxleyi* resulted in up-regulation of metacaspase gene expression in contrast to the noninfected cells.

Secondly, algal hosts may have ways to escape viral infection. *Phaeocystis* for example, has a polymorphic life cycle with flagellated unicellular and non-motile cells that are embedded in colonies. It appears that cells in colonies grow faster (Veldhuis *et al.* 2005) and are well protected against grazing as well as viral infection (Ruardij *et al.* 2005; Jacobsen *et al.* 2007), which strongly adds to the dense blooming of the species in spring (Brussaard *et al.* 2007). The contribution of colonies to single cells reduces sharply upon nutrient depletion or reduction of irradiance (Brussaard *et al.* 2005a). At such moment, the many single cells released from the colonial mucus matrix become readily infected, promoting the decline of the bloom (Ruardij *et al.* 2005; Baudoux *et al.* 2006). Another example of bloom-forming algae escaping viral infection comes from laboratory research where Thyrhaug and colleagues (2003) showed that a stable coexistence between algal host and virus could be established that was not due to resistance of the host to infection. Inhibitors seem to be released upon cell lysis of the host that results in reduction of infection of other algal cells of the same species. Stable coexistence of *E. huxleyi* and virus was even pronounced after adding filtered lysate. The authors hypothesized that defective interfering particles (mutant viruses with partially deleted genomes that require coinfection with wild-type virus to be able to replicate in host) may have been present before filtration that would have inhibited the production of normal wild-type EhVs.

Resistance to viral infection is of course the ultimate escape to viral control of the algal host population. A specific geographical population of an algal species tends to be genetically rich (e.g. Medlin *et al.* 1996), which potentially underlies intraspecies specificity for viral infection (e.g. Schroeder *et al.* 2002). Despite this genetic diversity in algal host and virus clones at the start of a of an *Emiliania huxleyi* bloom in the Norwegian fjords, only a few virus genotypes eventually were responsible for the demise of the bloom (Schroeder *et al.* 2003). Amazingly, this diversity was stable over years and the same host and virus genotypes dominated (Martínez Martínez *et al.* 2007). Quite the opposite, the combination of host and virus clones characterizing a series of *Heterosigma akashiwo* blooms in a semi-enclosed basin in northern Hiroshima, Japan, changed year by year (Tomaru *et al.* 2004b). Tarutani and co-workers (2006) could not detect virus adsorption to the resistant algal strains of *H. akashiwo*. Viral infection influenced not only quantity (biomass) but also quality (clonal composition during bloom) of the *H. akashiwo* population during a bloom. Tomaru and coworkers (2007) reported a similar situation during blooms of *Heterocapsa circulisquama*. A high variation in vulnerability to viral infection may affect the blooming period when algal growth-limiting nutrients that are regenerated through cell lysis become also available to non-infected strains of the same algal host species. During the bloom disintegration period, *H. akashiwo* clones that were resistant to most of the HaV clonal isolates became dominant (Tarutani *et al.* 2000). During the bloom, these algal cells were only a minor component, which may be explained by reduced competitive fitness as compared to the host clones that are sensitive to viral infection. To our knowledge, there is no comprehensive study that relates viral resistance to the physiological characteristics of the bloom-forming algal host.

Effects on ecosystem functioning and biogeochemical fluxes

Viruses infecting algae do not only cause disease and death of their hosts, but also play a critical role in aquatic geochemical cycles (Wilhelm and Suttle 1999; Ruardij *et al.* 2005; Suttle 2007). The virus-mediated lysis of their unicellular hosts results in the release of cellular compounds into the surrounding water (Gobler *et al.* 1997). These organic nutrient-rich compounds are readily utilized by heterotrophic bacteria, sharply enhancing the often carbon-limited bacterial production (Brussaard *et al.* 1996a; Gobler *et al.* 1997; Bratbak *et al.* 1998a; Brussaard *et al.* 2005b). A theoretical ecosystem model demonstrated that 6 to 26% of the photosynethetically fixed organic carbon was recycled back to dissolved organic matter by viral lysis (Wilhelm and Suttle 1999). This model, however, assumes a steady-state situation and thus holds best for the non-blooming situations found in open ocean and the summer season in coastal zones. Algal blooms do not represent steady-state situations. Field studies as well as a mathematical ecosystem model including a virus module revealed that during the wane of dense blooms (when large amounts of particulate organic

matter accumulate) very high percentages of primary production can be recycled through viral lysis (Brussaard *et al.* 1995; Bratbak *et al.* 1998a; Brussaard *et al.* 2005b; Ruardij *et al.* 2005). The released cellular organic carbon could account for the entire bacterial production. Concurrently with the enhanced bacterial activity, the bacterial community composition changed (Larsen *et al.* 2001; Brussaard *et al.* 2005b). The biogeochemical net effect of viral activity is thus diversion of organic matter from transfer to higher trophic levels through grazing (classical food web) towards microbe-mediated recycling processes through viral lysis (Suttle 2007). Enhanced activity of the so called microbial food web results also in regeneration of potential algal growth-limiting nutrients (e.g. P and N), affecting competition between and succession of the remaining phytoplankton species (Brussaard *et al.* 1996a; Gobler *et al.* 1997; Ruardij *et al.* 2005). Viruses are found to be catalysts of global biogeochemical cycles, playing a role in the recycling of nutrient elements as well as the flux of carbon to the deep ocean (biological pump; Suttle 2007).

An additional potentially interesting effect of viral lysis of algal blooms that is related to global scale processes is the release of DMSP. Eukaryotic algae that synthesize DMSP (e.g. bloom-forming species *Emiliania* and *Phaeocystis*), a nonvolatile DMS precursor, appear to be a principal source of DMS (Malin *et al.* 1998; Archer *et al.* 2001). DMS is believed to affect cloud cover and climate change. Rapid release of DMSP concurred with virally induced cell lysis of *P. pouchetii* (Malin *et al.* 1998), and the subsequently increased DMS concentrations were most likely due to the microbial agents that transfer DMSP to DMS (Hill *et al.* 1998). However, more (bloom-forming) algal model systems need to be investigated to determine the degree of impact of viruses on global sulphur cycling.

CONCLUDING REMARKS

Algal bloom viruses are highly diverse, and although the first algal bloom virus isolates seem to indicate that they typically consisted of large dsDNA genomes, recently also much smaller ssRNA and ssDNA viruses infecting algal bloom species are brought into culture. It becomes more and more apparent that many of these algal bloom viruses should be classified as representatives of new virus genera or even families. The present review illustrates undoubtedly that the ocean is a true treasure of novel viruses and that, thus far, we only scratched the surface. Especially the large genome-sized phycodnaviruses seem reservoirs of primarily unidentified or new genes (Wilson *et al*. 2005a; Dunigan *et al*. 2006). Other genes reveal functions more common to plants and animals or related to bacteria, archaea or to the dsDNA Mimivirus, which suggests the occurrence of horizontal gene transfer between viruses from different families (e.g. Nagasaki *et al.* 2005b; Wilson *et al.* 2005a).

A challenge for the future of algal bloom virology will be to establish proper phylogenetic relationships. Clearly more than one gene at the time needs to be compared. The characterization of new algal bloom viruses is an important area of further study; not only from the genomics perspective, but also to better understand the ecological relevance of these different viruses infecting relevant and often socio-economic important algal bloom species. Of crucial importance will be the synthesis of virology, (meta)genomics, diversity and biogeochemistry.

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