Vol. 118: 275–282, 1995 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published March 9

Viruses infecting the marine Prymnesiophyte *Chrysochromulina* spp.: isolation, preliminary characterization and natural abundance

Curtis A. Suttle, Amy M. Chan

Marine Science Institute, The University of Texas at Austin, PO Box 1267, Port Aransas, Texas 78373-1267, USA

ABSTRACT: Sixty-four natural virus communities were concentrated from seawater collected from 3 locations in Texas (USA) coastal waters (Gulf of Mexico, 27° 31' N, 96° 18' W; Aransas Pass, 27° 50' N, 97° 02' W; Laguna Madre, 27° 30' N, 97° 18' W) and screened for the presence of lytic pathogens which infect the marine Prymnesiophyte (Haptophyte) *Chrysochromulina brevifilum*. Viruses were detected in 16 of the samples and ranged in abundance from 2 to 688 infectious units l^{-1}. The pathogens were detected at the 3 locations, but not on all dates, from December through June when water temperatures were less than 28°C. A clonal isolate of the virus (CbV-PW1) was obtained by determining the concentration of the infectious agent by a most-probable-number assay and adding 0.2 of an infective unit into each of 20 exponentially growing cultures, removing an aliquot from a culture which lysed and repeating the procedure. The isolate also caused lysis of *C. strobilus*, but did not lyse 8 other isolates of *Chrysochromulina* or 5 other genera of Prymnesiophytes that were screened. The double-stranded DNA virus is a polyhedron of about 145 to 170 nm in diameter with a heavily staining central region that is distinct from the capsid. The appearance of the virions is associated with a granular region in the cytoplasm that does not appear within uninfected cells. Ultimately, viral production results in disruption of the organelles, lysis of the cell and release of the virus particles. Although the number of viruses produced per lytic event is presently unknown we have counted more than 320 virus particles in a single ultrathin section of an infected cell. These results suggest that viruses are likely important in regulating *Chrysochromulina* populations in the sea and may be the reason that blooms of the genus are relatively rare and ephemeral.

KEY WORDS: Algal virus · *Phycodna viridae* · Prymnesiophyte · *Chrysochromulina*

INTRODUCTION

Prymnesiophytes (Haptophytes) are arguably among the most important groups of phytoplankton in the sea; they are global in distribution, and include coccolithophorids such as *Emiliania huxleyi* and toxic bloom formers such as *Chrysochromulina polylepis* and *Prymnesium parvum*. *Chrysochromulina* is a particularly cosmopolitan genus which is found in fresh and marine waters and which can comprise >50% of the photosynthetic nanoplanktonic cells in the ocean (Estep & MacIntyre 1989). Although generally present in diverse phytoplankton assemblages, Prymnesiophytes and even *Chrysochromulina* spp. will form dense blooms on occasion. Some of these blooms produce toxins which have deleterious effects on fisheries (Shilo 1982, Estep & MacIntyre 1989). The most notable example occurred in 1988 when a large bloom (ca 60 000 km^2) of *C. polylepis* caused enormous losses to commercial fisheries in Scandinavia (Dundas et al. 1989, Dahl et al. 1989).

Virus-like particles (VLPs) have been observed in numerous genera of phytoplankton representing a large number of algal classes (Van Etten et al. 1991), including *Chrysochromulina* sp. (Manton & Leadbeater 1974). Also, the demise of blooms of *Emiliania huxleyi* in mesocosm experiments has been associated with the appearance of VLPs within the cells while high concentrations of similar particles were present in the water column (Bratbak et al. 1993). Moreover, it

has recently been realized that viruses infecting phytoplankton can be widespread and abundant in the sea. Lytic pathogens infecting a variety of important marine phytoplankton have been found in natural viral communities concentrated from seawater (Suttle et al. 1990, 1991). For example, viruses infecting the marine prasinophyte *Micromonas pusilla* (Mayer & Taylor 1979, Cottrell & Suttle 1991, 1994) and the cyanobacteria of the genus *Synechococcus* (Suttle & Chan 1993, 1994, Waterbury & Valois 1993) have been found in both coastal and offshore marine environments and can occur at concentrations greater than 10^5 ml^{-1}. Not only are viruses which infect phytoplankton abundant, but other evidence suggests that they can cause significant mortality in natural phytoplankton communities. Proctor & Fuhrman (1990) found that ca 1 to 3% of *Synechococcus* cells contained visible virus particles, and although they likely overestimated the impact of viruses (Waterbury & Valois 1993), their results still indicate that viral lysis of *Synechococcus* cells is of common occurrence. Moreover, estimates based on virus removal rates and contact rates between cyanophages and *Synechococcus* also imply that infection of phytoplankton by viruses frequently occurs (Suttle & Chan 1993, 1994, Waterbury & Valois 1993, Suttle 1994).

Given that virus infection of phytoplankton appears to be relatively common and that *Chrysochromulina* spp. is of wide-spread occurrence and ecological significance, we designed experiments to determine if lytic viruses that infect members of this genus can be readily isolated from seawater. In this paper we report the first data of which we are aware on the isolation and characterization of viruses which infect Prymnesiophytes, and present data on the abundance of these viruses in the coastal waters of Texas, USA.

MATERIALS AND METHODS

Phytoplankton cultures and growth conditions. The phytoplankton used in this study were obtained from culture collections at the University of Texas at Austin (*Chrysochromulina brevifilum* UTEX LB 985; *C. chiton* UTEX LB 982; *C. strobilus* UTEX LB 981; *Emiliania huxleyi* UTEX LB 1016; *Isochrysis galbana* UTEX LB 987; *Pavlova lutheri* UTEX LB 1293; *Prymnesium parvum* UTEX LB 995; *Pseudoisochrysis paradoxa* UTEX LB 1988) and the Provasoli-Guillard Center for Culture of Marine Phytoplankton (*Chrysochromulina* sp. CCMP289; *C. ericina* CCMP281 and CCMP282; *C. herdlensis* CCMP284; *C. polylepis* CCMP285, CCMP286 and CCMP287). Cultures were grown in microwave-sterilized f/2-enriched seawater (Keller et al. 1988) supplemented with 10 nM sodium selenite under continuous irradiance of ~35 µmol quanta m^{-2} s^{-1} photosynthetically active radiation. Growth was monitored by *in vivo* chlorophyll fluorescence, an approximate measure of cell biomass. All experiments were performed at 20°C with the exception of *C. polylepis*, which was grown at 14°C.

Sample collection. Natural communities of viruses were concentrated from seawater essentially as outlined previously (Suttle et al. 1991). Briefly, samples of seawater (20 to 215 l) were collected from the Gulf of Mexico (27° 31' N, 96° 18' W; 8 samples), Aransas Pass (27° 50' N, 97° 02' W; 43 samples) and Laguna Madre (27° 30' N, 97° 18' W; 13 samples) and filtered through 142 mm diameter glass-fiber (nominal pore size 1.2 µm) and polyvinylidine difluoride (0.22 or 0.45 µm pore size) membrane filters. The remaining particulate matter in the filtrate was concentrated 236- to 1415-fold using a 30 000 MW-cutoff ultrafiltration membrane.

Enumeration of infective viruses. Aliquots from these concentrated natural virus communities were added to duplicate exponentially growing cultures. Each culture received the equivalent number of viruses that would be present in 250 ml of seawater, assuming that the viruses were concentrated with 100% efficiency. Growth in these cultures was compared to control cultures, to which nothing was added, by daily monitoring of *in vivo* chlorophyll fluorescence using a Turner Designs fluorometer. For those concentrates which caused lysis of the cultures, the titer of the infective agent was determined by a most-probable-number (MPN) assay in liquid culture (Suttle & Chan 1993). Duplicate serial dilutions of each concentrate were added to exponentially growing 5 ml cultures of the alga, with 8 replicates at each dilution. The cultures were monitored daily for evidence of cell lysis. Cultures in which lysis did not occur after 21 d were propagated into fresh exponentially growing cultures and monitored for another 14 d. Cultures that did not lyse after propagation were scored as negative for the presence of lytic viruses. The number of cultures in which lysis occurred, or did not occur, was scored, and the concentration of infective units determined by a BASIC program (Hurley & Roscoe 1983). Transmission electron microscopy (TEM) was used to confirm the presence of the viral pathogen in the lysates at the highest dilutions.

Isolation, cloning and purification of the virus. A clonal isolate of the virus (CbV-PW1) was obtained by adding 0.2 infective units of a titered virus concentrate to 20 exponentially growing cultures of *Chrysochromulina brevifilum*. An aliquot was removed from a single culture in which lysis occurred and the entire procedure was repeated (Cottrell & Suttle 1991). Large-scale cultures of CbV-PW1 were prepared by adding 0.25 ml of a culture lysate to 2.5 l of exponentially growing *C. brevifilum* cells (virus:host ratio ca 0.1). After complete

lysis of the culture (ca 7 d later), the lysate was filtered through glass fiber (nominal pore size 0.8 µm) and polyvinylidine difluoride (0.22 µm pore size) filters and stored at 4°C. The viruses were collected from the filtered lysate and concentrated by ultrafiltration using a 30 000 MW-cutoff ultrafiltration membrane (Amicon S1Y30) followed by pelleting in an ultracentrifuge (146 000 × *g*, 2.5 h, 20°C). The pellet was gently resuspended in 30‰ seawater and further purified by centrifugation (70 950 × *g*, 20 min, 20°C) through a sucrose step gradient (10, 20, 30, 40 % w/v in 30‰ seawater). A single band containing infective viruses was recovered from the gradient and the band material diluted about 30-fold with sterile ultrafiltered seawater. The viruses were finally harvested by centrifugation (146 000 × *g*, 3 h, 20°C) and the pellet resuspended in sterile seawater.

Transmission electron microscopy. Sucrose-gradient purified CbV-PW1 were adsorbed to carbon-coated copper grids by floating a grid on a drop of virus suspension for 1 h. The grid was stained with 1 % uranyl acetate and photographed at 80 kV using a Philips EM301 transmission electron microscope. Virus particle diameters were estimated from photographic images of negatively stained virus particles.

Ultrathin sections of viruses within *Chrysochromulina brevifilum* were prepared as follows. An inoculum of viruses was added to exponentially growing cells and fixed in glutaraldehyde (1 % final concentration) about 12 h before lysis of the cultures was expected to occur. The infected cells were stored overnight at 4°C and gently harvested by centrifugation (3000 × *g*, 30 min, 4°C) the next day. The cell pellet was enrobed in 4 % agar, cut into 1 to 2 mm pieces, fixed overnight in 5 % glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.0, with 0.25 M sucrose), post-fixed 4 h in 1 % osmium tetroxide (in 0.1 M cacodylate buffer, pH 7.0), dehydrated in a graded acetone series, embedded in Eponate and sectioned. Ultrathin sections were stained with 2 % uranyl acetate, lead citrate, and photographed at 80 kV using TEM.

Host range. The host range of CbV-PW1 was tested by adding 0.5 ml of 0.22 µm filtered lysate (about 10^6 infective viruses) to duplicate 5 ml exponentially growing cultures of the potential host. The cultures were monitored daily for evidence of lysis by measuring *in vivo* chlorophyll fluorescence. Fluorescence readings were compared to control cultures to which nothing was added. Six genera of Prymnesiophyceae, including 9 other *Chrysochromulina* spp., were tested as potential hosts. For *C. polylepis*, a parallel experiment was done using *C. brevifilum* at 14°C. Cultures that had not lysed after 14 d in the stationary growth phase were considered to be unsuitable hosts for this virus. Cultures in which lysis occurred were 0.22 µm filtered and an aliquot introduced into another exponentially growing culture to ensure that the pathogen could be propagated.

RESULTS AND DISCUSSION

In this paper we report the first isolation and characterization of a virus which infects a Prymnesiophyte and present data on the abundance of these viruses in the coastal waters of Texas. Viruses infecting *Chrysochromulina brevifilum* were detected at all locations, but not on all dates, from December through June when water temperatures were less than 28°C. Viruses were found in approximately 25 % of the samples that were screened and ranged in abundance from 2 to 688 infectious viruses l^{-1} (Fig. 1). It is possible that the concentration of infectious viruses was underestimated because viruses could have been lost during the prefiltration and ultrafiltration procedures; however, there was no difference in the concentration of viruses (ca 10^6 infectious units ml^{-1}) in unfiltered culture lysate when compared to lysate that had been filtered through a variety of membrane filters: 0.22 and 0.45 µm pore-size polyvinylidine (Durapore); 0.20 µm

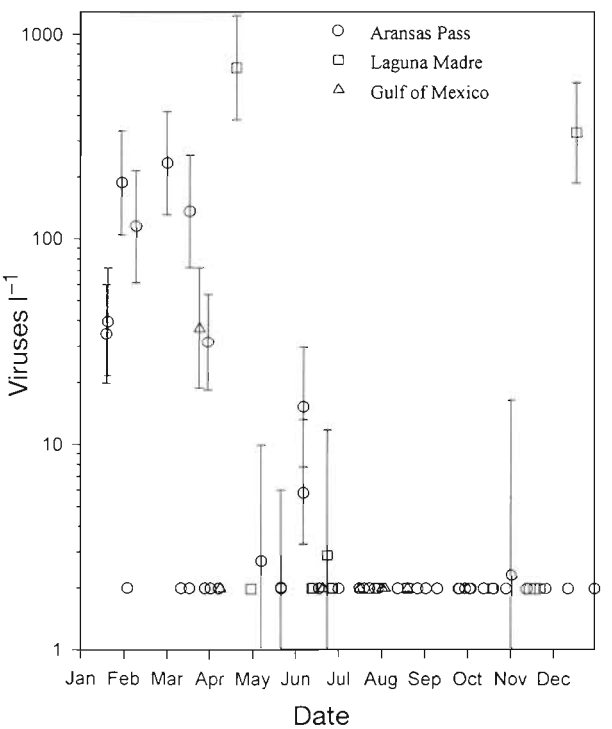
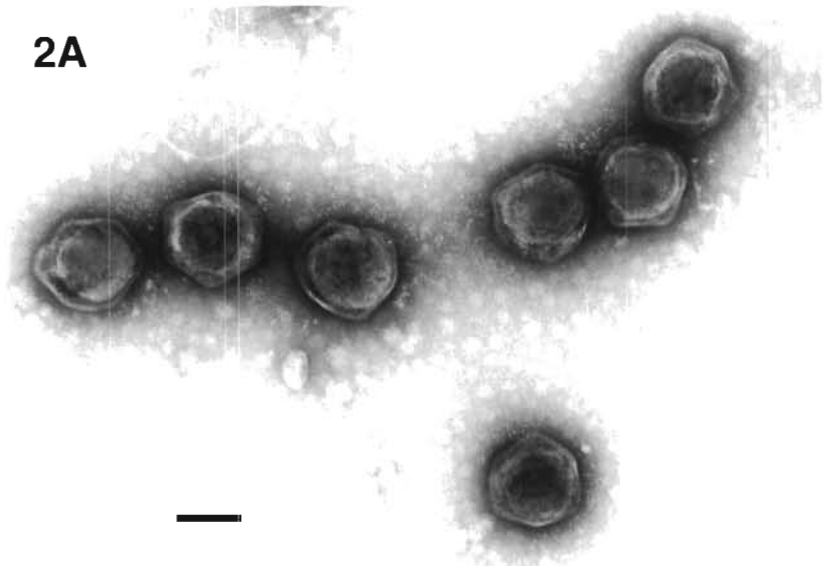


Fig. 1 Concentration (± SD) of viruses which infected and lysed *Chrysochromulina brevifilum* in coastal waters of Texas between 27 July 1992 and 30 March 1994. Where error bars are not shown the concentration of viruses was below the limit of detection (2 l^{-1})

Fig. 2. Electron micrographs of viruses which infect *Chrysochromulina* spp. (A) Negative-stained preparation of CbV-PW1 that had been purified on a sucrose gradient; (B) ultrathin section of viruses within the viroplasm. Scale bars = 100 nm ►



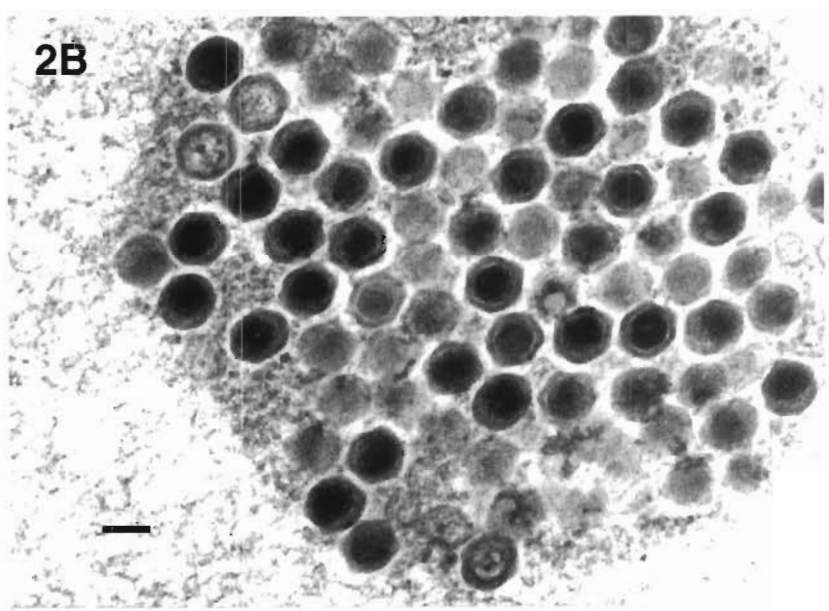
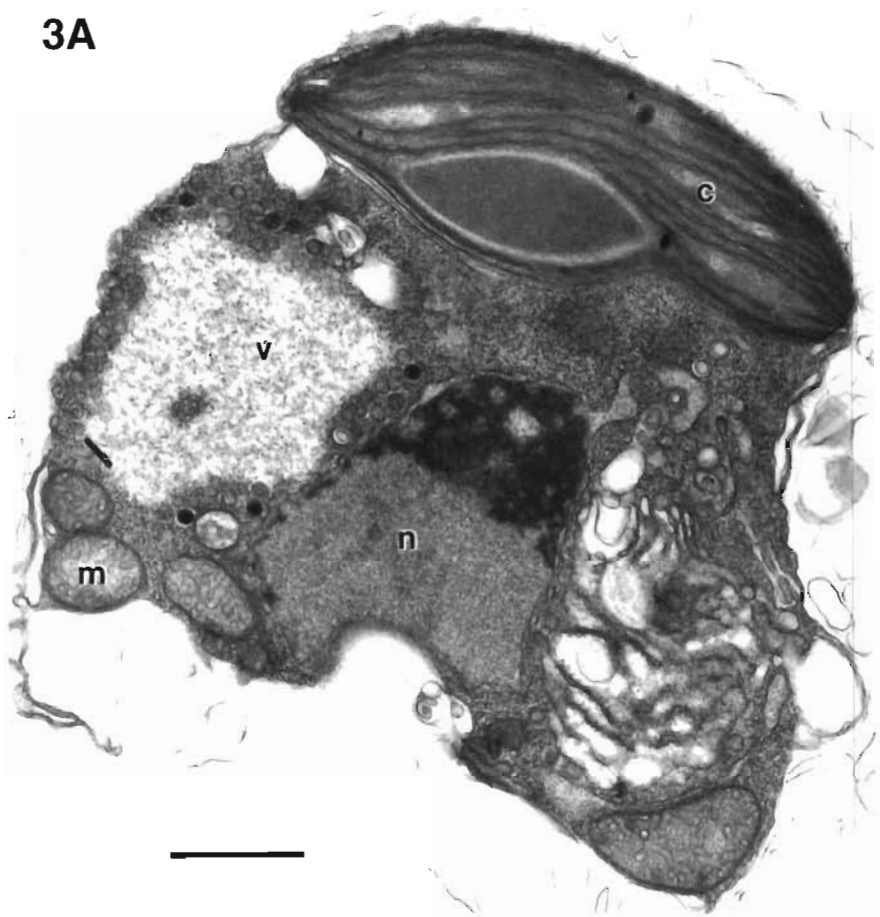
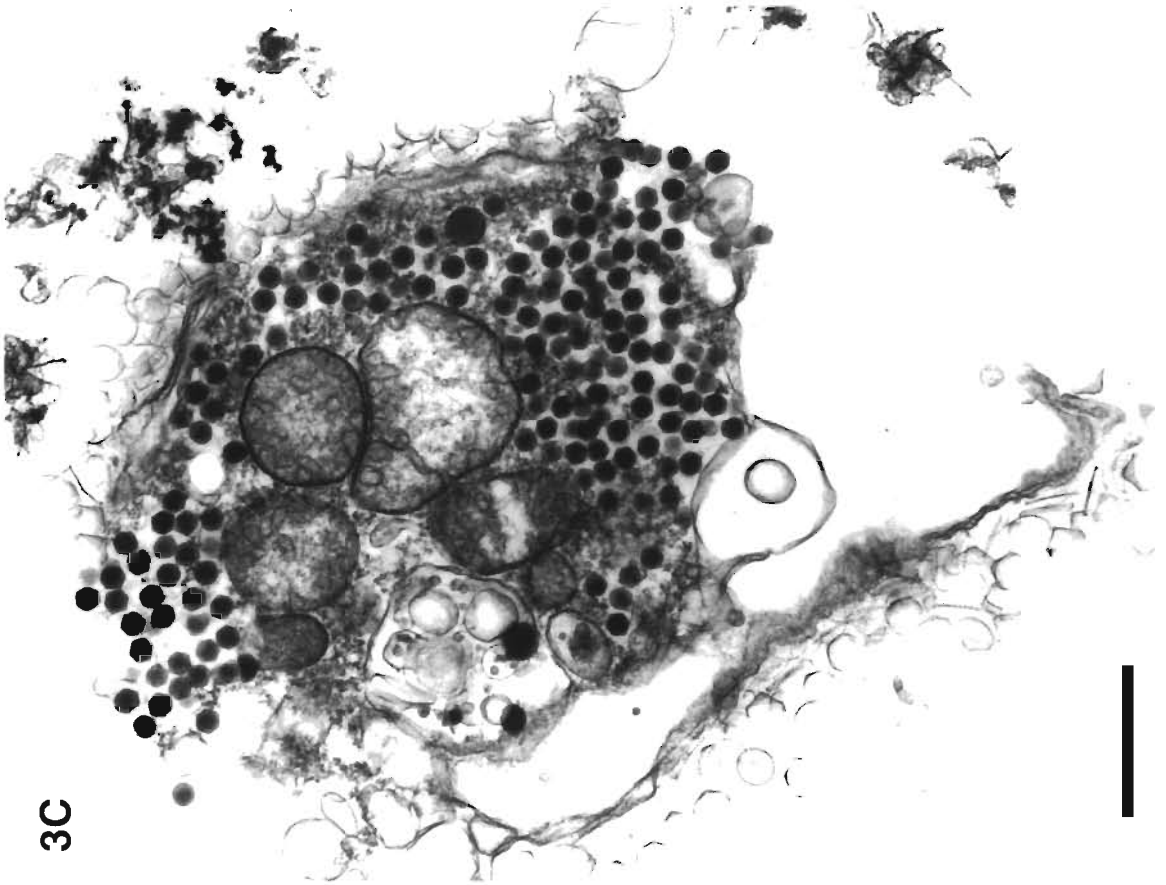
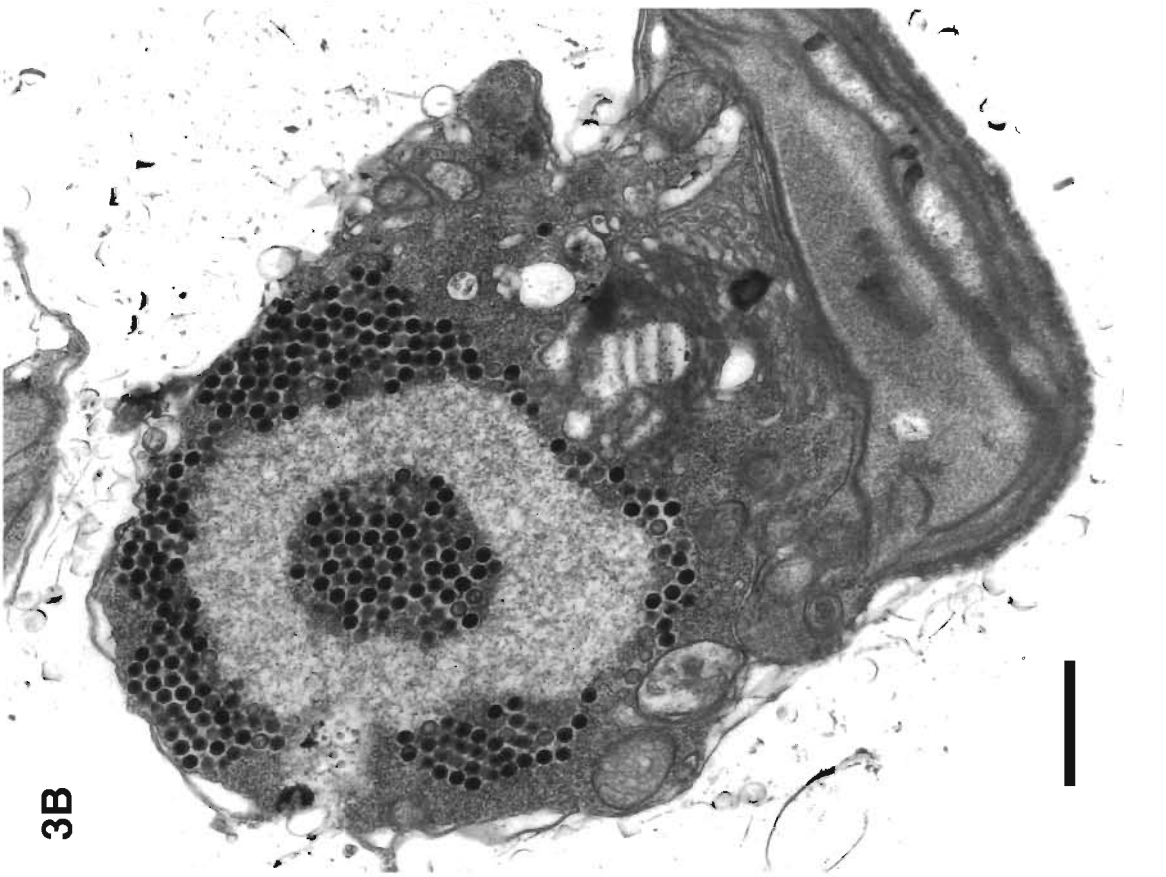


Fig. 3. *Chrysochromulina brevifilum*. Electron micrographs of ultrathin sections. (A) Early stage of viral infection showing the cytoplasmic site of virus replication; c: chloroplast, m: mitochondria, n: nucleus, v: viroplasm; (B) infected cell with >320 virus particles located within a distinct region of the cytoplasm; and (C) immediately prior to cell lysis. Scale bars = 1.0 µm ▼





3C



3B

pore-size polycarbonate (Poretics); 1.2 µm nominal pore-size glass fiber (MSI) (data not shown). The highest titers of viruses occurred during April in Laguna Madre, a large (425 km^2) and shallow (mean depth ca 1 m) semi-enclosed marine lagoon. However, infectious viruses were detected most often in Aransas Pass, a channel which connects the Gulf of Mexico to a large bay system behind a barrier island. Viruses were also detected (37 l^{-1}) in 1 of 8 samples collected at the offshore station in the Gulf of Mexico. The reasons for the temporal and spatial distribution of viruses infecting *C. brevifilum* is unknown, but is likely related to the abundance of cells which they infect. Viruses infecting *Micromonas pusilla* were also seasonally variable (Cottrell & Suttle 1994) and were most abundant when water temperatures were coolest. In contrast, viruses infecting marine *Synechococcus* are most abundant when water temperatures are warmest (Suttle & Chan 1993, 1994, Waterbury & Valois 1993). The abundance of cyanophages is related to the concentration of *Synechococcus* and it seems likely that viruses infecting *C. brevifilum* and *M. pusilla* are also related to the abundance of the hosts which they infect.

Addition of a sucrose-gradient-purified clonal isolate of the virus (CbV-PW1) (Fig. 2A) to an exponentially growing culture of *Chrysochromulina brevifilum* caused cell lysis, and resulted in the amplification of the virus, thereby fulfilling Koch's postulates. The virus infects *C. brevifilum* and *C. strobilus*, but did not cause lysis of 8 other isolates of *Chrysochromulina* or 5 other genera of Prymnesiophytes that were screened (see 'Materials and methods' for a list of the isolates screened). This assay is quite sensitive and lysis will eventually occur in cultures of hosts which are very inefficient at propagating the virus. Restriction enzyme digests of the purified viral DNA (data not shown), as well as staining with the fluorochrome DAPI (4, 6-diamidino-2-phenylindole) indicated that the virus possesses a double-stranded DNA genome.

The virion is tailless and about 145 to 170 nm in diameter with a heavily staining central region that is distinct from the capsid (Fig. 2A, B). It is hexagonal in cross section, suggesting icosahedral symmetry. Manton & Leadbeater (1974) described virus-like particles in *Chrysochromulina mantoniae* as being 22 nm in diameter. However, according to the published micrographs the particles were 220 nm in diameter, suggesting that additional species of *Chrysochromulina* are susceptible to infection by similar viruses. An electron micrograph of *C. brevifilum* in the early stage of infection clearly demonstrates that virus replication occurs in the cytoplasm (Fig. 3A). The nucleus is intact and exists apart from the viroplasm. The viruses appear to be associated with a lightly staining viroplasm that consists of a fibrillar matrix (Fig. 3A, B). Numerous ribosomes appear to be inside and around the viroplasm. Ultimately, viral production results in disruption of the organelles, lysis of the cell and release of the virus particles (Fig. 3C). Although the number of viruses produced per lytic event is unknown we have counted more than 320 virus particles in a single ultrathin section of an infected cell (Fig. 3B), giving a minimum estimate of the burst size. The icosahedral morphology, double-stranded DNA genome, and the cytoplasmic site of virus assembly are properties this virus has in common with other algal viruses (Mayer & Taylor 1979, Meints et al. 1986).

The only other viral pathogens of unicellular algae that have been isolated infect 2 groups that are distantly related to *Chrysochromulina*. These are the viruses which infect the prasinophyte *Micromonas pusilla* (Mayer & Taylor 1979, Cottrell & Suttle 1991) and *Chlorella*-like algae which are endosymbiotic in *Paramecium* and *Hydra* (Van Etten et al. 1982, Reisser et al. 1988, Zhang et al. 1988). Based on partial sequence analysis of the DNA polymerase gene of CbV-PW1 (data not shown), this virus appears to be closely related to the viruses that infect *M. pusilla* and *Chlorella*-like algae, and probably belongs to the virus family Phycodnaviridae (Van Etten & Ghabrial 1991). Although few systems are known, there is strong circumstantial evidence that viruses infecting unicellular algae are important components of marine ecosystems. Evidence from electron microscopy suggests that viruses infect a broad range of eukaryotic phytoplankton (Dodds 1979, Van Etten et al. 1991) and may even be involved in the termination of coccolithophorid blooms (Bratbak et al. 1993). Moreover, Emiliani (1993a, b) argues the fossil record is consistent with the spread of viral pathogens being responsible for mass extinctions of coccolithophorids and foraminiferans. Also, the virus-size fraction causes lysis of a variety of phytoplankton and is able to strongly suppress photosynthesis and the growth of natural phytoplankton communities (Suttle et al. 1990, Suttle 1992, Peduzzi & Weinbauer 1993).

The presence of these viruses should have a strong regulatory effect on the *Chrysochromulina* populations which they infect and would likely prevent bloom formation when present. It is not necessary for a virus to be present in high concentrations or to infect a broad range of host species in order to be important in regulating phytoplankton populations in nature. Given that the spread of a virus infection is highly dependent on host density (Wiggins & Alexander 1985, Murray & Jackson 1992) it seems unlikely that a phytoplankton species could achieve the high densities associated with blooms in the presence of a lytic virus.

Furthermore, if other viruses can be isolated which infect toxic bloom formers, such pathogens might ultimately serve as biological control agents. Phytoplankton blooms should be particularly amenable to control by a biological control agent such as a virus, and may be a reason why persistent blooms are an unusual phenomenon in nature. The comment is occasionally raised that biological control of phytoplankton blooms by viruses is not feasible because resistant cells will arise under the strong selective pressure of a lytic virus. Although resistant cells might be present they would typically comprise a very small proportion of a population. Hence, the majority of cells in a bloom would likely be susceptible to lysis. This would provide the opportunity for other non-blooming phytoplankton to increase in abundance and replace the bloom-forming species. Moreover, virus adsorption is often mediated by attachment to highly conserved sites, such as transport proteins, on the surface of the host cell. Mutations at these sites can make the resistant cells competitively inferior (Szmelcman & Hofnung 1975), therefore, in the absence of the pathogen there will be selective pressure to return to cells that are susceptible to infection.

Our results suggest that viruses are likely important in regulating *Chrysochromulina* populations in the sea and a reason that bloom events are relatively rare and often ephemeral. G. E. Hutchinson in his seminal paper 'Paradox of the Plankton' (Hutchinson 1961) posed the question, 'Why are phytoplankton communities so diverse?' As viruses will spread rapidly through abundant host populations, viruses may be one of the primary mechanisms for maintaining high species diversity in planktonic communities. Given the diversity of viral pathogens that seem to be present in the sea perhaps a more puzzling question is 'How is it that phytoplankton populations are able to form blooms?'

Acknowledgements. We thank H. R. DeYoe for help with the ultrathin sections, and K. Rodda for technical assistance. J. A. Zeikus kindly provided phytoplankton from the University of Texas culture collection, and D. Stockwell obtained seawater from Laguna Madre. This research was supported by grants from the National Science Foundation (OCE-9018833), the U.S. Office of Naval Research (N00014-92-J-1676), and from NOAA through Texas A&M Sea Grant (NA-16RG0457-01). Contribution no. 917 of the Marine Science Institute

LITERATURE CITED

Bratbak, G., Egge J. K., Heldal, M. (1993). Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Mar. Ecol. Prog. Ser. 93: 39–48

Cottrell, M. T., Suttle, C. A. (1991). Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, *Micromonas pusilla*. Mar. Ecol. Prog. Ser 78: 1–9

Cottrell, M. T., Suttle, C. A. (1994). Strain specificity of *Micromonas pusilla* viruses and the effect on estimating the concentration of infective *M. pusilla* viruses in seawater. EOS 75: 167

Dahl, E., Lindahl, O., Paasche, E., Throndsen, J. (1989). The *Chrysochromulina polylepis* bloom in Scandinavian waters during spring 1988, In: Cosper, E. M., Bricelj, V. M., Carpenter, E. J. (eds.) Novel Phytoplankton Blooms. Springer-Verlag, New York, p. 383–405

Dodds, J A. (1979). Viruses of marine algae. Experientia 35: 440–442

Dundas, I., Johannessen, O. M., Berge, G., Heimdal, B. (1989). Toxic algal bloom in Scandinavian waters, May–June 1988. Oceanography 2: 9–14

Emiliani, C. (1993a). Extinction and viruses. Biosystems 31: 155–159

Emiliani, C. (1993b). Viral extinctions in deep-sea species. Nature 366: 217–218

Estep, K. W., MacIntyre, F. (1989). Taxonomy, life cycle, distribution and dasmotrophy of *Chrysochromulina*: a theory accounting for scales, haptonema, muciferous bodies and toxicity. Mar. Ecol. Prog. Ser. 57: 11–21

Hurley, M. A., Roscoe, M. E. (1983). Automated statistical analysis of microbial enumeration by dilution series. J. appl. Bact. 55: 159–164

Hutchinson, G. E. (1961). The paradox of the plankton. Am. Nat. 95: 137–145

Keller, M. D., Bellows, W. K., Guillard, R. R. L. (1988). Microwave treatment for sterilization of phytoplankton culture media. J. exp. mar. Biol. Ecol. 117: 279–283

Manton I., Leadbeater, B. S. C. (1974). Fine-structural observations on six species of *Chrysochromulina* from wild Danish marine nanoplankton, including a description of *C. campanulifera* sp. nov. and a preliminary summary of the nanoplankton as a whole. Biol. Skr. 20: 1–26

Mayer, J. A., Taylor, F. J R. (1979). A virus which lyses the marine nanoflagellate *Micromonas pusilla*. Nature 281. 299–301

Meints, R. H., Lee, K., Van Etten, J. L. (1986). Assembly site of the virus PBCV-1 in a *Chlorella*-like green alga: ultrastructural studies. Virology 154: 240–245

Murray, A. G., Jackson, G. A. (1992). Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. Mar. Ecol. Prog. Ser. 89: 103–116

Peduzzi, P., Weinbauer, M. G. (1993). Effect of concentrating the virus-rich 2–200 nm size fraction of seawater on the formation of algal flocs (marine snow). Limnol. Oceanogr. 38: 1562–1565

Proctor, L. M., Fuhrman, J. A. (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343: 60–62

Reisser, W., Burbank, D. E., Meints, S. M., Meints, R. H., Becker, B., Van Etten, J. L. (1988). A comparison of viruses infecting two different *Chlorella*-like green algae. Virology 167: 143–149

Shilo, M. (1982). The toxic principles of *Prymnesium parvum*, In: Carmichael, W. (ed.) The water environment. Plenum, New York, p. 37–47

Suttle, C. A. (1992). Inhibition of photosynthesis in phytoplankton by the submicron size fraction concentrated from seawater. Mar. Ecol. Prog. Ser. 87: 105–112

Suttle, C. A. (1994). The significance of viruses to mortality in aquatic microbial communities. Microb. Ecol. 28: 237–243

Suttle, C. A., Chan, A. M. (1993). Marine cyanophages infecting oceanic and coastal strains of *Synechococcus*: abundance, morphology, cross-infectivity and growth characteristics. Mar. Ecol. Prog. Ser. 92: 99–109

Suttle, C. A., Chan, A. M. (1994). Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl. environ. Microbiol. 60: 3167–3174

Suttle, C. A., Chan, A. M., Cottrell, M. T. (1990). Infection of phytoplankton by viruses and reduction of primary productivity. Nature 347: 467–469

Suttle, C. A., Chan, A. M., Cottrell, M. T. (1991). Use of ultrafiltration to isolate viruses from seawater which are pathogens of marine phytoplankton. Appl. environ. Microbiol. 57: 721–726

Szmelcman, S., Hofnung, M. (1975). Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage Lambda receptor. J. Bacteriol. 124: 112–118

Van Etten, J. L., Ghabrial, S. A. (1991). Phycodnaviridae, in: classification and nomenclature of viruses. Arch. Virol. Suppl. 2: 137–139

Van Etten, J. L., Lane, L. C., Meints, R. H. (1991). Virus and viral-like particles of eukaryotic algae. Microb. Rev. 55: 586–620

Van Etten, J. L., Meints, R. H., Kuczmarski, D., Burbank, D. E., Lee, K. (1982). Viruses of symbiotic *Chlorella*-like algae isolated from *Paramecium bursaria* and *Hydra viridis*. Proc. natl. Acad. Sci. U.S.A. 79: 3867–3871

Waterbury, J. B., Valois, F. W. (1993). Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl. environ. Microbiol. 59: 3393–3399

Wiggins, B. A., Alexander, M. (1985). Minimum bacterial density for bacteriophage replication: implication for significance of bacteriophages in natural ecosystems. Appl. environ. Microbiol. 49: 19–23

Zhang, Y., Burbank, D. E., Van Etten, J. L. (1988). *Chlorella* viruses isolated from China. Appl. environ. Microbiol. 54: 2170–2173

This article was presented by J. Fuhrman (Senior Editorial Advisor), Los Angeles, California, USA

Manuscript first received: August 17, 1994
Revised version accepted: October 21, 1994