

Algal Virus Workshop  
Abstracts  
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## **Biology of brown algal viruses in experimental cultures and natural habitats.**

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Many brown algal species are infected by viruses with genomes of double stranded DNA. We maintain clonal cultures of healthy and infected isolates comprising seven species from five genera: Ectocarpus, Feldmannia, Hincksia, Pilayella and Myriotrichia. All viruses are distinct taxonomic entities, and highly host specific with few exceptions. Our brown algal viruses are systemic and latently present in every cell of an infected host. They become virulent in the reproductive structures and render the host sterile. A fragment of the Ectocarpus virus genome coding for a coat protein is used as a virus specific indicator to detect infected plants with a PCR amplification technique. With this method 42 out of 97 clonal isolates from all oceans and continents were found to contain viral DNA in a cryptic state. In an epidemiological study we followed an Atlantic and a Pacific Ectocarpus population over 26 months. Between 60 and 100% of the Ectocarpus plants in both habitats contain viral DNA. Furthermore, two different virus genotypes were found to co-exist in the Canary Island habitat. Easiness of infection, vertical passage to daughter generations by vegetative and sexual reproduction, and virus elimination by Mendelian segregation in the host's meiosis suggest a highly dynamic interaction between host and pathogen, including the possibility of lateral gene transfer.

## Host-virus interactions in brown algae: ways to suppression of virus replication and escape from infection

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In all brown algal dsDNA viruses investigated so far, the virus genome is intimately associated with the host genome and goes through an extended latency period. Virus propagation is restricted to the presumptive reproductive organs of the host, resulting in infertility. The mechanisms underlying the latency status and activation of virus replication are unknown, but are apparently related to the initiation of sporo- or gametogenesis. In several virus-host systems, infected thalli may partly suppress virus formation and resume formation of normal reproductive cells, thus producing simultaneously virus particles and functional spores or gametes. The ability to suppress virus formation is variable in the different virus-host systems, and most strongly pronounced in heterologous infections. An additional mechanism of escape from virus infection occurs in sexual species like *Ectocarpus siliculosus*, where the virus genome may be eliminated by Mendelian meiotic segregation. The information available on recovery from virus infections will be summarized and discussed. In addition, a new brown algal DNA virus, the *Pilayella littoralis* virus (PlitV-1), will be described.

## **Viral infections of the brown alga, *Feldmannia***

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A persistent dsDNA virus (FsV) infection in the algal genus *Feldmannia* occurs within the sporophyte meiotic sporangia that normally produce zoospores. No trace of virus is evident in vegetative cells of sporophyte, and gametophytes produce no virus at all. CHEF electrophoresis demonstrates two virus genome size-classes of 158 and 178 which map as circles. Repetitive sequences (173 bp) were found in virus DNA. In an encrypted sequence of FsV, a large (50 kbp) repeat insert occurs within a serine/threonine protein kinase gene. Both the major capsid protein and the DNA polymerase gene have been isolated and characterized. Two transcriptionally active open reading frames which contain an ATP binding site and a "RING" zinc finger motif were found. The capsid protein is related to that of the Iridoviruses and less so to African Swine fever virus. Here we will describe the insertion sites of replicationally active FsV integrated within the host genome. The viral insertion site occurs in the viral *Bam*HI-fragment A (B26.0A) of small genome size class viruses. The viral genomes of both size classes open at the precise base pair, but insertion of each is at different, but related, places of the host genome. These sites have been cloned and sequenced.

## **Virus assembly in *Hincksia hincksiae***

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The filamentous brown alga *Hincksia hincksiae* can be infected by a large icosahedral double-stranded DNA virus, HincV-1. The virus is latently present in somatic cells of the host and is replicated only in the presumptive reproductive cells. Virus formation was studied by DAPI staining, tubulin immunofluorescence and transmission electron microscopy. The first indication of infection is a lack of cytokineses in the virus producing cells resulting in multinucleate cells, whereby the microtubular cytoskeleton does not seem to be affected. Replication of viral DNA begins in the nuclei, which increase in size and eventually disintegrate. Capsid formation starts in the cytoplasm, maturation of the virus particles takes place in a mixed cyto-/nucleoplasm after nuclear breakdown. Capsids bud from the edge of cisternae which are possibly modified ER aggregated to virus assembly centres. The internal membrane of the capsid is thus derived from the ER. When assembled the particles appear empty, the nucleoprotein core seems to be packaged subsequently through an opening in the capsid. Several fine structural features related to virus formation are described. A comparison is made with other brown algal host-virus systems.

## **Molecular investigations of the *Ectocarpus siliculosus* Virus EsV**

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The marine brown alga *Ectocarpus siliculosus* is frequently infected by a virus that multiplies in modified gametangia and sporangia of the host. EsV particles are characterized by an icosahedral multilayered capsid enclosing a circular double stranded DNA of about 320 kb which is interrupted by single-stranded regions. Based upon one-dimensional SDS-PAGE analysis, the virus contains at least 50 proteins ranging in molecular mass from 20 to > 100 kDa, one of which is the glycoprotein gp-1. In an attempt to better understand the molecular aspects of the EsV infection cycle we have focused our investigations on structural proteins and the state of viral DNA during the latent phase. Several capsid proteins were identified by immunological screening of a ZAP Express expression library of EsV DNA with an antiserum raised to structural proteins. The corresponding genes were partially sequenced and mapped by Southern blots. Computer-assisted search did not reveal homologies to known protein virus sequences, supporting the idea that brown algal viruses are a phylogenetically distinct group. Furthermore, we are now able to isolate high molecular alga DNA by pulsed-field agarose electrophoresis. Polymerase chain reaction with virus specific primers confirms the integration of the viral DNA in the host genome.

## **Unusual lifestyle of giant *Chlorella* viruses.**

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Many large (150 - 190 nm in diameter), polyhedral, plaque-forming viruses that replicate in certain isolates of unicellular, eukaryotic, chlorella-like green algae have been isolated and partially characterized in the past 15 years. The viral particles contain at least 50 structural proteins and a lipid component located inside the outer glycoprotein capsid. The 330,740 base pair genome of the prototype virus PBCV-1 has been sequenced and is predicted to encode 377 protein encoding genes and ten tRNA genes. Many of these viral encoded genes were unexpected and have never been found in a virus genome. In addition to their large genome size, the chlorella viruses have at least three other unusual properties. i) The viruses encode multiple DNA methyltransferases and DNA site-specific (restriction) endonucleases. ii) PBCV-1 virions, like many viruses, contain glycoproteins. However, unlike other viruses that contain glycoproteins, PBCV-1 encodes at least part, if not its entire, glycosylation machinery. iii) PBCV-1 has two different types of introns; a self-splicing intron in a transcription factor-like gene and a splicesomal processed type of intron in its DNA polymerase gene. In addition, one of the tRNA genes is predicted to contain a small intron.

# Characterisation of a Microalgal Virus Isolated from Irish Waters and Initial Genome Studies

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In an attempt to isolate algal viruses indigenous to inner Galway Bay (W coast of Ireland), concentrates of the 20-200 nm particulate fraction from surface waters were added to cultures of phytoplankton species typically observed in the bay. Lysis was observed in a culture of *Pyramimonas orientalis* (Prasinophyceae). Electron micrographs of the algal cells showed structural damage and the presence of polygonal-shaped particles. Filtrates (<200 nm) of lysed cultures were used to inoculate further cultures to confirm that lysis was caused by a tail-less virus with a capsid diameter of  $74 \pm 7.9$  nm (average  $\pm$  standard deviation). These characteristics are similar to those previously described for the Phycodnaviridae. Restriction enzyme and nuclease studies showed the viral nucleic acid to be single-stranded DNA. PCR and RAPD (Random Amplified Polymorphic DNA) primers were used to randomly amplify regions (fragments) of the viral genome. Following subcloning and DNA sequencing of these fragments BLAST (Basic Local Alignment Search Tool) analyses were carried out. Initial results suggest similarity between the virus sequences and several known gene sequences of non-viral origin including protein-encoding genes. The development of a nucleic acid-based probe specific for the viral isolate is underway. This probe will be used for the study of virus/host dynamics in Galway Bay.

## **Algal-virus interactions in Swedish coastal waters: host specificity of viruses infecting the marine photosynthetic flagellate *Micromonas pusilla*.**

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Seawater sampled in the Skagerrak and Kattegat coastal waters, during the period October 1995 - September 1996 were screened for the occurrence of viruses lytic to marine microalgae. Viruses lytic to the photosynthetic marine picoflagellate *Micromonas pusilla* (Butcher) Manton & Parke (Prasinophyceae) were detected in all seawater samples screened. Evidence for viral lysis of any other of the 11 algal species tested were not obtained. Several viruses infecting different strains of *M. pusilla* were isolated. Ten isolated viruses which were tested for host-specificity were found to be species-specific to *M. pusilla* and even strain-specific to 1-3 of the 6 strains of *M. pusilla* used in the experiment. The abundance of viruses infectious to a *M. pusilla* strain isolated from the Oslofjord, Norway, was at least one order of magnitude higher (average  $2.5 * 10^5 \text{ l}^{-1}$ ) than viruses infecting two *M. pusilla* strains isolated from Gulf of Maine, USA (average  $2.2 * 10^4$  and  $4.6 * 10^3 \text{ l}^{-1}$ , respectively). This is the first time that strain specificity has been reported in MpV. The negative results in detecting lytic infection in the algal species other than *M. pusilla*, might have been due to the possibility of these algae already being latent infected.

## **Diversity of viruses as revealed by DNA polymerase gene sequence analysis.**

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Recent methodological developments have allowed the use of the polymerase chain reaction (PCR) to examine the diversity of algal virus isolates, and to infer taxonomic relationships among the isolates. DNA polymerase genes are highly conserved and are therefore suitable targets for PCR analysis of microbes that do not encode rRNA. As natural virus communities are largely made up of dsDNA viruses, and as many dsDNA algal viruses encode their own DNA polymerase, PCR primers can be designed to amplify fragments of these genes. This approach can also be used to examine the diversity of natural communities of viruses without the need for culture. This approach has been used to examine the genetic diversity in natural communities of viruses that infect phytoplankton. Algal-virus-specific primers were used to amplify polymerase fragments from natural virus samples, demonstrating the presence of a diverse community of viruses closely related to those that are known to infect phytoplankton. We have modified this approach by using denaturing gradient gel electrophoresis (DGGE) to rapidly analyze PCR products. DGGE will permit rapid and efficient fingerprinting of natural marine viral communities, and allow spatial and temporal differences in viral community structure to be examined.

## **Detection of *Phaeocystis*-virus by PCR**

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Current methods for detection and quantification of viruses are based either on titration, electron or epifluorescence microscopy. Titration requires an established host-virus system and time-consuming culturing, while microscopy lacks potential for specific detection. Ecological studies of algal viruses require reliable, specific techniques for detection and quantification.. To study the ecology of *Phaeocystis pouchetii* - PpV host virus system we are working on a competitive PCR approach, applying an internal standard template that allows specific and sensitive detection of the virus both in algal cultures and sea water samples. To develop a specific PCR for the *Phaeocystis* virus (PpV) we have used degenerate primers specific for phycodnaviridae, and sequenced the obtained PCR product to design specific primers for PpV. This approach also allows us to compare the sequenced strains with other well described algal viruses. This highly versatile methodological approach may be applied also to other culturable vira and possibly also to viral systems not yet isolated.

## **Characterization of virus infected phytoplankton cells by FCM**

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The significance of viruses infecting phytoplankton cells has received much attention the last years. Algal viruses have been detected in different algal species and enumeration in natural waters suggests that they play an important role in phytoplankton dynamics. Causing lysis of the algal cells, viruses will also indirectly affect the carbon and nutrient flow in the pelagic systems.

However, until now not much is known on the cellular characteristics of viral infected phytoplankton cells. We have used flow cytometry to analyse changes in cellular parameters during viral infection as compared to non-infected cells of two axenic phytoplankton species (*Phaeocystis pouchetii* and *Micromonas pusilla*). Differences in forward angle, side angle scatter and autofluorescence were observed for both algal species. The use of fluorescent dyes furthermore yielded information on DNA content and viability which is not easily obtained with other methods. The use of these techniques in this field of research is new and provides detailed information for the study of the ecophysiology of viral infected phytoplankton species.

## **Investigation of cyanophage diversity in phosphate-manipulated seawater mesocosms using denaturing gradient gel electrophoresis (DGGE).**

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Cyanophages (viruses that infect cyanobacteria) are abundant in the marine environment and are thought to be a significant factor in determining the population dynamics of members of the unicellular phycoerythrin-containing cyanobacteria of the genus *Synechococcus*. In an effort to use molecular techniques to characterise cyanophage populations, a conserved region from the cyanophage genome was identified in 3 genetically distinct marine cyanomyoviruses and sequence analysis revealed that they exhibited significant similarity to a gene encoding a capsid assembly protein (gp20) from the enteric coliphage T4. Comparison of these sequences permitted the design of PCR primers which specifically amplified a region of 165 bp from cyanomyovirus isolates tested. Denaturing gradient gel electrophoresis (DGGE) was then used to separate 165 bp DNA fragments from a range of different cyanomyovirus isolates which had been PCR-amplified together. DGGE was subsequently used to investigate the population structure of cyanophages during the course of a seawater mesocosm study. A large *Synechococcus* spp. bloom developed in one mesocosm enclosure which was maintained at a high N:P ratio, simulating phosphate-deplete growth conditions. Following phosphate addition to this enclosure, there was a large increase in estimated total virus numbers shortly before an apparent collapse of the *Synechococcus* bloom. DGGE analysis of the > 0.3  $\mu\text{m}$  fraction revealed that one cyanophage genotype disappeared following the addition of phosphate to the phosphate-deplete enclosure. It is tentatively suggested that lysogenic viruses were induced following phosphate addition to the phosphate-limited enclosures. Such observations further indicate that nutrient availability is responsible for the switch between lysogeny and lytic production.

# Dynamics, morphology and molecular characterisation of cyanophages infecting coastal strains of *Synechococcus* from the western Baltic Sea

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Cyanobacteria of the genus *Synechococcus* are important primary producers in the Sea. Viruses can be important to maintain diversity of microbial communities and for mineral cycling. In this study viruses of *Synechococcus* spp. from the Western Baltic Sea were investigated. Seventeen strains of phycoerythrin-containing *Synechococcus* were isolated from the Western Baltic Sea. The seasonal abundance of cyanophages infecting two of the strains was investigated in 1995 and 1996. The highest titers of *Synechococcus*-phages ( $2,5 \times 10^4 \text{ ml}^{-1}$ ) were found in September 1996, the lowest ( $<1 \text{ ml}^{-1}$ ) in spring and early summer 1996. There was a threshold of about  $10^4$  *Synechococcus* cells  $\text{ml}^{-1}$ , above which viral propagation was efficient. Several viruses infecting *Synechococcus* sp. were isolated in 1992/93 and 1995/96. The isolated phages belong to the group of *Siphoviridae*. At least one morphological type of phage belonging to the *Myoviridae* was additionally apparent in high concentration. Only one of the *Synechococcus*-strains was lysed by abundant cyanophages in 1992/93, whereas several *Synechococcus*-strains were lysed in 1995/96. The isolated viruses were described morphologically and by restriction length analysis.

These results indicate that viruses infecting *Synechococcus* spp. are an abundant and dynamic component in the Western Baltic Sea. The data emphasize further that they could be an important factor to maintain diversity of the *Synechococcus* population.

# Distributions and diversity of lysogenic cyanophages in marine cyanobacteria

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Recent studies suggest that cyanophages (viruses which infect cyanobacteria) play an important ecological role in marine environments. However, most known cyanophages are virulent types which kill the host shortly after infection. We found that considerably large numbers of marine cyanobacteria of section III isolated from different areas harbored lysogenized cyanophages; complete cell lysis was induced within several days after addition of mitomycin C (1  $\mu$ g/ml) or a brief UV-illumination in 16 strains out of 28. Occurrence of phage particles in cells prior to lysis induced by mitomycin C was confirmed in three *Phormidium* strains, *persicinum* (Provacoli strain), NIBB 1044 and NIBB1048, and *Trichodesmium* sp. NIBB1067. Cyanophages isolated from these strains were different in morphology, restriction fragment patterns of DNA and immunological characteristics. Some fraction of phages were in the lytic cycle even under the normal growth conditions; the fraction of phages in the lytic cycle varied depending on many factors including the culture conditions of the host cells. The possible role of lysogenic cyanophages on cyanobacterial populations in marine environment will be discussed.

# Observations on complete virus-induced lysis of a semi-natural community of filamentous cyanobacteria

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Experiments aimed at forcing change in bacterial community associated with cyanobacteria. Lake water from the shallow, highly eutrophic Lake Loosdrecht (The Netherlands) was brought into two Laboratory-Scale Enclosures (LSEs). The phytoplankton consisted almost exclusively of filamentous cyanobacteria, mainly '*Oscillatoria* c.f. *limnetica*'. After one week of adaptation (from winter to 'summer' conditions; the cyanobacterial biomass had about doubled), the LSEs received a high light dose during 4 days, while in one LSE also the mixing was interrupted. In both LSEs the cyanobacteria continued to grow well. After another week the high light during 4 days was repeated, and the mixing was interrupted in the other LSE. Again the cyanobacteria continued to grow, but at the end of this high light period lysis of the phytoplankton became apparent in one LSE, and within 24 h also occurred in the other. Within 48 h in both LSEs all species of filamentous cyanobacteria, including *Aphanizomenon flos-aquae* and *Oscillatoria agardhii*, had disappeared. Transmission electronmicroscopy of samples at the onset of lysis showed high numbers of at least two different cyanophages, occurring in suspension, attached to filaments and inside 'ghosts' of the cyanobacteria. In a new experiment the viral-induced collapse was reproduced. Ecological implications will be discussed.

## **Ecology of algal host-virus interactions.**

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Virus and virus like particles have been observed in about 50 different algal species but few algal-host-virus systems have been brought into culture and studied. The possible ecological significance of viruses has been inferred from the presence of algal cells containing virus like particles in natural communities, the presence of viruses in natural waters infecting specific algal populations and from studies of algal and virus population dynamics. Laboratory experiments with *Phaeocystis pouchetii* and the lytic virus PpV01 show that *P. pouchetii* is susceptible to virus infection in both exponential and stationary growth phase. Virus reproduction and cell lysis was not inhibited by nutrient (N or P) or light limitation, but inferior growth conditions caused a decrease in burst size of >50%. Viral infection allowed primary production in the cells to continue throughout most of the lytic cycle. During lysis of the algal cells the entire algal biomass was converted to DOC, which was efficiently utilized for bacterial biomass production.

## **Elevated production of dimethylsulfide resulting from viral infection of cultures of *Phaeocystis pouchetii*.**

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Emissions of dimethyl sulfide (DMS), the major volatile sulphur compound in seawater, account for a dominant fraction of the sulfur entering the atmosphere over the open oceans. DMS atmospheric oxidation products are the major source of sub-micron aerosol particles over remote marine areas, and influence global climate by scattering and absorbing radiation, and by affecting cloud albedo. The major precursor of DMS is dimethylsulfoniopropionate (DMSP), which is a compatible solute in many marine phytoplankton. Attention has focused recently on water column microbial processes that influence DMS production, with the aim of defining the key factors that control the quantity of DMS available for sea-air exchange. In this laboratory study we looked at the effects of viral lysis on dimethyl sulfide (DMS) and particulate and dissolved dimethylsulfoniopropionate (DMSPp and DMSPd) concentrations in batch cultures of *Phaeocystis pouchetii* infected with a strain specific viral isolate. After 20 hours DMS concentration had increased 4-fold over the levels observed in the control cultures and this increased to 8-fold 45 hours after viral particles were added. The results will be discussed in the context of current understanding of the biogeochemical cycle of DMS in seawater.

## Is Red Tide Disintegration Regulated by Viral Algicidity?

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*Heterosigma akashiwo* (Raphidophyceae) is one of the typical HAB (harmful algal bloom)-causing phytoplankton, which is distributed in coastal waters of subarctic and temperate areas of both northern and southern hemispheres. In 1993, it was found that the proportion of *H. akashiwo* cells containing VLPs (virus-like particles) specifically increased in the final stage of a red tide. This finding suggested that there could be some kind of relationship between the appearance of VLPs and the disintegration of a *H. akashiwo* red tide. We have succeeded in isolating 15 clones of a virus infecting and killing *H. akashiwo* (HaV: *Heterosigma akashiwo* virus) in 1996. Their morphological feature is highly comparable to that of the VLP observed in the natural red tide samples. Since the infectivity of HaV is highly specific to *H. akashiwo*, HaV has the potential to disintegrate *H. akashiwo* red tides. However, high diversity among both virus clones and host strains regarding viral infection (intra-species host specificity) was found. This result indicates that the interaction between viruses and hosts *in situ* appears more complicated than a simple host and pathogen relationship. HaV is quite unstable under simulated *in situ* environmental conditions (light and temperature). Thus, it is unclear how HaV survives, i.e. maintains its infectivity in the natural environment. These problems have to be solved in order to elucidate the role of HaV in the regulation of *H. akashiwo* red tides.

# Is diatom lysis in extensive mucilage of Adriatic Sea due to a viral infection ?

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The extensive mucilage appeared in the North Adriatic Sea in 1988, 1989, 1991 after a long period. These catastrophic events in the row enhanced studies on the origin of this phenomenon, which was described for the first time in the 1729. Different hypotheses have been proposed so far but not one can reproduce mucilage under laboratory conditions. The mucilage is chemically constituted by heterogeneous polysaccharides. This finding was related to a special algal exudation. It was recently demonstrated that the polysaccharide matrix was probably formed also from lysed cells of diatoms (Baldi et al., 1997). Intracellular polysaccharides such as carbon storage materials and polysaccharidic envelopes could contribute strongly to mucilage formation. Investigations were undertaken to demonstrate the presence of virus in the frozen and formaline stored mucilage in 1991 and in fresh sample of 1997.

The stored mucilage under buffered formaline was fixed and observed with a Zeiss EM 9A transmission electron microscope. Frozen mucilage was thawed and immediately stained with Yo-Pro-1 {4-[3-methyl-2,3-dihydro(benzo-1,3-oxazole)-2-methylmethylenedene]-1-(3'-trimethylammoniumpropyl)-quinoliniumdiiiodide} (Molecular Probes Inc.) as regards as Hennes and Suttle protocol (1995). The distribution fluorescence viral particles were than observed with scanning confocal laser microscopy (SCLM). Isolation of viral DNA and restriction enzymes analysis was also performed according to the method by Cottrell and Suttle (1991).

The TEM analyses of formaline stored mucilage showed the presence of virus-particles with appropriated geometric shape and size (~70 nm) in eukaryotic organisms producing intracellular holes. TEM images of virus particles were not conclusive since their shapes were not well preserved. The use of YO-PRO-1 molecular probe, to determine viral DNA particles was performed with SCLM microscopy. This technique showed the presence of many nucleic acid particles similar to virus in washed sample of frozen mucilage. Control analysis of sample of *Phaeocystis pouchetii* infected by PPV (kindly donated by M. Haldal) has been used as reference sample confirm that fluorescence particles could be virus. So a further technique was used on the basis of molecular biology protocol. The digestion of viral DNA with enzymes *BamHI* and *EcoRI* produced a restriction pattern if it was compared with standard lambda/*Hind III*. Spectrometric analysis of viral DNA at 260 nm and 280 nm showed a high impurity of our DNA preparation. The presence of polysaccharides make the DNA extraction more difficult. A high degradation of DNA molecules was observed more in the frozen samples than in the formaline sample. These results are encouraging for supporting the viral infection of diatoms.

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## **Modelling virus - phytoplankton interactions**

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Appropriately used, modelling can be a useful tool for unravelling phytoplankton - virus interactions. The applicability of modelling is assessed for key processes in viral ecology: physical transmission, retention of viability, host defences, behaviour within the host, and ecosystem scale effects. Viral transmission depends on simple physics and is highly suited to modelling. The continued viability of viruses during transmission depends on a variety of environmental factors; models of the effects of individual factors are presented. The potential host's defences that involve physical effects and ecological interactions are explored. The behaviour of viruses within their hosts depends upon biology, and so is complex; however, information is becoming available and preliminary modelling is practical. Finally, viruses can alter entire ecosystems by diverting fluxes of nutrients between pathways; budgets and simple steady-state models are used to estimate the viral role. In conclusion, many of the individual processes controlling the ecology of phytoplankton viruses can usefully be modelled (to varying degrees). Unfortunately, modelling the dynamic interaction of viruses with host populations is still only of limited practicality.

## **Distribution of virus infection in *Ectocarpus fasciculatus* on a rocky shore in Southern England.**

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*Ectocarpus fasciculatus* is a marine filamentous brown alga, and like many other members of the Ectocarpaceae, is known to be infected by a virus which is carried latently by vegetative cells and only expressed in the reproductive structures (sporangia). *E. fasciculatus*, in particular, has been selected for an ecological study into the distribution and transmission of virus infection, for the following reasons: (i) it is readily available on British shores, (ii) it forms almost exclusive epiphytic populations on the fronds of *Laminaria digitata*, (iii) details of its cell and molecular biology are well known, and (iv) the symptoms of infection are easily recognised in field populations. Peveril Point, Swanage, on the South Coast of England, has been chosen as the study site for: (i) it is a relatively small shore with well defined boundaries, (ii) it has a diversity of filamentous brown algae, and (iii) it has substantial epiphytic populations of *E. fasciculatus*. Sampling is currently being carried out at monthly intervals, and is randomised at three levels: within the *L. digitata* (basiphyte) population; on the selected basiphyte; and within the *E. fasciculatus* population. Preliminary results show that the levels of infection range between 1-5 % on differing parts of the shore. Work is now underway to monitor the spatial and temporal distribution of infection in this *E. fasciculatus* population, throughout the annual cycle.

## Several genes in *Chlorella* virus strain CVG-1 encode putative coatamers.

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We have isolated and characterized the major coat protein Vp49 and its corresponding gene from German *Chlorella* virus isolate CVG-1 (*Phycodnaviridae*). Three more open reading frames were identified and sequenced, which showed significant sequence similarity with Vp49. CVG-1 represents the European Pbi-subgroup of the *Phycodnaviridae* and neither infects nor attaches to *Chlorella* host strains of other subgroups. As this distinct host specificity may be linked to differences in capsid composition, the sequences were compared to data from virus isolates PBCV1 and CVK2, representing the NC64A-subgroup of the family. The major coat proteins have a high degree of homology and are similar in size, but differ in glycosylation. CVG-1 ORF8 corresponds well to a PBCV-1 gene similar in size and sequence, while ORF3 shows only weak homology to other virus genes. A fourth, incomplete CVG-1 ORF has the same aminoterminal as the major coat protein Vp49. The variability in certain regions of the major coat protein sequences could account for the viruses' host specificity. Moreover, they show that viruses of the different subgroups did evolve separately, probably parallel to their respective host algae.

## **Observations on viral infection in *Micromonas pusilla*.**

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An investigation on *Micromonas pusilla* virus has been started to assess the role of viral infection in the seasonal pattern of this species in the Gulf of Naples. The virus and its host have been quantified weekly at a coastal station around the spring bloom time over three consecutive years, using a serial dilution technique and epifluorescence microscopy, respectively. In some cases, we have observed that virus concentration increases once the host has attained a threshold concentration, whereas in other cases no relationships can be tracked.

A viral strain isolated from the gulf, MpV N1, was used to investigate virus-algal relationship. Recovery after massive lysis was often observed in infected cultures, which showed resistance to further infections with MpV N1. Resistance to MpV N1 was also observed in algal strains not previously infected in the lab. Southern Blot hybridization did not reveal the presence of viral genome in recovered *M. pusilla* DNA. The presence and relative importance of resistant strains in the natural environment should be assessed in order to understand the role of virus in the seasonal pattern of the species.