

**Marine Algal Viruses:
Review of Phycovirus and Cyanophage Biology and Ecological Significance**

Ian Hewson*

Department of Botany, University of Queensland, Brisbane, Australia

Report Submitted for partial fulfillment of BT490 Botany Honours IV for the award of PGB.Sc(Hons)

Word Count (Excluding References): 6,828

*Corresponding author

Mailing Address:

Marine Botany Group

Department of Botany

University of Queensland

QLD 4072

Australia

Tel: +61 7 3365 2529

Fax: +61 7 3365 7321

Email: i.hewson@botany.uq.edu.au

Abstract

Viruses specific to marine eukaryotic algae (phycoviruses) and cyanobacteria (cyanophages) are now recognised as ubiquitous in the world's oceans and specificity has been determined for many species of both micro- and macroalgae. Algae have evolved mechanical, chemical and genetic defences against infection, however viruses potentially regulate several metabolic processes in marine plants. Virus-induced physiological damage results in changes to the biomass, photosynthetic and nutrient uptake rates in both micro- and macroalgae. Use of lysis products resulting from viral infection is potentially an important nutrition of marine plants in oligotrophic systems. Viral infection may adversely effect the reproductive capability of both micro- and macroalgae, by causing lysis of mature cells and gametophytes, respectively. Toxin production by marine cyanobacteria may be dependant on lysogeny by temperate phages. Viruses potentially influence community composition in mixed-algal populations, and have been shown to be responsible for the termination of algal blooms. Marine phycoviruses are genetically diverse in terms of gene sequence and genome size, however their morphology is similar. The distribution of phycoviruses in marine environments strongly reflects that of hosts. The use of phycoviruses in tracking harmful algal blooms and the use of algal chemical defences in preventing or treating human disease represent promising areas of future research.

Keywords: Phycovirus, Cyanophage, Marine Virus, Microalgae, Macroalgae.

Table of Contents

Abstract & Keywords	2
1. Introduction	4
2. Summary of Known Phycoviruses	6
Prochlorophytes	6
Cyanobacteria	6
Phytoflagellates and Cocolithophores	7
Diatoms	7
Dinoflagellates	7
Rhodophyta (Red Algae)	8
Phaeophyta (Brown Algae)	8
Chlorophyta (Green Algae)	8
Symbiotic Algae	8
3. Effects on Metabolic Processes and Environmental Fluxes	12
Photosynthesis and Photosynthetic Apparatus	12
Nutrient Cycling	15
Aggregation/Formation of Algal Floccs	16
Growth and Reproduction	16
Toxin Production	17
4. Ecology of Algal Marine Viruses	19
Distribution of Phycoviruses and Cyanophages	19
Dynamics of Viruses and Virus-Like Particles in Algal Blooms	20
5. Diversity of Phycoviruses and Cyanophages	23
Genetic Diversity of Phycoviruses and Cyanophages	23
Effects of Virus Infection on Host Diversity	24
6. Role of Phycoviruses in Water Quality and Health Management	26
Anti-Viral Compounds Isolated from Algae	26
Use of Phycoviruses for Controlling Algal Blooms	26
Use of Phycoviruses in Water Quality Monitoring	26
Acknowledgements	27
References	29

1. Introduction

Viruses are ubiquitous in aquatic environments, with a typical surface water abundance of 10^{10} virus-like particles per litre (Fuhrman, 1999). Direct observation of marine viruses began in 1991, with the application of DAPI (4',6-diamidino-2-phenylindole) staining and epifluorescence microscopy (reviewed in Noble & Fuhrman, 1998) to seawater samples, however the study of phycoviruses and cyanophages has taken place well before direct observation of virus-like particles was possible (Safferman & Morris, 1963). It has been established that the majority of marine viruses infect bacteria (bacteriophages), however viruses infecting cyanobacteria (cyanophages) and eukaryotic algae (phycoviruses) have been shown to be abundant in the marine environment (reviewed in Fuhrman, 1999). Viruses of eukaryotic algae (primarily freshwater) have been reviewed extensively by VanEtten *et al.* (1991) and Reisser (1992), however to date there have been no specific reviews of marine algal viruses. This review summarises the current state of knowledge of marine phycoviruses and their effects on hosts. It is widely accepted that viruses are a significant factor in regulating the abundance of microalgae during normal conditions, and they have been included with light, nutrient availability, temperature, water motion and grazing (reviewed in Clayton & King (1995)) as factors which potentially limit algal growth and photosynthesis.

The identification of phycoviruses has been made possible with the techniques of plaque assay (first suggested for algae by Safferman & Morris, 1963), transmission electron microscopy, which provides morphological details of viruses (Bergh *et al.*, 1989; Bratbak *et al.*, 1990; Bratbak *et al.*, 1992; Paul *et al.*, 1993), flow cytometry (FCM) (Marie *et al.*, 1999) and direct observation with epifluorescence microscopy (Hara *et al.*, 1991; Noble & Fuhrman, 1998a), which may suggest potential hosts based on co-occurrence of distribution. High-molecular weight ultrafiltration technology has also allowed the study of algal virus pathogenicity by the addition of seawater concentrates to cultured and naturally occurring phytoplankton communities (Suttle *et al.*, 1990; Suttle *et al.*, 1991a; Suttle, 1992; Bratbak *et al.*, 1993; Peduzzi & Weinbauer, 1993a; Milligan & Cosper, 1994; Bratbak *et al.*, 1998; Nagasaki *et al.*, 1999). The suppressed biomass or photosynthetic rates of the culture or community indicates the presence of pathogenic viruses in the concentrate.

There are three types of infection cycle among phycoviruses (Figure 1). A lytic virus reproduces rapidly after infecting a cell, causing mortality of the host before it divides (Alberts *et al.*, 1994). This is common in both cyanobacteria and eukaryotic micro- and macroalgae. Lysogeny is an infection where the viral replication rate matches the dividing rate of the host (Prescott *et al.*, 1993). Viruses causing a lysogenic infection in cyanobacteria (temperate phages) are induced by environmental stimuli (Jiang & Paul, 1994). Viruses causing a lysogenic [latent] infection in eukaryotic algae may use chloroplast, mitochondrial or nuclear genetic systems (Alberts *et al.*, 1994). Lysogeny in eukaryotic algae is therefore more complex than in prokaryotic algae. A third type of infection, where the progeny virus release is sub-lethal (chronic) has not been studied in the marine environment (Fuhrman, 1999).

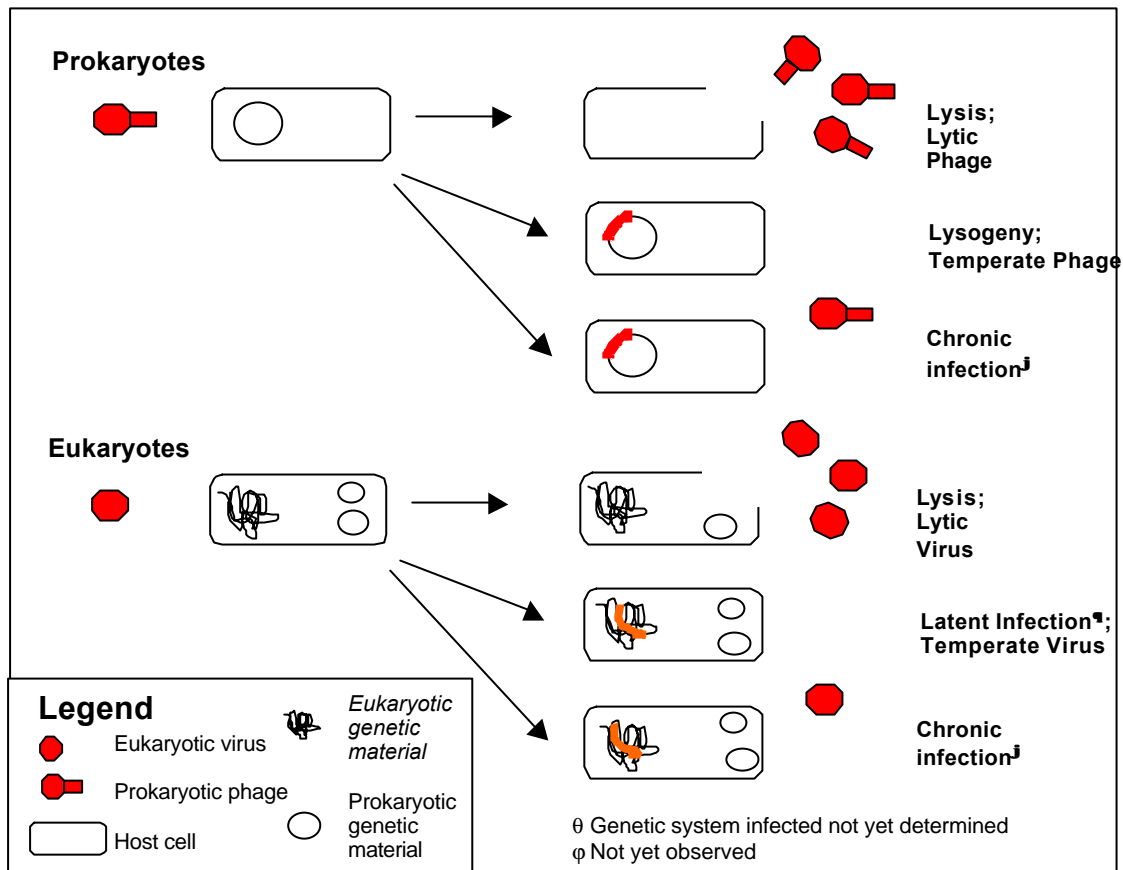


Figure 1 : Comparison of prokaryotic and eukaryotic infection cycles. Eukaryotic infection cycles are more complicated than in prokaryotes because there are three genetic systems present, whilst bacteria contain one. Lytic infection of chloroplast genome may have less effect on eukaryotic cell function than an infection of the nuclear genome. Lytic infection of prokaryotic genome normally results in lysis of host (adapted from Fuhrman & Suttle (1993)).

2. Summary of Known Phycoviruses and Cyanophages

Phycoviruses have been isolated from most groups of marine algae (Table 1). It is evident that phycoviruses of prokaryotes are primarily binal (tailed-contraction and non-contraction- with a polyhedral capsid) (Figure 2a), while those of higher taxonomic levels are icosohedral (polyhedral head lacking a tail) (Figure 2b). Phycoviruses of macroalgae show greater diversity, and include rod-shaped and *potyvirus*-shaped (both helical) morphology (Figures 2c). Exceptions to these generalisations are *Aureococcus anophagefferens* (Milligan & Cosper, 1994) and *Platymonas* sp. (Pearson & Norris, 1974a) which have binal phages.

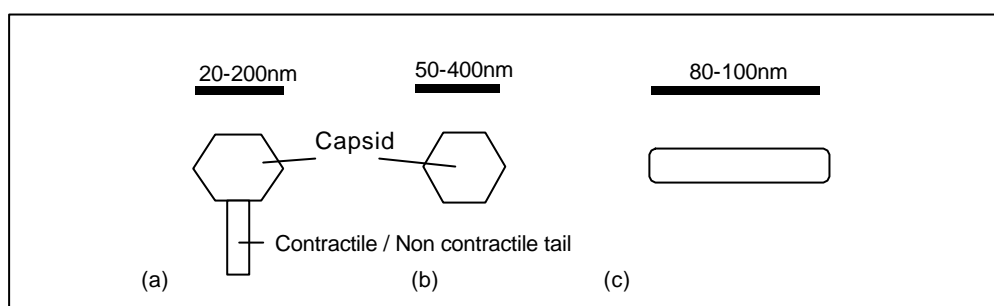


Figure 2: Morphology of different types of algal viruses
 (a) binal (prokaryote bacteriophage) (b) icosohedral (eukaryotic phycovirus) (c) helical (macroalgal phycovirus) (after Prescott, 1993).

Prochlorophytes

Prochlorophytes are oxygenic prokaryotes containing chlorophyll *a* and *b* (Prescott *et al.*, 1990) which are found primarily in oceanic deep chlorophyll maxima (Campbell *et al.*, 1994; Vaultot *et al.*, 1995; Hess *et al.*, 1996; Shimada *et al.*, 1996). There are presently no reports of phages of *Prochlorococcus*, however it is likely that they exist for several reasons. In pelagic environments, virus-like particles and *Prochlorococcus* have overlapping abundance maxima (Marie *et al.*, 1999). Additionally, *Prochlorococcus* have high genetic diversity spatially (Scanlan *et al.*, 1996; Urbach & Chisholm, 1998) which has been linked [in other prokaryotes] with high rates of transformation by marine viruses (Jiang & Paul, 1998). Study of bacteriophage activity on cultured *Prochlorococcus* to date has been limited by the sensitivity of this algae to changes in light and temperature and weak chlorophyll fluorescence (N. Mann, pers comm; Marie *et al.*, 1999)

Cyanobacteria

Marine cyanophages have been isolated from cultures of *Synechococcus* spp. (Suttle & Chan, 1993), *Phormidium persicinum* (Ohki & Fujita, 1996), *Phormidium ubcinatum* (Bisen *et al.*, 1986) and *Trichodesmium* spp. (Ohki, 1999). While those specific to unicellular cyanobacteria are lytic, those of filamentous cyanobacteria include temperate phages which can be induced by exposure to mytomycin C or Ultraviolet (UV) light (Ohki & Fujita, 1996).

Phytoplankton and Cocolithophores

Phycoviruses specific to phytoplankton and cocolithophores have been isolated and identified for *Emiliana huxleyii* (Bratbak *et al.*, 1993), *Aureococcus anophagefferens* (Milligan & Cosper, 1994), *Micromonas pusilla* (Suttle *et al.*, 1990), *Phaeocystis pouchetii* (Jacobsen *et al.*, 1996), *Rhodomonas* sp. (Suttle *et al.*, 1990), *Heterosigma akashiwo* (Nagasaki & Yamaguchi, 1996), *Hymenomonas carterae* (Pienaar, 1976), *Chrysochromulina* spp. (Suttle & Chan, 1995), *Cryptomonas* sp. (Pienaar, 1976), *Pyramimonas orientalis* (Pienaar, 1976) and *Platymonas* sp. (Pearson & Norris, 1974a). This group of organisms is extremely well studied as many species are red- or brown-tide causing organisms [hence have importance to economic losses or human health] (Clayton & King, 1995), or form predictable blooms (Bratbak *et al.*, 1993), facilitating their study.

Diatoms

To date there have been no reports of isolation and characterisation of diatom phycoviruses, although it has been demonstrated that cultures of an unknown centric and pennate diatom, and *Navicula* sp. had reduced biomass after addition of high molecular weight concentrates of seawater (Suttle *et al.*, 1990; Suttle *et al.*, 1991b). Additionally, Bratbak *et al.* (1990) observed concurrent maxima in abundance of *Skeletonema costatum* and virus like particles. The latter study is confounded by the presence of a large concentration of heterotrophic bacteria which may be the primary hosts. While it is likely that diatom-specific viruses exist as diatoms have a high abundance in many marine environments, it has been suggested that most diatoms are resistant to infection due to comparatively low contact rates with free virus-like particles (Murray & Jackson, 1992). Diatoms also exude mucilage, supporting the growth of bacteria, which intercept viruses (Murray, 1995).

Dinoflagellates

There are few reports of infection of dinoflagellates by viruses, and recent research on the red-tide dinoflagellate *Gymnodinium breve* suggests that they are substantially unaffected or that viruses present in cultures infect mutualistic bacteria (J. Paul, pers. comm.). Virus-like particles have been observed in the freshwater dinoflagellate *Gyrodinium resplendens* (Franca, 1976), however the presence of virus-particles in *Phalacrocoma favus* has recently been shown to be the result of phagocytation and ingestion (Hallegraeff & Lucas, 1988). It is plausible that the viruses observed in *G. resplendens* were vacuolated, as the viral matrix was surrounded by a plasma membrane. Lohuis & Miller (1998) demonstrated that *Amphidinium* sp. can be genetically transformed by plasmids (which are similar to viruses except they replicate autonomously), suggesting that virus infection of this species is possible. Dinoflagellates, like diatoms, have comparatively low contact rates with viruses, hence have potentially lower infection rates than smaller phytoplankton and bacteria (Murray & Jackson, 1992).

Rhodophyta (Red Algae)

There have recently been several reports of viruses specific to the marine red algae, including *Gracilaria epihippisor* (Apt & Gibor, 1991), *Audouinella saviana* (Pueschel, 1995) and *Porphyridium purpureum* (Chapman & Lang, 1973). Many species of red algae have been found to have no phycoviruses, which is possibly due to large concentrations of antiviral sulfated polysaccharides found in red algal cell walls (Clayton & King, 1995).

Phaeophyta (Brown Algae)

There are several isolated phycoviruses of brown algae, specific to *Ectocarpus siliculosus* (Easton *et al.*, 1997), *Ecklonia* spp. (Mueller & Stache, 1992), *Ecklonia radiata* (Mueller, 1990), *Feldmannia simplex* (Mueller & Stache, 1992), *Laminaria* sp. (Mueller & Stache, 1992), *Sorocarpus uvaeformis* (Oliveira & Bisalputra, 1978), *Hincksia hincksiae* (Wolf *et al.*, 1998), *Streblonia* sp. (Claire & West, 1977), *Chorda tomentosa* (Toth & Wilce, 1972) and *Pylaiella littoralis* (Markey, 1974). Brown algae cell walls contain sulfated polysaccharides (Clayton & King, 1995), however their concentration is generally less than in the Rhodophyta.

Chlorophyta (Green Algae)

Study of green algae is mostly limited to freshwater unicellular microalgae, for example *Chlorella* sp., *Chlorococcum* sp., and *Cylindrocapsa* sp. (reviewed in VanEtten *et al.*, 1991). Marine green algae have been shown to contain antiviral substances including sulphated polysaccharides, for example sulphated galactose in *Ulva lactuca* (Ivanova *et al.*, 1994).

Symbiotic Algae

O'Brien *et al.* (1984) suggests that there is an absence of lytic or temperate phages in symbiotic cyanobacteria and *Prochloron*. Despite a lack of direct evidence, it is possible that viruses of symbiotic algae exist.

Coral bleaching is a phenomenon where cnidarian corals expel their symbiotic dinoflagellates (*Symbiodinium* spp.), resulting in the loss of colour in animal tissues (Glynn, 1991). These events have been correlated to increases in sea-surface temperature (Hoegh Guldberg, 1999). Coral bleaching has also been linked to the production of adhesins by parasitic *Vibrio* AK-1 bacteria (Toren *et al.*, 1998), however the pathogenicity of this has been shown only for the coral *Oculina patagonica*. UV radiation has been shown to result in photosynthetic membrane damage in zooxanthellae inhabiting corals which lack UV-blocking mucus, which is attributable to nicking or damage to DNA (Lesser, 1996). Recently, genetic transformation of *S. microadriaticum* has been demonstrated (Lohuis & Miller, 1998) suggesting that virus infection of symbiotic dinoflagellates is possible. The high levels of solar radiation and therefore high incident UV light on coral reefs and the responses of zooxanthellae to these conditions (Lesser, 1996), suggests that viruses potentially forming latent infections in zooxanthellae may play a role in coral bleaching. If this is the case, temperature or salinity changes may be the environmental stimuli required for the switch

between lysogeny and lysis. However, corals such as *Heliopora coerulea* have been found to contain aglycons which may have an antiviral effect (Tanaka *et al.*, 1993).

Table 1: Summary of reported phycoviruses and cyanophages isolated from marine algae, arranged by taxonomic level. Evidence of infection; [A] Transmission electron microscopy, [B] Overlapping distribution of viruses/ algae [C] Infection of culture.

Division	Marine Species Infected	Evidence	Capsid Morphology	Capsid Diameter	Tail Morphology	Reference
Prochloron	None Known	-	-	-	-	[1]
Prochlorophyta; Prochlorophyceae	<i>Prochlorococcus</i> spp. ^λ	B	Unknown	Unknown	Unknown	[2]
Cyanophyta; Cyanophyceae	<i>Synechococcus</i> spp.	A,B	Polyhedral	50-65nm	Non-contractile tail, 230nm long	[3]
	<i>Phormidium perniscidium</i>	A	Polyhedral	40nm [∅]	Non-contractile tail, 300nm long	[4]
	<i>Trichodesmium</i> sp.	A	Polyhedral	50nm [∅]	Untailed	[5]
Cryptophyta; Prymnesiophyceae	<i>Emiliana huxleyii</i>	A,B	Polyhedral	180-200nm	Untailed	[6]
	<i>Aureococcus anophagefferens</i>	A,C	Polyhedral	50-70nm	Non-contractile tail, 80-100nm long	[7]
	<i>Phaeocystis pouchetii</i>	A,C	Polyhedral	130-170nm	Untailed	[8]
	<i>Chrysochromulina</i> spp.	A,C	Polyhedral	145-170nm	Untailed	[9]
Cryptophyceae	<i>Hymenomonas carterae</i>	A	Polyhedral	57-63nm	Untailed	[10]
	<i>Rhodomonas</i> sp. ^φ	C	Unknown	Unknown	Unknown	[11]
	<i>Cryptomonas</i> sp.	A	Polyhedral	100-240nm	Tailed and Untailed	[12]
Raphidophyceae	<i>Heterosigma akashiwo</i>	A,C	Polyhedral	200nm [∅]	Untailed	[13]
Bacillariophyceae	<i>Skeletonema costatum</i> ^λ	B	Unknown	Unknown	Unknown	[14]
	Unknown Pennate ^φ	C	Unknown	Unknown	Unknown	[15]
	Unknown Centric ^φ	C	Unknown	Unknown	Unknown	[16]
	<i>Navicula</i> sp. ^φ	C	Unknown	Unknown	Unknown	[17]
Dinophyceae	<i>Gyrodinium resplendens</i> ^φ	A	Polyhedral	35nm [∅]	Untailed	[18]
Rhodophyta	<i>Gracilaria epihippisor</i>	A	Polyhedral	80nm [∅]	Untailed	[19]
	<i>Audouinella saviana</i>	A	Rod-Shaped	1000nm [∅]	-	[20]
	<i>Porphyridium purpureum</i>	A	Polyhedral	40nm [∅]	Untailed	[21]
Phaeophyta	<i>Ectocarpus siliculosus</i>	A	Polyhedral	130nm [∅]	Untailed	[22]
	<i>Ectocarpus fasciculatus</i>	A	Polyhedral	131-135nm	Untailed	[23]
	<i>Ecklonia radiata</i>	A	Rod-shaped <i>Potyvirus</i> -like	280nm [∅] 700-900nm	-	[24]
	<i>Feldmannia simplex</i>	A	Polyhedral	112-130nm	Untailed	[25]
	<i>Laminaria</i> sp.	A	Polyhedral	170nm [∅]	Untailed	[26]
	<i>Sorocarpus uvaeformis</i>	A	Polyhedral	166-173nm	Untailed	[27]
	<i>Hincksia hincksiae</i>	A	Polyhedral	135-150nm	Untailed	[28]
	<i>Chorda tomentosa</i>	A	Polyhedral	170nm [∅]	Untailed	[29]
	<i>Pylaiella littoralis</i>	A	Polyhedral	130-170nm	Untailed	[30]
	<i>Streblonema</i> sp.	A	Polyhedral	135-150nm	Untailed	[31]
	Chlorophyta; Prasinophyceae	<i>Micromonas pusilla</i>	A,C	Polyhedral	104-118nm	Untailed
<i>Platymonas</i> sp.		A	Polyhedral	55nm [∅]	Untailed	[33]
<i>Pyramimonas orientalis</i>		A	Polyhedral	60-200nm	Tailed	[34]

^λ Phycophages highly likely or under current investigation; ^φ Phycophage infection not demonstrated or infection response may be due to confounding factors; [∅] Average diameter (range not available).

References: [1] (O'Brien *et al.*, 1984) [2] (Mann, 1999) [3] (Suttle & Chan, 1993) [4] (Ohki & Fujita, 1996) [5] (Ohki, 1999) [6] (Bratbak *et al.*, 1993) [7] (Milligan & Cosper, 1994) [8] (Jacobsen *et al.*, 1996) [9] (Suttle & Chan, 1995) [10] (Pienaar, 1976) [11] (Suttle *et al.*, 1990) [12] (Pienaar, 1976) [13] (Nagasaki & Yamaguchi, 1996) [14] (Bratbak *et al.*, 1990) [15] (Suttle *et al.*, 1990) [16] (Suttle *et al.*, 1990) [17] (Suttle *et al.*, 1991) [18] (Franca, 1976) [19] (Apt & Gibor, 1991) [20] (Pueschel, 1995) [21] (Chapman & Lang, 1973) [22] (Mueller *et al.*, 1990) [23] (Mueller & Stache, 1992) [24] (Easton *et al.*, 1997) [25] (Mueller & Stache, 1992) [26] (Oliveira & Bisalputra, 1978) [27] (Wolf *et al.*, 1998) [28] (Claire & West, 1977) [29] (Toth & Wilce, 1972) [30] (Markey, 1974) [31] (Claire & West, 1977) [32] (Cottrell & Suttle, 1991) [33] (Pearson & Norris, 1974b) [34] (Moestrup & Thomsen, 1974)

3. Effects on Metabolic Processes and Environmental Fluxes

Viral infection of marine plant cells results in damage or disintegration of tissues, which is analogous to damage caused by viruses in higher plants. Viral infection in terrestrial plants has been shown to result in: nucleolus deformation; abnormal membrane systems on mitochondria; small vesicles bound to cristae and within mitochondrial space; damage or irregular arrangement of chloroplasts; cell wall thickening and the formation of virus crystalline structures in the cytoplasm (Matthews, 1981). While these abnormalities have not been directly observed in marine algae, similar physiological and morphological damage can be inferred by effects on metabolic processes, and from symptoms observed in macroalgae.

Photosynthesis and Photosynthetic Apparatus

Viruses infecting algal cells may detrimentally affect the photosynthetic apparatus of their host. This has been inferred from studies of biomass reduction, however it has also been documented that cyanobacterial photosynthetic membranes become envaginated when infected, resulting in thylakoid deformation and destruction (Martin & Benson, 1988). It has been demonstrated that virus reproduction is not dependant on phosphorylation in cyanobacteria (Sherman & Haselkorn, 1971), indicating that virus replication can occur when photosynthetic membranes are destroyed.

The majority of research on the effects of viral infection on photosynthetic biomass (chlorophyll *a*) employ techniques of ultrafiltration to concentrate seawater which is then added to the host organism, and the response monitored by changes to *in vitro* or *in vivo* fluorescence. Concentrates have primarily been added to axenic culture (Suttle *et al.*, 1990; Peduzzi & Weinbauer, 1993a; Milligan & Coper, 1994; Nagasaki *et al.*, 1999). This technique has been used to prove pathogenicity of viruses through iterated treatment of axenic culture with the lysate of previous assays (ie inoculum transfer).

The effects of viral infection on photosynthesis of freshwater algae is reviewed by Balachandran *et al.* (1997), and it can be assumed that these effects also occur in marine algae. Virus-induced damage to Photosystem II (through changes in protein translation sequence) causes breakdown of the electron transport chain to Photosystem I, resulting in the production of toxic oxygen species (O^{\cdot}). These oxygen species cause damage to photosynthetic membranes unless eliminated by photochemical (q_p) or non-photochemical quenching (NPQ; such as by emitting heat or increasing the pool of the xanthophyll pigment zeaxanthin (Demmig Adams & Adams, 1992)). Research of the freshwater chlorophyte *Chlorella* sp., showed that viral infection results in an increase in NPQ and consequent reduction in both the variable fluorescence (F_v ; which is defined as the maximum excitable fluorescence minus initial fluorescence or $F_m - F_o$) divided by the maximum excitable fluorescence (F_v/F_m ; a measurement of physiological stress), and in initial fluorescence (F_o ; which is commonly used as a measure of chlorophyll *a*) (Rohozinski *et al.*, 1995; Seaton *et al.*, 1996) (Figures 3a and 3b). However when photoinhibition (due to strain on photoprotection systems) occurs, an increase in F_o and decrease in F_v/F_m is observed due to saturation of NPQ (Figure 3c)

(Balachandran *et al.*, 1997). Several studies (Suttle *et al.*, 1990; Milligan & Coper, 1994) which use F_0 as a measure of chlorophyll *a*, have not taken this into consideration. The increases in F_0 observed after initial decline may be a result of NPQ saturation, despite the argument that increases in F_0 on concentrate addition is due to the growth of resistant or uninfected algae (Suttle, 1992).

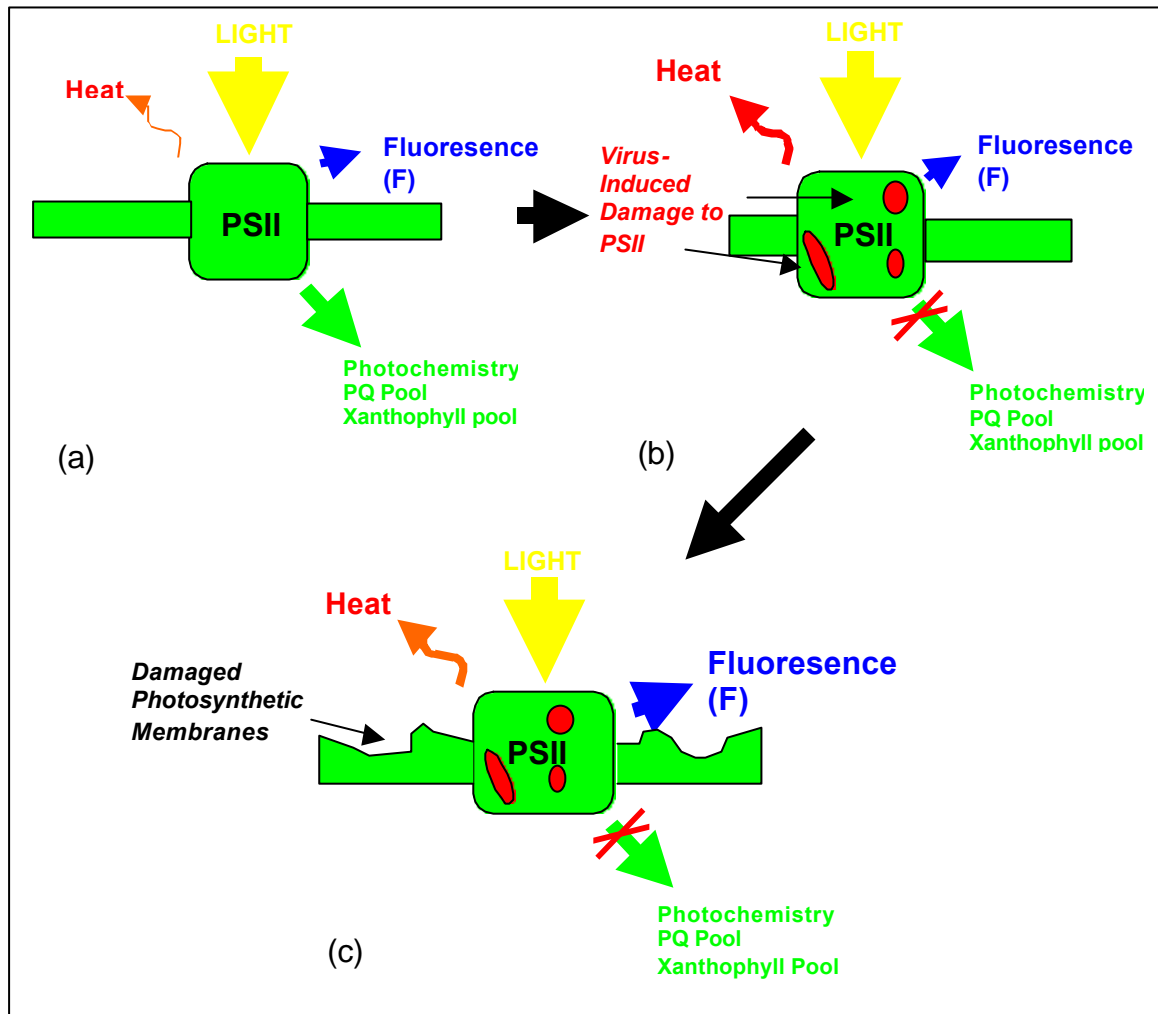


Figure 3: Effect of viral infection on photosynthetic apparatus (a) Photosystem II functioning in uninfected chloroplast (b) Photosystem II functioning in infected chloroplast before photoinhibition occurs (c) Photosystem II functioning in infected chloroplast after photoinhibition occurs. Direction of large black arrows indicated progression of infection. Adapted from Purves *et al.* (1995) with information from Balachandran *et al.* (1997).

In microalgal cultures, enrichment of the ambient virus population has been observed to reduce F_0 (Suttle *et al.*, 1990) (Table 2). Viral concentrate addition to natural communities and cultures of microalgae suggest that viruses exert substantial influence on primary production (Suttle, 1992). Changes observed in relative carbon fixation rate between uninfected phytoplankton communities and samples to which seawater concentrates had been added show that viruses reduced photosynthesis on average by 55% (Suttle, 1992) (Table 3). Similarly seawater concentrate additions to cultured microalgae reduced carbon fixation rates by up to 78% (Suttle *et al.*, 1990;

Suttle *et al.*, 1991a). One noted drawback of the ultrafiltration- concentration technique of seawater is that other bioactive agents (such as humic and fulvic acids) in seawater which may inhibit photosynthesis are also speculated to be concentrated. However, autoclaved ultrafiltrate concentrates have been added as control samples with little effect on either photosynthesis or *in vitro* fluorescence (Milligan & Cosper, 1994).

Table 2: Summary of reported decreases in biomass with viral concentrate addition. Methods used [A] *In vitro*F₀ [B] Cell Enumeration [C] Chlorophyll a (acetone extraction). Response times for increases in brackets.

Alga	Decrease in Biomass (%)	Increase in Biomass (%)	Time of response (hrs)	Method Used	Reference
Infections of Culture					
<i>Heterosigma</i>	-99.9 ^φ	-	48	B	[8]
<i>Akashiwo</i>					
<i>Navicula</i> sp.	-99.97	-	144	A	[3]
<i>Micromonas pusilla</i>	-95	-	175	A,B	[4]
	-99.09		44	A,B	[5]
Pennate diatom	-94	-	175	A	[2]
Centric diatom	-80	-	135	A	[1]
<i>Chroococcoid</i> cyanobacteria	-64	-	95	A	[9]
<i>Rhodomonas</i> sp.	-55	-	190	A	[6]
<i>Aureococcus anophagefferens</i>	-33	-	288	A	[7]
Median	-94	-	144	-	-
Infections of Natural Communities					
Aurisina, (Italy)	-90	60	125 (125)	C (C)	[13]
Port Aransas (USA)	-39	627	23 (145)	A (A)	[10]
Aurisina (Italy)	-	28	210	C	[11]
Northern Adriatic sea (Italy)	-	67	225	C	[12]
Median	-65	347	168 (135)	-	-

^φControl treatment not sampled, change inferred from uninfected original concentration .

References: [1] (Suttle *et al.*, 1990) [2](Suttle *et al.*, 1990) [3](Suttle *et al.*, 1991) [4](Suttle *et al.*, 1990) [5](Cottrell & Suttle, 1991) [6](Suttle *et al.*, 1990) [7](Milligan & Cosper, 1994) [8](Nagasaki *et al.*, 1991) [9](Suttle *et al.*, 1990) [10](Suttle, 1992) [11](Weinbauer & Peduzzi, 1995) [12](Peduzzi & Weinbauer, 1993) [13](Peduzzi & Weinbauer, 1993)

Table 3: Summary of reported decreases in photosynthesis with viral concentrate addition. Photosynthetic rates for all studies are determined by ¹⁴C-bicarbonate uptake rates.

Alga	Photosynthetic reduction (%)	Time of response (hrs)	Reference
Infections in Culture			
<i>Synechococcus</i> sp. Strain BBC-1	-100	26	[1]
Infections of Natural Communities			
Port Aransas (USA)	-78	4	[2]
Port Aransas (USA)	-10 to -60	8	[3]
Median	-78	8	-

References: [1](Suttle & Chan, 1993) [2](Suttle *et al.*, 1990) [3](Suttle, 1992)

The only technique used to date to determine the effects of virus infection on productivity in natural communities or cultures has been the ^{14}C -tracer method. Recently developed methods, including Pulse Amplitude Modulated (PAM) fluorometry (White & Critchley, 1999) and oxygen micro-sensors (DeBeer, in press) are potentially useful tools for phycovirus research.

Nutrient Cycling

The viral lysis of host cells, subsequent nutrient release and bacterial uptake of lysis products is potentially an important nutrient cycle in oligotrophic, pelagic environments (reviewed in Fuhrman, 1999). Viral lysis accounts for a significant percentage of daily mortality of *Synechococcus* spp. (Suttle & Chan, 1994), and lysis of eukaryotic algae (*Emiliana huxleyi*) can account for up to 28% of total daily mortality (Bratbak *et al.*, 1993), suggesting viruses play a significant role in nutrient release from microalgae.

Biomass, photosynthesis and nutrient uptake rates of marine plants are limited by bioavailable nitrogen, phosphorus and carbon (Clayton & King, 1995). The lysis of prokaryotic cells results in the release of amino acids (which contain mainly nitrogen and phosphorus) (Fuhrman, 1999). Viral-mediated lysis products of eukaryotic algae have been shown to contain large amounts of bioavailable nutrients (Gobler *et al.*, 1997). Uptake of lysis products may account for the stimulation and proliferation of more resistant species of algae (Figure 3).

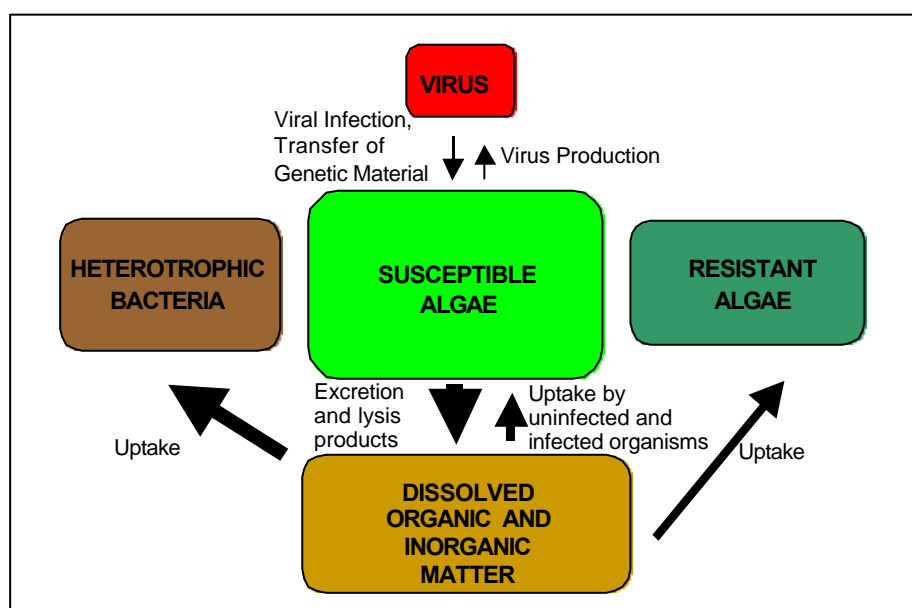


Figure 4: Conceptualised nutrient uptake/release model for prokaryotic and eukaryotic microalgae. Size of arrows is proportional to hypothesised rate of transfer of products. Direction of arrows is from source to sink. Adapted from information in Fuhrman (1999).

Prokaryotes

It is reasonable to expect that lytic cyanophage infection does not reduce the hosts ability to take up nutrients as viral replication is dependant on the [partial] functioning of the prokaryotic host cell. Low phosphate availability has been shown to result in lysogeny by *Synechococcus* sp. viruses,

however when phosphate enrichment occurs, prophages are induced which results in host death (Wilson *et al.*, 1996; Wilson *et al.*, 1998). This suggests that uptake of nutrients by prokaryotic host cells is required for viral replication.

Eukaryotes

Viral lysis of eukaryotic algal cells results in the release of large amounts of bioavailable nutrients (Gobler *et al.*, 1997). Prokaryotic organisms, such as heterotrophic bacteria are observed to succeed the collapse of eukaryotic algal blooms which has been attributed to their high uptake rates of lysis products (Bratbak *et al.*, 1998). After a spring bloom of diatoms (primarily *Sketetonema costatum*), heterotrophic bacteria were observed to live in the remains of the dead phytoplankton (Bratbak *et al.*, 1990). A similar phenomenon was observed during a spring bloom of phytoplankton in a coastal microcosm (Guixa-Boixereu *et al.*, 1999). The role of lysis product uptake by resistant phytoplankters in mixed communities to which seawater concentrates were added has been hypothesized in previous studies (Suttle, 1992), [however in this study the observed F_o increase may also be the result of NPQ and photoinhibition (Balachandran *et al.* 1997)].

Emiliana huxleyii viruses increased in abundance when phosphate (but not nitrogen oxides or urea) concentrations increased in a mesocosm experiment (Bratbak *et al.*, 1993). This suggests that phycoviruses of *E. huxleyii* form a latent infection within the host until nutrient enrichment occurs (similar to the phenomenon observed in *Synechococcus* sp. (Wilson *et al.*, 1996; Wilson *et al.*, 1998)), hence viral production may be dependant on nutrient uptake by the algal host. The reports of phosphorus (in the form of orthophosphate) stimulation of virus production (Wilson *et al.*, 1996) may be due to the relatively large phosphorus to nitrogen ratio of nucleic acids in viruses (Fuhrman, 1999).

Aggregation / Formation of Algal Flocs

The formation of algal flocs or aggregates is an important process in pelagic ecology which results in increased nutrient regeneration and primary production in aggregates (Gotschalk & Alldredge, 1989). Aggregates occur when phytoplankton are 'glued' together by amorphous mucus and is most common when there is a significant proportion of chain-forming plankton species present (Gotschalk & Alldredge, 1989). Increasing viral abundance in aquaria has been shown to increase the time for aggregate formation (presumably due to delayed phytoplankton growth) and also increases the total number of aggregates formed after approximately 200 hours (Pezuzzi & Weinbauer, 1993a). The authors argued that this was due to the increased abundance of uninfected algae, aggregating on the lysis products of infected algae.

Growth and Reproduction

Marine microalgae reproduce asexually by binary fission (in prochlorophytes and cyanobacteria) and gene transfer occurs by transformation or transduction (Prescott *et al.*, 1993), or by a

combination of meiosis (resulting in gametes) and mitosis (cell division; asexual) in eukaryotes (Clayton & King, 1995). Infection of prokaryotes by viruses results in either a total loss of reproductive ability (in the case of lytic infection) or in no loss until induction by environmental stimuli (in the case of lysogenic infection) (Jiang & Paul, 1996; Wilson *et al.*, 1996; Wilson *et al.*, 1998).

Little is known of the effects of viruses on eukaryotic algal reproduction. Viruses have been argued to have difficulty infecting diatoms due to organic extrusion through areoli (supporting bacterial growth which intercepts viruses)(Murray, 1995). The only life cycle stage at which diatoms lack a silicious exoskeleton is during gametogenesis, hence it is possible that phycoviruses have adapted to target this stage. This may also apply to thecate dinoflagellates which rarely undergo meiosis (Minguez *et al.*, 1994). Dinoflagellate genetic material is difficult to modify by virus infection as it is permanently condensed (Spector, 1984) and thymidine is replaced by hydroxymethyl-uracil (Steele & Rae, 1980). Dinoflagellate chloroplast DNA also comprises multiple DNA circles (Zhang *et al.*, 1999) which is more difficult to infect than a single genome.

There has been more research on the effects of viral infection on the reproduction of macroalgae than in microalgae. Macroalgae reproduce by a process of mitosis (asexual budding) and meiosis (gametogenesis) (Clayton & King, 1995). The brown algae *Ectocarpus siliculosus* has viruses specific to both adult plants (Kuhlenkamp & Mueller, 1994) and gametophytes which may result in reduced protoplast formation in the latter (Mueller *et al.*, 1990). This suggests that viral infection detrimentally affects reproductive potential in *E. siliculosus*.

Toxin Production

The production of toxins by cyanobacteria is well documented (reviewed in Martin & Benson (1988)). Production of toxins by other bacteria, such as enterotoxins in *Escherichia coli* (Marcello *et al.*, 1994) and cholera toxin in *Vibrio cholerae* (Waldor & Mekalanos, 1996) has been shown to be the result of temperate bacteriophage infection. The large number of cyanophages isolated (Martin & Benson, 1988) and the large occurrence of lysogeny in filamentous cyanobacteria (Ohki & Fujita, 1996; Ohki, 1999) suggests it is possible that cyanotoxins are produced as a result of lysogeny. Vance (1977) found that lysis and progeny virus release in toxic strains of *Microcystis aeruginosa* could be induced by Mytomycin C, but no progeny viruses were released from non-toxic strains. Lysogenic infection may explain intraspecies differences in cyanobacterial toxicity.

Resistance to Infection

Resistance to viral infection is well studied in higher terrestrial plants, however is virtually unstudied in the marine environment. The relative susceptibility of different algal groups (both known and hypothetical) is given in Figure 5.

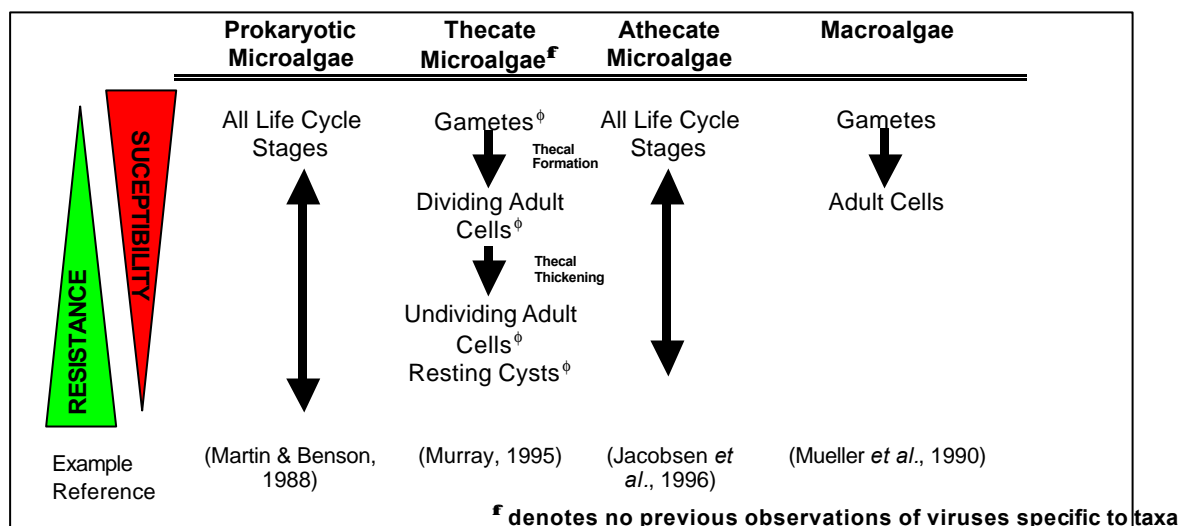


Figure 5: Known and hypothetical resistance of algal groups to infection (resistance based on presence of antiviral defence). Prokaryotic microalgae (cyanobacteria) and athecate microalgae are susceptible throughout life cycle, whilst thecate microalgae and macroalgae have varying susceptibility depending on life cycle stage.

Mechanical Resistance

Mechanical defences against viral infection have primarily been modelled in diatoms. It has been demonstrated that extrusion of mucous through silica frustules attracts bacteria which may intercept viruses (Murray, 1995). However, the phenomena of extrusion is not observed in other algal divisions. Other possible mechanical defenses against virus infection include thick, impermeable cell walls (eg silica frustules in diatoms, particularly as resting spores) or thecal plates (eg calcium carbonate plates in coccolithophores).

Chemical Resistance

Chemical defences against viral infection are poorly studied in microalgae, although it is conceivable that cyano- and dinotoxins have anti-viral effects. Anti-viral compounds, such as sulphated polysaccharides (Mouhim & Hours, 1995) have been isolated in macroalgae, primarily in red algae (where carageenans are in greatest concentration of all macroalgae (Clayton & King, 1995)) but in smaller concentrations in brown and green algae (Ivanova *et al.*, 1994 ; Clayton & King, 1995).

Genetic Resistance

Little is known of genetic resistance against viral infection in either macroalgae and microalgae. However it has been demonstrated that some strains of *Synechococcus* sp. are resistant to viral infection by some cyanophage clones, while susceptible to other clones (Suttle & Chan, 1993). Genetic resistance to infection by *Synechococcus* spp. has been speculated to allow coexistence of a large cyanophage abundance and their hosts (Waterbury & Valois, 1993). Genetic resistance to viral infection is a potentially promising area of future marine virus research.

4. Ecology of Algal Marine Viruses

Distribution of Phycoviruses and Cyanophages

There has been limited study of the distribution of phycoviruses (as distinct from bacterio- and cyanophages) in the marine environment. The majority of phycovirus research has used overlapping abundances (co-occurrence) of virus-like particles and microalgae (Bratbak *et al.*, 1990; Marie *et al.*, 1999), or isolation of phytoplankton from natural communities and then infection with seawater concentrates (Suttle *et al.*, 1990; Suttle *et al.*, 1991a; Suttle, 1992; Milligan & Cosper, 1994; Weinbauer & Peduzzi, 1995; Nagasaki *et al.*, 1999) to determine ambient distribution.

Pelagic Algal Virus Distribution

In samples collected from the Mediterranean Sea, virus like particles had maximum abundance of approximately 6×10^6 cells mL^{-1} at a depth of 100m coincident with the maximum *Synechococcus* sp. concentration (1×10^4 cells mL^{-1}) and *Prochlorococcus* sp. abundance (1×10^5 cells mL^{-1}) (Marie *et al.*, 1999). Heterotrophic bacteria do not change in abundance with depth. While it is likely that the majority of viruses in this study are bacteriophages (due to the high abundance of bacteria compared with other potential hosts), the slightly greater abundance of virus-like particles at maxima of cyanobacterial abundance suggests that substantial numbers of cyanophages are present at 100m. By contrast, in the photic zone (0-50m), phytoplankton concentration was greatest (6×10^3 cells mL^{-1}) while viral abundance was lowest (1.5×10^6 cells mL^{-1}). This suggests that algal viruses are in lowest abundance within this zone, as bacteria are the most probable hosts. A similar pattern was observed by Cochlan *et al.* (1993), where chlorophyll *a* abundance was not correlated to virus-like particle abundance in the photic zone of neritic waters off Texas (USA) and in estuarine waters in the Gulf of Bothnia (Sweden).

Other descriptions of phycovirus distribution focus on a single alga. The natural abundance of a virus infecting the prymnesiophyte *Chrysochromulina* sp. was studied by Suttle & Chan (1995). They found this virus could be detected at three stations in the Gulf of Mexico, however not during all seasons sampled. Phycovirus distribution in vertical profile reflected that of the host.

Similarly, a virus lysing *Synechococcus* spp. was found at three stations in the Gulf of Mexico, however their infectivity varied with the strain of *Synechococcus* assayed (Suttle & Chan, 1993). A strong correlation was found between cyanophages and host abundance with depth (Suttle & Chan, 1994). Seasonally, the abundance of cyanophages (estimated by most-probable number calculations) was correlated with the abundance of hosts.

Neritic Algal Virus Distribution

A survey of the Chesapeake Bay (USA) reported viruses with genomes larger than 200kbp (argued to be mostly phycoviruses) had an erratic distribution along a transect from North-South that could not be attributed to algal abundances (Wommack *et al.*, 1999b). However seasonally,

viruses in this category had greatest abundance in the spring-summer period, which corresponds to an overall increase in algal biomass (Clayton & King, 1995).

Algal Virus Distribution in Arctic Environments

Recently research has been focussed on viral existence in extreme environments, including the arctic and southern oceans (Simon *et al.*, 1996; Kepner *et al.*, 1998), due to their regional importance to global productivity (Clayton & King, 1995). The abundance of virus-like particles in freshwater antarctic lakes has demonstrated that production by microalgae and bacteria is potentially lost through viral infection in extreme environments (Kepner *et al.*, 1998). Similarly, Maranger *et al.* (1994) found that marine viruses exist both on and within arctic sea ice, their distribution corresponding primarily to that of sea ice algae (phytoplankton living within sea ice).

Benthic Algal Virus Distribution

Benthic microalgae (algae living on- or within sediments, also referred to as microphytobenthos) are now recognised as abundant and highly productive in most coastal environments (reviewed in Hartig *et al.* (1998) and Heil *et al.*, submitted). There is evidence that benthic phycoviruses exist. Along a transect from mangroves to a coral reef crest in Florida Bay (USA), viral abundance decreases initially before increasing toward the reef crest (Paul *et al.*, 1993). Study of a similar transect from the Fitzroy River to Heron Island (Great Barrier Reef) found that benthic microalgae follows the same trend (Heil *et al.*, 1999 submitted). There is extremely high species diversity in benthic microalgae (MacIntyre *et al.*, 1996), which may be due to genetic transformation by viruses (Jiang & Paul, 1998).

Dynamics of Viruses and Virus-Like Particles in Algal Blooms

Phytoplankton blooms terminate when available resources (eg nutrients and photosynthetically active radiation) limit algal growth, or when grazing exceeds the reproductive rate of algae (Clayton & King, 1995). While the crash of many blooms can be attributed to these limitations, there is mounting evidence that viruses may play a significant role in terminating blooms of microalgae and macroalgae.

An analogy to viral infection and inhibition of growth may be drawn to both nutrient availability and grazing. Nutrient limited algae are unable to divide, hence growth is delayed. Since lytic viral infection results in an inability to divide, the limiting factor is viral infection. However, viral infection is also comparable to grazing as the host cell is destroyed (as opposed to a delay in growth) and lysis products are recycled back into the environment.

Mixed Diatom Bloom

Study of a spring bloom of the chain-forming diatom *Skeletonema costatum*, and of a mixed phytoplankton bloom in a mesotrophic microcosm, showed that the abundance of virus-like particles increased after the bloom collapse (Bratbak *et al.*, 1990 and Guixa-Boixereu *et al.*, 1999

respectively). There are three possible explanations for this observation: Lytic viruses of the phytoplankton are responsible for the bloom termination; Temperate phages in the associated bacteria become lytic on the bloom onset; Or latent infections in the microalgae are induced by the bloom conditions. It is also possible that the increase in virus-like particles corresponds to the increase in heterotrophic bacteria which consume lysis products of the phytoplankton. Transmission electron microscopy images showed that the majority of virus-like particles in this study were untailed, suggesting they are eukaryotic algal viruses. The first study pre-dated the study of Suttle *et al.* (1990) which found that primary production and biomass of phytoplankton was inhibited by concentrates from seawater. Exponentially-growing cultures of several phytoplankton species (including centric and pennate diatoms, along with two phytoflagellate and one cyanobacterial species) showed similar decreases in biomass with addition of seawater concentrates to those observed in the *Skeletonema costatum* bloom. Similar results have been reported in axenic cultures of *Aureococcus anophagefferens* (Milligan & Cosper, 1994) and *Heterosigma akashiwo* (Nagasaki *et al.*, 1999) both species that cause economically and ecologically significant blooms.

Emiliana huxleyii Bloom

Emiliana huxleyii blooms are not limited by available nutrients (Bratbak *et al.*, 1993), therefore termination of blooms must occur due to other factors. Between 25 and 100% of total *E. huxleyii* mortality on bloom collapse can be attributed to phycovirus infection, suggesting this is the primary limiting factor on the algal bloom (Bratbak *et al.*, 1993).

'Red' and 'Brown Tides'

The presence of large numbers of phycoerythrin-containing flagellates has been termed 'red tides', due to the discolouration of waters. Red tide organisms include dinoflagellates (eg *Pfisteria piscidia* (Burkholder *et al.*, 1992)), and various phytoflagellate taxa (eg *Heterosigma akashiwo* (Nagasaki & Yamaguchi, 1996)). Brown tides occur when waters are discoloured by the presence of coccolithophorids (eg *Aureococcus anophagefferens* (Milligan & Cosper, 1994)). The dynamics of virus like particles during red and brown tides has not been previously studied, however viruses capable of lysing red and brown tide-causing flagellates have been isolated (Milligan & Cosper, 1994; Nagasaki & Yamaguchi, 1996).

Trichodesmium Blooms

Blooms of the tropical cyanobacteria *Trichodesmium* sp. terminate suddenly (within a few days) which has been shown to result in nutrient release which is taken up by successional dinoflagellate blooms (reviewed in Capone *et al.* (1998)). *Trichodesmium* sp. bloom collapse has been attributed to nutrient limitation or copepod grazing, however Ohki (1999) isolated a temperate phage of *Trichodesmium* sp. which became lytic when subjected to mutagen (mytomycin C) addition. She argues that this cyanophage may be responsible for the collapse of *Trichodesmium* spp. blooms and release of nutrients, which is consistent with the model (Figure 3) proposed in this review. This

is also consistent with a resistant species (in this case dinoflagellates) succeeding the collapse of a susceptible species (*Trichodesmium* sp.).

Macroalgal blooms

The dynamics of viruses in blooms of macroalgae has not been studied to the same extent as microalgae, however it is of potential interest as several macroalgal blooms are observed to terminate suddenly. Blooms of the macroscopic cyanobacterium *Lyngbya majuscula* in Moreton Bay, Australia, terminate in short periods, or when subjected to environmental instability (Dennison & Abal, 1999). *Cladophora* sp. has also been shown to lyse quickly in at least two geographically remote locations (Moriches Bay, New York and Peel-Harvey estuary, Australia) (Valiela *et al.*, 1997).

The death of large areas of kelp (*Ecklonia radiata*) in coastal New Zealand has been associated with the presence of virus like particles (Easton *et al.*, 1997). This remains the only definitive report of viruses potentially regulating the abundance of a marine macrophyte.

Modelling the Role of Viruses in Algal Blooms

The role of viruses in the termination of algal blooms (*Emiliana huxleyii*, *Micromonas pusilla* and *Noctiluca scintillans*) was modeled by Beltrami & Carroll (1994) however the latter has no known phycoviruses. The model also assumes that all viruses are lytic and does not consider latent infections, which have been inferred in the species modelled (Bratbak *et al.*, 1993; Cottrell & Suttle, 1995). Nevertheless the predicted dynamics of an infected monospecific phytoplankton bloom shown in this model strongly resembles that observed in natural blooms.

5. Diversity of Algal Marine Viruses

Genetic Diversity of Phycoviruses and Cyanophages

Most viruses are species-specific (Fuhrman, 1999), therefore changes in viroplankton diversity may reflect changes in phyto- and bacterioplankton community diversity (Wommack *et al.*, 1999a). Traditionally, viral genetics have been studied using gene sequencing and amplification (Prescott *et al.*, 1993; Alberts *et al.*, 1994), however, the use of pulsed-field gel electrophoresis (Wommack *et al.*, 1999a) and to a limited extent flow-cytometry (Marie *et al.*, 1999) have been used to elucidate the genetic diversity of marine phyco- and cyanophages.

Gulf of Mexico

The first study of phycovirus genetics (Chen *et al.*, 1996) reported at least 18 distinct phycovirus populations in Gulf of Mexico phytoplankton populations. This study used algal specific polymerase chain reaction primers to sequence DNA polymerase genes in marine viruses concentrated from seawater. To date this remains the only published study using PCR techniques on natural viroplankton communities, however hybridisation has been used previously (Cottrell & Suttle, 1995) on natural samples to determine relativity of viruses of *Micromonas pusilla* to other algal viruses.

Chesapeake Bay

Changes in phytoplankton communities potentially due to viral infection and lysis are reflected in changes in virus morphology and size over the same time. Pulsed-field gel electrophoresis has been used to analyse virus populations in the Chesapeake Bay (Wommack *et al.*, 1999a). It is suggested that changes in abundance of large (>247kbp) viruses over one year is correlated to changes in algal abundance. No taxonomic data is available (phytoplankton community composition was not determined in this study) however changes in viral diversity (from 16 to 7 bands of similar-size DNA) shows that viruses potentially regulate phytoplankton structure.

Pulse-Field Gel Electrophoresis measures the size of viral genomes (in kbp) and not the sequence of genes. The technique does not distinguish between two or more genetically distinct populations that have approximately the same size genome. Blotting of the gels and sequencing of similar sized DNA would potentially elucidate the genetic diversity of viral populations and overcome this problem.

Mediterranean Sea

The first reported use of flow-cytometry to enumerate viruses in natural seawater samples alluded to the potential use of this technique in distinguishing between populations of virus-like particles based on their fluorescence and side-scatter signatures (Marie *et al.*, 1999). This study indicates that only two populations of marine viruses are distinguishable in the Mediterranean Sea.

Macroalgal viruses

There is no published information on the diversity of macroalgal viruses, however it is possible free macroalgal viruses in the water column have been included in previous studies (such as Wommack *et al.* (1999b).

Effects on Virus Infection on Host Diversity

The effects of phycovirus infection on host genetic diversity is poorly understood. It has been suggested that viruses mediate transformation of unicellular heterotrophic and photosynthetic bacteria, hence increasing their genetic diversity (Prescott *et al.*, 1993; Jiang & Paul, 1998), however the role of viruses in genetic transformation of eukaryotic algae has not been studied. The high species diversity of phytoplankton in areas of low nutrient availability (Hutchinson's Paradox of the Plankton (Hutchinson, 1961) has been argued to be due to transformation by phycoviruses (Fuhrman, 1999).

It has been observed that some divisions of algae have more phycoviruses than others. In a mixed community infection of one species may favour another species by the release of lysis products (Gobler *et al.*, 1997).

The dynamics of phytoplankton, bacteria and virus-like particles observed during a spring bloom of *Skeletonema costatum* (Bratbak *et al.*, 1990) showed that virus-like particle abundance maxima coincided with maximum diatom and heterotrophic bacteria abundances. The abundance of phycoerythrin-containing cyanobacteria increased marginally after the bloom collapse, suggesting viral-mediated unicellular phytoplankton succession occurred when cyanobacteria took up the lysis products of the diatoms.

Increasing viral abundance by 250% has been shown to result in slower growth of a mixed-diatom community (Peduzzi & Weinbauer, 1993b). The diatom community in this study was not identified, however the abundance of heterotrophic bacteria and flagellates was initially stimulated by increased viral abundance, then after 200 hr (coinciding with a diatom bloom) growth rates of microalgae approximated mortality rates (Peduzzi & Weinbauer, 1993b). Viruses therefore may play an important role in regulating the ratio of heterotrophs to autotrophs.

Little is known of the effects of phycoviruses on macroalgal community composition. It is plausible that marine viruses exert influence on macroalgal community structure analogous to their influence on microalgal community structure, by infecting susceptible species and thereby providing resources (eg nutrients or space) for resistant species. Viruses may help explain the loss of macroalgal diversity and subsequent bloom of a single chemically resistant alga (eg *Ulva lactuca* blooms in the Mediterranean Sea (Runca *et al.*, 1996) and in Bramble Bay (Australia) (Abal *et al.*, 1998) and *Caulerpa taxifolia* blooms off the coast of Monaco (Hill *et al.*, 1998)).

6. Role of Phycoviruses in Water Quality and Ecosystem Health Management

Anti-viral Compounds Isolated from Algae

The isolation and characterisation of sulphated polysaccharides shown to have strong antiviral effects is an area of promising phycovirus research. Antiviral compounds isolated from algae include chondroitin, dermatan sulphate, heparin, hyaluronic acid, dextran sulphate, pentosan sulphate, pucoidan, k-carragenan, l-carragenan, chitin and glucosamin-6-sulphate (Mouhim & Hours, 1995). The most common of these compounds, the carageenans, have been shown to reduce the infectivity of human-health degrading viruses, such as *Herpes simplex* and the human immunodeficiency virus (HIV) (Bentler *et al.*, 1993). It has also been shown that grazers may be transferred the immunity by sequestering these compounds from their food source (Bentler *et al.*, 1993).

Use of Phycoviruses for Controlling Algal Blooms

The identification of viruses capable of lysing algal blooms, particularly those of 'nuisance' or harmful algal bloom species have attracted recent interest. The discovery of phycoviruses specific to *Aureococcus anophagefferens*, which causes brown tides (Milligan & Coper, 1994) and *Heterosigma akashiwo* which causes red tides (Nagasaki *et al.*, 1999) recommend the further study of phycoviruses as algicides.

The introduction of biocidal pathogens into natural systems has numerous counter-arguments. Viruses infective to one species of phytoplankton may be added to phytoplankton communities on a small scale to reduce biomass of the target organism without affecting other microalgae present (Milligan & Coper, 1994; Nagasaki *et al.*, 1999). However, there is speculation that the target organisms may become genetically resistant to viral infection, which would result in blooms which are unlimited by viruses in natural communities. The crash of one 'nuisance' species, demonstrated in mixed culture to result in the bloom of other (potentially harmful) species, due to uptake of lysis products of the first boom (Suttle, 1992; Milligan & Coper, 1994; Nagasaki *et al.*, 1999)

Use of Phycoviruses in Water Quality Monitoring

Monitoring of marine viruses (including bacteriophages) are presently incorporated into a handful of water quality monitoring strategies, for example the Southern California Bight 1998 Regional Marine Monitoring Survey (Noble, 1999). However the viruses observed in most strategies are those specific to human-disease causing bacteria, such as coliphages specific to *Escherichia coli*. The use of phycoviruses in water quality monitoring, for example for the remote detection of harmful algal blooms, or for the detection of temperate phages causing toxin production in cyanobacteria remain potentially useful areas for future research.

Acknowledgments

The authour wishes to thank Jed Fuhrman, Gunnar Bratbak, Alexander Murray, Cynthia Heil, Judy O'Neil, William Dennison, Kath Chaston and Andrew Watkinson for their assistance with editing this review. The authour also wishes to thank Nicholas Mann and John Paul for their conversations on current research.

List of Tables

Table 1a – Summary of reported phycoviruses and cyanophages isolated from marine algae, arranged by taxonomic level.

Table 2 – Summary of reported decreases in biomass with viral concentrate addition

Table 3- Summary of reported decreases in photosynthesis with viral concentrate addition

List of Figures

Figure 1 – Comparison of prokaryotic and eukaryotic infection cycles.

Figure 2- Morphology of different types of algal virus

Figure 3 – Effect of viral infection on photosynthetic apparatus

Figure 4 – Conceptualised nutrient uptake / release model for prokaryotic and eukaryotic microalgae

Figure 5- Known and hypothetical resistance of algal groups to infection

References

- Abal, E. G., Holloway, K. M. & Dennison, W. C. 1998. *Interim Stage II Scientific Report*. The University of Queensland, Brisbane.
- Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. 1994. *Molecular Biology of The Cell*. Garland Publishing, New York.
- Apt, K. E. & Gibor, A. 1991. The ultrastructure of galls on the red alga *Gracilaria epihippisor*. *Journal Of Phycology* **27**, 409-413.
- Balachandran, S., Hurry, V. M., Kelley, S. E., Osmond, C. B., Robinson, S. A., Rohozinski, J., Seaton, G. G. R. & Sims, D. A. 1997. Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. *Physiologia Plantarum* **100**, 203-213.
- Beltrami, E. & Carroll, T. O. 1994. Modelling the role of viral disease in recurrent phytoplankton blooms. *Journal of Mathematical Biology* **32**, 857-863.
- Bentler, J. A., McKee, T. C., Fuller, R. W., Tischler, M., Cardellin, J. H. F., Sander, K. M., McCloud, T. G. & Boyd, M. R. 1993. Frequent occurrence of HIV-inhibitory sulphated polysaccharides in marine invertebrates. *Antiviral Chemistry and Chemotherapy* **4**, 167-172.
- Bergh, O., Borsheim, K. Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. *Nature* **340**, 467-468.
- Bisen, P. S., Audholia, S., Bhatnagar, A. K. & Bagchi, S. N. 1986. Evidence for lysogeny and viral resistance in the cyanobacterium *Phormidium uncinatum*. *Current Microbiology* **13**, 1-6.
- Bratbak, G., Egge, J. K. & Heldal, M. 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. *Marine Ecology Progress Series* **93**, 39-48.
- Bratbak, G., Haslund, O. H., Heldal, M., Naess, A. & Roeggen, T. 1992. Giant marine viruses? *Marine Ecology Progress Series* **85**, 201-202.
- Bratbak, G., Heldal, M., Norland, S. & Thingstad, T. F. 1990. Viruses as partners in spring bloom microbial trophodynamics. *Applied and Environmental Microbiology* **56**, 1400-1405.
- Bratbak, G., Jacobsen, A. & Heldal, M. 1998. Viral lysis of *Phaeocystis pouchetii* and bacterial secondary production. *Aquatic Microbial Ecology* **16**, 11-16.
- Burkholder, J. M., Noga, E. J., Hobbs, C. W., Glasgow, H. B. & Smith, S. A. 1992. New "phantom" dinoflagellate is the causative agent of major estuarine fish kills. *Nature* **358**, 407-410.
- Campbell, L., Nolla, H. A. & Vaultot, D. 1994. The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnology and Oceanography* **39**, 954-961.
- Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B. & Carpenter, E. J. 1998. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276**, 1221-1229.
- Chapman, R. L. & Lang, N. J. 1973. Virus-like particles and nuclear inclusions in the red alga *Porphyridium purpureum* (Bory) Drew et Ross. *Journal of Phycology* **9**, 117-122.
- Chen, F., Suttle, C. A. & Short, S. M. 1996. Genetic diversity in marine algal virus communities as revealed by sequence analysis of DNA polymerase genes. *Applied and Environmental Microbiology* **62**, 2869-2874.
- Claire, J. W. L. & West, J. A. 1977. Virus-Like Particles in the Brown Alga *Streblonema*. *Protoplasma* **93**, 127-130.
- Clayton, M. N. & King, R. J. 1995. *Biology of Marine Plants*. Longman, Melbourne.
- Cochlan, W. P., Wikner, J., Steward, G. F., Smith, D. C. & Azam, F. 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environment. *Marine Ecology Progress Series* **92**, 77-87.
- Cottrell, M. T. & Suttle, C. A. 1991. Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eucaryotic marine phytoplankter, *Micromonas pusilla*. *Marine Ecology Progress Series* **78**, 1-9.
- Cottrell, M. T. & Suttle, C. A. 1995. Genetic diversity of algal viruses which lyse the photosynthetic picoflagellate *Micromonas pusilla* (Prasinophyceae). *Applied and Environmental Microbiology* **61**, 3088-3091.
- DeBeer, D. 1999. Use of micro-electrodes to measure in situ microbial activities in biofilms, sediments and microbial mats. *Submitted to Molecular Microbial Ecology Manual*, In Prep.
- DemmigAdams, B. & Adams, W. W. I. I. I. 1992. Photoprotection and other responses of plants to high light stress. *Annual Reviews in Plant Physiology and Plant Molecular Biology* **43**, 599-626.
- Dennison, W. C. & Abal, E. G. 1999. *Moreton Bay Study: A scientific basis for the healthy waterways campaign*. Healthy Waterways, Brisbane.
- Easton, L. M., Lewis, G. D. & Pearson, M. N. 1997. Virus-like particles associated with dieback symptoms in the brown alga *Ecklonia radiata*. *Diseases of Aquatic Organisms* **30**, 217-222.
-

- Franca, S. 1976. On the presence of virus-like particles in the dinoflagellate *Gyrodinium resplendens*. *Protistologica* **12**, 435-430.
- Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399**, 541-548.
- Fuhrman, J. A. & Suttle, C. A. 1993. Viruses in marine planktonic systems. *Oceanography* **6**, 51-63.
- Glynn, P. W. 1991. Coral reef bleaching in the 1980s and possible connections with global warming. *Trends in Ecology and Evolution* **6**, 175-179.
- Gobler, C. J., Hutchins, D. A., Fisher, N. S., Cosper, E. M. & SanudoWilhelmy, S. A. 1997. Release and bioavailability of C,N,P,Se and Fe following viral lysis of a marine chrysophyte. *Limnology and Oceanography* **42**, 1492-1504.
- Gotschalk, C. C. & Alldredge, A. L. 1989. Enhanced primary production and nutrient regeneration within aggregated marine diatoms. *Marine Biology* **103**, 119-129.
- Guixa-Boixereu, N., Lysnes, K., Pedros-Alio, C., 1999 Viral Lysis and Bacterivory during a Phytoplankton Bloom in a Coastal Water Microcosm. *Applied and Environmental Microbiology* **65** 1949-1958
- Hallegraeff, G. M. & Lucas, I. A. N. 1988. The marine dinoflagellate genus *Dinophysis*: Photosynthetic, neritic and non-photosynthetic, oceanic species. *Phycologia* **27**, 25-42.
- Hara, S., Terauchi, K. & Koike, I. 1991. Abundance of viruses in marine waters: assessment by epifluorescence and transmission electron microscopy. *Applied and Environmental Microbiology* **57**, 2731-2734.
- Hartig, P., Wolfstein, K., Lippemeier, S. & Colijn, F. 1998. Photosynthetic activity of natural microphytobentos populations measured by fluorescence (PAM) and ¹⁴C-tracer methods: A comparison. *Marine Ecology Progress Series* **166**, 53-62.
- Heil, C. A., Chaston, K. A., Jones, A., Bird, P., Costanzo, S., Longstaff, B. & Dennison, W. C. 1999. Benthic Microalgae in coral reef sediments. *Submitted to Coral Reefs In prep.*
- Hess, W. R., Partensky, F., Van, D. S. G. W. M., Garcia, F. J. M., Borner, T. & Vaultot, D. 1996. Coexistence of phycoerythrin and a chlorophyll a/b antenna in a marine prokaryote. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 11126-11130.
- Hill, D., Coquillard, P., DeVaugelas, J. & Meinesz, A. 1998. An algorithmic model for invasive species: Application to *Caulerpa taxifolia* (Vahl) C. Agardh development in the North-Western Mediterranean Sea. *Ecological Modelling* **109**, 251-265.
- HoeghGuldberg, O. 1999. *Climate Change, Coral Bleaching and the Future of the World's Coral Reefs*. Greenpeace, Sydney.
- Hutchinson, G.E. 1961 The paradox of the plankton *American Naturalist* **45**, 137-145
- Ivanova, V., Rouseva, R., Kolarova, M., Serkedjieva, J., Rachev, R. & Manolova, N. 1994. Isolation of a polysaccharide with antiviral effect from *Ulva lactuca*. *Preparative Biochemistry* **24**, 83-97.
- Jacobsen, A., Bratbak, G. & Heldal, M. 1996. Isolation and characterization of a virus infecting *Phaeocystis pouchetii* (Prymnesiophyceae). *Journal of Phycology* **32**, 923-927.
- Jiang, S. C. & Paul, J. H. 1994. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. *Marine Ecology Progress Series* **104**, 163-172.
- Jiang, S. C. & Paul, J. H. 1996. Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. *Marine Ecology Progress Series* **142**, 27-38.
- Jiang, S. C. & Paul, J. H. 1998. Gene transfer by transduction in the marine environment. *Applied and Environmental Microbiology* **64**, 2780-2787.
- Kepner, R. L., Wharton, R. A. & Suttle, C. A. 1998. Viruses in Antarctic lakes. *Limnology and Oceanography* **43**, 1754-1761.
- Kuhlenkamp, R. & Mueller, D. G. 1994. Isolation and Regeneration of Protoplasts from Healthy and Virus-infected Gametophytes of *Ectocarpus siliculosus* (Phaeophyceae). *Botanica Marina* **37**, 525-530.
- Lesser, M. P. 1996. Elevated temperature and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnology and Oceanography* **41**, 271-283.
- Lohuis, M. R. & Miller, D. J. 1998. Genetic transformation of dinoflagellates (*Amphidinium* and *Symbiodinium*): expression of GUS in microalgae using heterologous promoter constructs. *The Plant Journal* **13**, 437-435.
- MacIntyre, H. L., Geider, R. J. & Miller, D. C. 1996. Microphytobenthos: The ecological role of the secret garden of unvegetated, shallow-water marine habitat. I. Distribution, abundance and primary production. *Estuaries* **19**, 186-201.
-

- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, N.W.T., Canada. *Marine Ecology Progress Series* **111**, 121-127.
- Marcello, A., Loregian, A., Palu, G. & Hirst, T. R. 1994. Efficient extracellular production of hybrid *E.coli* heat-labile enterotoxin B subunits in a marine vibrio. *FEMS Microbiology Letters* **117**, 47-52.
- Marie, D., Brussaard, C. P. D., Thyraug, R., Bratbak, G. & Vaulot, D. 1999. Enumeration of marine viruses in culture and natural samples by flow cytometry. *Applied and Environmental Microbiology* **65**, 45-52.
- Markey, D. R. 1974. A possible virus infection in the brown alga *Pylaeiella littoralis*. *Protoplasma* **80**, 223-232.
- Martin, E. & Benson, R. 1988. Phages of Cyanobacteria. In *The Bacteriophages* (Calendar, R., eds). Plenum Press, New York, pp. 607-645.
- Matthews, R. E. F. 1981. *Plant Virology*. Academic Press, New York.
- Milligan, K. L. D. & Cosper, E. M. 1994. Isolation of virus capable of lysing the Brown Tide microalga, *Aureococcus anophagefferens*. *Science Washington D C* **266**, 805-807.
- Minguez, A., Franca, S. & Moreno, D. D. L. E. S. 1994. Dinoflagellates have a eukaryotic nuclear matrix with lamin-like proteins and topoisomerase II. *Journal of Cell Science* **107**, 2861-2873.
- Moestrup, O. & Thomsen, H. A. 1974. An ultrastructural study of the flagellate *Pyramimonas orientalis* with particular emphasis on Golgi apparatus activity and the flagellar apparatus. *Protoplasma* **81**, 247-269.
- Mouhim, R. F. & Hours, M. 1995. Les activites antivirales des polysaccharides sulfates. *Acta Botanica Gallica* **142**, 125-130.
- Mueller, D. G. 1990. Mendelian segregation of a virus genome during host meiosis in the marine brown alga *Ectocarpus siliculosus*. *Journal Of Plant Physiology* **137**, 739-743.
- Mueller, D. G., Kawai, H., Stache, B. & Lanka, S. 1990. A virus infection in the marine brown alga *Ectocarpus siliculosus* (Phaeophyceae). *Botanica Acta* **103**, 72-82.
- Mueller, D. G. & Stache, B. 1992. Worldwide occurrence of virus-infections in filamentous marine brown algae. *Helgolaender Meeresuntersuchungen* **46**, 1-8.
- Murray, A. 1995. Phytoplankton exudation: Exploitation of the microbial loop as a defence against algal viruses. *Journal of Plankton Research* **17**, 1079-1094.
- 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. *Marine Ecology Progress Series* **89**, 103-116.
- Nagasaki, K., Tarutani, K. & Yamaguchi, M. 1999. Growth characteristics of *Heterosigma akashiwo* virus and its possible use as a microbiological agent for red tide control. *Applied and Environmental Microbiology* **65**, 898-902.
- Nagasaki, K. & Yamaguchi, M. 1996. Intra-species host specificity of HaV (*Heterosigma akashiwo* virus) clones. *Aquatic Microbial Ecology* **14**, 109-112.
- Noble, R. T. & Fuhrman, J. A. 1998a. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology* **14**, 113-118.
- Noble, R. T. & Fuhrman, J. A. 1998b. Use of SYBR Green I rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology* **14**, 113-118.
- O'Brien, T. L., Macleod, R. & Maclean, M. C. 1984. Absence of lytic virus in 2 species of symbiotic algae within the sea anemone *Anthopleura xanthogrammica* (Coelenterata: Anthozoa). *Transactions Of The American Microscopical Society* **103**, 228-232.
- Ohki, K. 1999. A possible role of temperate phage in the regulation of *Trichodesmium* biomass. *in press* , .
- Ohki, K. & Fujita, Y. 1996. Occurrence of a temperate cyanophage lysogenizing the marine cyanophyte *Phormidium persicinum*. *Journal of Phycology* **32**, 365-370.
- Oliveira, L. & Bisalputra, T. 1978. A virus infection in the brown alga *Sorocarpus uvaeformis*(Lyngbye) Pringsheim (Phaeophyta, Ectocarpales). *Annals of Botany* **42**, 439-445.
- Paul, J. H., Rose, J. B., Jiang, S. C., Kellogg, C. A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. *Applied and Environmental Microbiology* **59**, 718-724.
- Pearson, B. R. & Norris, R. E. 1974a. Intranuclear virus-like particles in the marine alga *Platymonas* sp. (Chlorophyta, Prasinophyceae). *Phycologia* **13**, 5-9.
- Pearson, P. B. & Norris, R. E. 1974b. Intranuclear virus-like particles in the marine lga *Platymonas* sp. (Chlorophyta, prasinophyceae). *Phycologia* **13**, 5-9.
-

- Peduzzi, P. & Weinbauer, M. G. 1993a. Effect of concentrating the virus-rich 2-200-nm size fraction of seawater on the formation of algal flocs (marine snow). *Limnology and Oceanography* **38**, 1562-1565.
- Peduzzi, P. & Weinbauer, M. G. 1993b. The submicron size fraction of seawater containing high numbers of virus particles as bioactive agent in unicellular plankton community successions. *Journal of Plankton Research* **15**, 1375-1386.
- Pienaar, R. N. 1976. Virus-like particles in three species of phytoplankton from San Juan Island, Washington. *Phycologia* **15**, 185-190.
- Prescott, L. M., Harley, J. P. & Klein, D. A. 1993. *Microbiology*. Wm. C. Brown Publishers, Dubuque.
- Pueschel, C. M. 1995. Rod-shaped virus-like particles in the endoplasmic reticulum of *Audouinella saviana* (Acrochaetales, Rhodophyta). *Canadian Journal of Botany* **73**, 1974-1980.
- Purves, W. K., Orians, G. H. & Heller, H. C. 1995. *Life: The Science of Biology*. Sinauer Associates, Salt Lake City.
- Reisser, W. 1992. Interactions of eukaryotic algae and viruses. In *Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored* (Reisser, W., eds). Biopress Ltd, Bristol, pp. 746.
- Rohozinski, J., Patil, P. N. & Seaton, G. G. R. 1995. Infectivity of algal viruses studied by chlorophyll fluorescence. *Journal of General Virology* **76**, 2859-2862.
- Runca, A., Bernstein, A., Postma, L. & DiSilvio, G. 1996. Control of macroalgae blooms in the Lagoon of Venice. *Ocean and Coastal Management* **30**, 235-257.
- Safferman, R. S. & Morris, M. E. 1963. Algal Virus: Isolation. *Science* **140**, 679-680.
- Scanlan, D. J., Hess, W. R., Partensky, F., Newman, J. & Vaultot, D. 1996. High degree of genetic variation in *Prochlorococcus* (Prochlorophyta) revealed by RFLP analysis. *European Journal of Phycology* **31**, 1-9.
- Seaton, G. G. R., Hurry, V. M. & Rohozinski, J. 1996. Novel amplification of non-photochemical chlorophyll fluorescence quenching following viral infection in *Chlorella*. *FEBS Letters* **389**, 319-323.
- Sherman, L. A. & Haselkorn, R. 1971. Growth of the blue-green algae virus LPP-1 under conditions which impair photosynthesis. *Virology* **45**, 739.
- Shimada, A., Maruyama, T. & Miyachi, S. 1996. Vertical distributions and photosynthetic action spectra of two oceanic picophytoplankters, *Prochlorococcus marinus* and *Synechococcus* sp. *Marine Biology Berlin* **127**, 15-23.
- Simon, M., Hennes, K. & Rosenstock, B. 1996. Bacteria, Viruses and Marine Snow. *Berichte fur Polarforschung* **1996**, 107-112.
- Spector, D. L. 1984. *Dinoflagellates*. Academic Press, Orlando.
- Steele, R. E. & Rae, P. M. M. 1980. Ordered distribution of modified bases in the DNA of a dinoflagellate. *Nucleic Acid Research* **8**, 4709-4725.
- Suttle, C. A. 1992. Inhibition of photosynthesis in phytoplankton by the submicron size fraction concentrated from seawater. *Marine Ecology Progress Series* **87**, 105-112.
- Suttle, C. A. & Chan, A. M. 1993. Marine cyanophages infecting oceanic and coastal strains of *Synechococcus*: Abundance, morphology, cross-infectivity and growth characteristics. *Marine Ecology Progress Series* **92**, 99-109.
- Suttle, C. A. & Chan, A. M. 1994. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Applied and Environmental Microbiology* **60**, 3167-3174.
- Suttle, C. A. & Chan, A. M. 1995. Viruses infecting the marine prymnesiophyte *Chrysochromulina* spp.: Isolation, preliminary characterization and natural abundance. *Marine Ecology Progress Series* **118**, 275-282.
- 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. 1991a. Use of ultrafiltration to isolate viruses from seawater which are pathogens of marine phytoplankton. *Applied and Environmental Microbiology* **57**, 721-726.
- Suttle, C. A., Chan, A. M. & Fuhrman, J. A. 1991b. Dissolved free amino acids in the Sargasso Sea: uptake and respiration rates, turnover times, and concentrations. *Marine Ecology Progress Series* **70**, 189-199.
- Tanaka, J. I., Ogawa, N., Liang, J. & Higa, T. 1993. Helioporins: Bioactive diterpenes from the Blue Coral *Heliopora coerulea*. *Tetrahedron* **49**, 811-822.
- Toren, A., Landau, L., Kushmaro, A., Loya, Y. & Rosenberg, E. 1998. Effect of temperature on adhesion of *Vibrio* Strain AK-1 to *Oculina patagonica* and on coral bleaching. *Applied and Environmental Microbiology* **64**, 1379-1384.
-

- Toth, R. & Wilce, R. T. 1972. Viruslike particles in the marine alga *Chorda tomentosa* Lyngbye (Phaeophyceae). *Journal of Phycology* **8**, 126-130.
- Urbach, E. & Chisholm, S. W. 1998. Genetic diversity in *Prochlorococcus* populations flow cytometrically sorted from the Sargasso Sea and Gulf Stream. *Limnology and Oceanography* **43**, 1615-1630.
- Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D. & Foreman, K. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* **42**, 1105-1118.
- Vance, B. D. 1977. Prophage induction in toxic *Microcystis aeruginosa* NRC-1. In *Meeting of Phycology Society of America* (eds) , , pp. 405.
- VanEtten, J. L., Lane, L. C. & Meints, R. H. 1991. Viruses and viruslike particles of eukaryotic algae. *Microbiological Reviews* **55**, 586-620.
- Vaulot, D., Marie, D., Olson, R. J. & Chisholm, S. W. 1995. Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. *Science Washington D C* **268**, 1480-1482.
- Waldor, M. K. & Mekalanos, J. J. 1996. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* **272**, 1910-1914.
- Waterbury, J. B. & Valois, F. W. 1993. Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. *Applied and Environmental Microbiology* **59**, 3393-3399.
- Weinbauer, M. G. & Peduzzi, P. 1995. Effect of virus-rich high molecular weight concentrates of seawater on the dynamics of dissolved amino acids and carbohydrates. *Marine Ecology Progress Series* **127**, 245-253.
- White, A. J. & Critchley, C. 1999. Rapid light curves: A new fluorescence method to assess the state of the photosynthetic apparatus. *Photosynthesis Research* **59**, 63-72.
- Wilson, W. H., Carr, N. G. & Mann, N. H. 1996. The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. *Journal of Phycology* **32**, 506-516.
- Wilson, W. H., Turner, S. & Mann, N. H. 1998. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effects on DMSP and DMS production. *Estuarine, Coastal and Shelf Science* **46**, 49-59.
- Wolf, S., Maier, I., Katsaros, C. & Mueller, D. G. 1998. Virus assembly in *Hincksia hincksiae* (Ectocarpales, Phaeophyceae): An electron and fluorescence microscopic study. *Protoplasma* **203**, 153-167.
- Wommack, K. E., Ravel, J., Hill, R. T., Chun, J. & Colwell, R. R. 1999a. Population dynamics of Chesapeake Bay viroplankton: Total-community analysis by pulsed-field gel electrophoresis. *Applied and Environmental Microbiology* **65**, 231-240.
- Wommack, K. E., Ravel, J., Hill, R. T. & Colwell, R. R. 1999b. Hybridization analysis of Chesapeake Bay viroplankton. *Applied and Environmental Microbiology* **65**, 241-250.
- Zhang, Z., Green, B. R. & Cavalier-Smith, T. 1999. Single gene circles in dinoflagellate chloroplast genomes. *Nature* **400**, 155-159.
-