

## **Dynamics of Marine Viruses along Eutrophication Gradients in Australian Subtropical Estuaries**

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Viruses are recognised as ubiquitous and numerically abundant components of marine ecosystems, however most of the understanding of viral dynamics is based on water column studies, particularly in the oceanic realm. There has been limited study of viral abundance and ecological role in sediments, or along estuarine gradients in coastal marine ecosystems. Hence, viral dynamics were investigated in both water column and sediments along estuarine gradients in two subtropical ecosystems in Queensland, Australia; a eutrophic estuary (Brisbane River / Moreton Bay; 27° S, 153° E) and an oligotrophic estuary (Noosa River; 26°S, 153°E). Virus, bacteria and microalgae from water and sediment samples were enumerated following filtration through a 0.02 µm Al<sub>2</sub>O<sub>3</sub> filter using a SYBR Green I staining technique. Benthic viral abundance ranged from 10<sup>7</sup> –10<sup>9</sup> particles mL<sup>-1</sup>, bacterial abundance ranged from 10<sup>7</sup> –10<sup>8</sup> cells mL<sup>-1</sup>, and microalgal abundance ranged from 10<sup>4</sup> –10<sup>5</sup> cells mL<sup>-1</sup>. Water column abundances for all microorganisms were 10 –1000 fold lower than sediment abundances. Benthic virus abundance correlated strongly to water column virus abundance ( $r^2 = 0.80$ ), total suspended solids ( $r^2 = 0.73$ ) and virus/bacteria ratio ( $r^2 = 0.71$ ). These correlations suggest that viruses sorbed to suspended material in the water column may settle out and contribute to benthic virus population. Virus production was measured by a time course increase of virus abundance in seawater filtered (0.22 µm) to remove viruses but not bacteria. Virus production was highest in eutrophic portions of Brisbane River and addition of inorganic nutrients (NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup> + SiO<sub>3</sub> + PO<sub>4</sub><sup>-3</sup>) stimulated virus production rates at all stations. These results suggest inorganic nutrient availability plays a key role in viral dynamics. Manipulations of virus abundance were used to test for viral effects on microalgal growth and photosynthesis. Virus abundance was enhanced by tangential flow centrifugation and the resulting high molecular weight concentrate was added to seawater and sediments. Species composition of the benthic microalgal flora was affected by concentrate additions after 7 d both in eutrophic and oligotrophic experiments. In addition, photosynthesis was reduced and photoinhibition increased in eutrophic experiments. These results suggest that viral infection may play a significant role in benthic microalgal populations, particularly in eutrophic regions. Virus abundances, production rates and effects on microalgal communities were strongly influenced by nutrient gradients in the subtropical estuaries studied. These results indicate that the effects of eutrophication in many of the world's estuaries may be mediated by viruses, highlighting the importance of considering viral dynamics in microbial communities.

**Keywords:** Virus, sediment, microalgae, phytoplankton, estuaries, eutrophication

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## INTRODUCTION

The importance of estuaries in the flux of matter from terrestrial to marine environments and their proximity to major urban centres has made these ecosystems loci of current research. Estuaries often contain both salinity and eutrophication gradients as a result of nutrient rich freshwater input from rivers, which are diluted by oceanic exchange, particularly in temperate regions (Valiela, 1995). Australian estuaries differ from those in many other geographic locations as they are characterised by comparatively low flow rates (due to low average rainfall) and low nutrient concentrations (Bowen *et al.*, 1996). However, several estuaries on the East coast of Australia are eutrophied as a result of urban runoff and sewage outfalls (Dennison & Abal, 1999).

Marine viruses are now recognised as ubiquitous and abundant components of aquatic ecosystems. Virus-like particles (VLP; viruses which have not been cultured to identify hosts) typically have abundances between  $10^4$  to  $10^7$  particles  $\text{mL}^{-1}$  in surface waters of the marine environment (Fuhrman, 1999). Viral infection has been shown to affect photosynthesis and biomass in phytoplankton (Suttle *et al.*, 1990; Suttle, 1992; Milligan & Cosper, 1994; Nagasaki *et al.*, 1999), production and abundance of bacteria (Proctor & Fuhrman, 1992; Mathias *et al.*, 1995; Middelboe *et al.*, 1996) and has been implicated in genetic exchange in prokaryotic (Jiang & Paul, 1998) and potentially eukaryotic organisms (Thingstad *et al.*, 1993). Viruses have also been implicated in the decline of phytoplankton blooms (Bratbak *et al.*, 1990; Bratbak *et al.*, 1993) and may be a source of nutrition for heterotrophic nanoflagellates (Gonzales & Suttle, 1993).

Previous studies of subtropical estuarine and neritic viroplankton (pelagic virus) communities (Torella & Morita, 1979; Bergh *et al.*, 1989; Cochlan *et al.*, 1993; Cochran & Paul, 1998) have shown that viruses in surface waters have an average abundance of between  $10^4$  to  $10^8$  particles  $\text{mL}^{-1}$ , however the lower range of these values only considered viruses larger than  $0.22 \mu\text{m}$  (Torella & Morita, 1979). The

abundance of viruses has been largely correlated to the distribution of bacteria, which are the most numerous and therefore most probable hosts. However there is mounting evidence that hosts other than bacteria exist in neritic environments. Suttle *et al.* (1990) showed that viruses specific to various microalgae were present in high-molecular weight seawater concentrates from estuarine waters, while several studies have detected viruses specific to the cyanobacteria *Synechococcus* spp. and the microalgae *Chrysochromulina* spp. in neritic viroplankton (Suttle *et al.*, 1991; Suttle & Chan, 1993; Milligan & Cosper, 1994; Suttle & Chan, 1995).

Coupling of benthic and water column processes is well established in shallow-water estuarine systems (Valiela, 1995). Nitrogen and phosphorus concentrations in estuarine sediments exceed those in the water column in estuaries by several orders of magnitude, and flux of nutrients from sediments to the water column often provides nutrients for planktonic organisms (Valiela, 1995). In addition, the benthos is commonly a sink for settled or adsorbed particulate or dissolved material and is consequently enriched with benthic micro-organisms. Anthropogenic inputs enhance sediment nutrient content which has been shown to result in either increased abundance of autotrophs (e.g. diatoms and cyanobacteria) and heterotrophs (e.g. bacteria and thraustochytrid fungi) (Tufail, 1987), or with excessive nutrient loading decreased abundance of both autotrophs and heterotrophs due to sediment anoxia (Rybarczyk *et al.*, 1996).

The components of marine benthic microbial communities comprise benthic microalgae (BMA), heterotrophic bacteria, non-oxygenic photosynthetic bacteria (eg green and purple sulfur bacteria), thraustochytrid fungi and many higher taxonomic groups (Tufail, 1987; Valiela, 1995). Viruses have also been previously shown to have a high abundance in marine sediments (Paul *et al.*, 1993; Maranger & Bird, 1996; Steward *et al.*, 1996). These studies have stimulated interest in the role of viruses in benthic microbial processes, however to date there have been no reports on their possible effects on benthic hosts.

Bacteria are the most abundant organisms in sediments (Valiela, 1995). However, it has been demonstrated recently that benthic microalgae have substantial biomass and abundance in marine sediments, particularly in oligotrophic areas with low water column light attenuation (Heil *et al.*, submitted). Benthic microalgae are diverse assemblages of autotrophs (MacIntyre *et al.*, 1996) that play significant roles in the ecology of Australian estuaries. They contribute substantially to primary production and hence oxygenation of sediments, as well as potentially providing a large food source for invertebrates and fish (Kendrick *et al.*, 1996; Kendrick *et al.*, 1998; Light & Beardall, 1998). The abundance of benthic microalgae in Australian subtropical estuaries ranges from 20-200 mg m<sup>-2</sup> which frequently exceeds the abundance of phytoplankton in overlying waters (1-10 mg m<sup>-2</sup> (Dennison & Abal, 1999)), and their photosynthetic rate exceeds other component flora in some areas (Heil *et al.*, submitted, Hewson, unpub. data.).

Mortality of microalgae (benthic microalgae and phytoplankton) has been attributed to grazing, burial or resource exhaustion (Fenchel & Staarup, 1971; Blanchard, 1990; Valiela, 1995). However a recent study has shown that phytoplankton photosynthesis is inhibited by up to 78% with the addition of high-molecular weight seawater concentrates which are rich in viruses with capsids larger than 3 nm (Suttle, 1992). In addition, various microalgae including the pennate diatom, *Navicula* sp. (which is the most common benthic pennate diatom in estuarine sediments (Underwood *et al.*, 1998; Hewson *et al.*, unpub. data) have been assayed in culture with high molecular weight concentrates from seawater that was collected offshore. In these experiments the high molecular weight concentrate additions reduced diatom biomass by up to 99% compared with cultures to which high molecular weight concentrate was not added (Suttle *et al.*, 1991). This suggests that virus infection may be a common source of mortality in both benthic microalgae and phytoplankton.

Despite several previous reports of virus abundance in marine (eg Bergh *et al.*, 1989; Bratbak *et al.*, 1990) and freshwater environments (eg Klut & Stockner, 1990; Maranger & Bird, 1996; Kepner *et al.*, 1998; Tapper & Hicks, 1998), there have been no comprehensive reports of the distribution of viruses along estuarine salinity or

eutrophication gradients. Similarly, there have been few reports on the spatial distribution of benthic marine viruses, and none on their effects on co-occurring hosts

Abundance of viruses, bacteria and microalgae were studied in the Brisbane River/ Moreton Bay estuary, and the Noosa River estuary which are located on the East coast of Australia. The aims of the present study were: to determine the distribution of benthic and pelagic marine viruses in these estuaries; their production in the water column along eutrophication gradients; and to determine the effects of high molecular weight concentrates on benthic and pelagic microbial communities from oligotrophic and eutrophic areas of Moreton Bay.

## **MATERIALS / METHODS**

### ***Description of Study Sites***

Moreton Bay (27° S, 153° E) is a shallow embayment (Figure 1a), characterised by long residence time of seawater (>70d in western bay) and distinct nutrient and salinity gradients emanating from four rivers (Dennison & Abal, 1999). The Brisbane River (Figure 1b), which is the major river discharging into the bay, receives sewage outfall from 13 treatment facilities, located from approximately 65km upriver to the river mouth (Dennison & Abal, 1999). Vertical mixing of the water column is primarily wind-driven, however mixing due to tidal currents is also common in upriver sections of the estuary.

In contrast, the Noosa River (26°S, 153°E) (Figure 1c) is a well flushed, oligotrophic estuarine system). The river seasonally receives organic matter loading from pine plantations in the catchment, however there is no sewage treatment outfall and the majority of nutrient input is agricultural runoff. Mixing in the Noosa River is primarily driven by tidal currents; however in the larger estuarine embayments (Lakes Weyba, Cooroibah and Cootharabah) wind-driven mixing may be common (Coles & Greenwood, 1983). Oceanic swell may affect downriver stations, however swells are largely dissipated by an extensive, shallow (< 2 m at Mean High Water Springs (MHWS)) sill at the river mouth.

### ***Field Sampling***

Sampling was carried out in the Brisbane River in November 1999; in Western Moreton Bay in December 1999; and the Eastern Bay and Coral Sea in February 2000 using a variety of research vessels. Sampling in the Noosa River was conducted on in August 1999, and experiments with high molecular weight concentrates were conducted from shore in March 2000. Aquaria experiments were conducted at the

Department of Botany, University of Queensland. Virus production experiments took place at the Moreton Bay Research Station, Dunwich, North Stradbroke Island in February 2000.

Water samples were collected in acid-washed and seawater-rinsed polyurethane buckets and placed into either sterile polycarbonate bottles, 50 mL centrifuge tubes or clear 5 L cubic carboys until analysis. Benthic sampling was conducted by either SCUBA diving or snorkelling, however at some stations water depth or strong currents prevented divers entering the water. At these stations a modified Van Veen grab operated by hand was used to obtain sediments with minimal disturbance to sediment profile. Salinity, temperature, conductivity and dissolved oxygen measurements were made at each station, and samples for dissolved nutrient analyses (0.45 µm filtered) and microalgal community analysis were collected from each station.

### ***Enumeration of Micro-organisms***

#### *1. Viruses and Bacteria*

##### Water Column Virus and Bacteria Enumeration

Seawater (50 mL) was collected in centrifuge tubes for viral and bacterial enumeration. Samples were fixed immediately with 3.5 % formalin and stored at 4 °C until analysis, which was normally completed within 1 mo of sample collection. Viruses and bacteria were enumerated by nucleic acid staining (SYBR Green I) and epifluorescence microscopy (Noble & Fuhrman, 1998). Sample volumes from 0.5 to 3.0 mL were filtered through Anodisc Al<sub>2</sub>O<sub>3</sub> membrane filters (pore size 0.02µm; backed by 0.8µm Millipore type AA filter) and dried immediately on the surface of tissue paper. These were then placed on drops of 1:10000-diluted SYBR Green I (Molecular Probes, Inc.; in 0.02 µm-filtered distilled water) and stained in darkness for 15 min. After drying, filters were placed on glass slides, and drops of 1:1 diluted

phosphate buffer saline (PBS; 120 mM NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.5) and glycerol containing 0.1% p-phenylenediamine were added as antifade and mounting agents. Bacteria and viruses were counted under blue light excitation at x100 magnification on an Olympus BX-60 epifluorescence microscope, while green light excitation was used to count unicellular cyanobacteria and small photosynthetic protists. A minimum of 200 cells of each organism were counted in between 1 and 100 eyepiece micrometer grids in independent duplicate samples.

### Benthic Virus and Bacteria Enumeration

Benthic samples were collected using a 50 mL syringe with the needle end cut off as a sediment corer. Samples were collected from the top 2 cm of sediment and were extruded into 50 mL sterile centrifuge tubes. Samples over a vertical profile were collected by syringe cores that were kept vertical until sectioning with a steel razor blade in the laboratory. Benthic samples were extracted by placing sediments in 35 mL 0.02 µm-filtered PBS which were agitated on a shaker table for 30 min. At some stations where sediments were composed of fine silty muds, the extract was centrifuged at 1000 x g for 10 min to remove suspended sediment. It was assumed that centrifugation did not remove substantial numbers of viruses or bacteria as their mass is unaffected by even strong gravitational forces (Prescott *et al.*, 1993). The supernatant was processed and counted as above with SYBR Green I staining.

Bacteria samples were prepared by filtering acridine orange-stained samples (volumes of between 0.5 to 2 mL) through 0.22 µm irgalan black-stained polycarbonate filters (Osmonics) followed by repeated rinsing with 0.22 µm filtered seawater to remove excess stain. Bacteria were enumerated with epifluorescence microscopy under green light excitation at 100 x magnification (Parsons *et al.*, 1985).

## Electron Microscopy of Viruses

Transmission electron microscopy was used to estimate capsid diameter and morphology (tailed or untailed) of viruslike particles as previously described (Steward *et al.*, 1996). High molecular weight concentrates (to a factor of approximately 50 x ambient assuming 100% efficiency) were prepared using Amicon Centriprep (molecular weight cut off 30,000) centrifugal ultrafilters at 3,000 x *g* for 30 min. Aliquots (100 µL) of high molecular weight concentrate were fixed with 3.5% EM grade glutaraldehyde and harvested directly by ultracentrifugation in a Beckman airfuge at 100,000 x *g* for 30 min onto carbon stabilised formvar-coated 200-mesh copper grids. These were rinsed once in 0.02 µm-filtered milliQ water to remove salts and stained with 4% NH<sub>4</sub>MO<sub>4</sub> for 30 s. Grids were then air-dried and observed in a JEOL 1010 electron microscope at 80 kV accelerating voltage and 80,000 x magnification. Morphology and size of > 50 viruslike particles was determined in three randomly selected grid divisions. The small volume harvested did not allow reliable enumeration of viruslike particles as the number of VLP per grid division seldom exceeded 70.

## *2. Microalgae*

Whole water phytoplankton samples (50 mL) were allowed to settle for 24 h and the supernatant removed to a final volume of 5 mL. Phytoplankton were then enumerated using bright field microscopy at 10 x to 40 x magnification in a Sedgwick-Rafter slide. Benthic microalgae cores (2 cm depth) were resuspended in 0.22 µm-filtered seawater and aliquots of supernatant placed in a Palmer-Molloney slide and enumerated. More than 200 cells (total) of microalgae were counted in each sample in volumes of 0.1 mL for benthic microalgae and 1 mL for phytoplankton. Microalgae were identified to genus level (Tomas 1997).

### *3. Microalgal Pigments*

Chlorophyll-*a* samples were analysed by grinding 2 cm deep sediment cores or GF/F filters (through which a known volume of seawater had been filtered) in 90% acetone (Parsons *et al.*, 1985). Samples were allowed to extract for 24 h at  $-20^{\circ}\text{C}$  and were then centrifuged at  $3000 \times g$  for 25 min to remove flocculated sediment and subcellular components. Absorbance at 630, 647, 664 and 750 nm were measured on a Pharmacia spectrophotometer and chlorophyll-*a* calculated using acidified absorbances at 664 and 750 nm to correct for phaeopigments (Parsons *et al.* 1985).

### ***Chemical and Physical Parameters***

#### *1. Nutrient Analyses*

Nutrient analyses for total ammonium ( $\text{NH}_4^+$ ), total nitrogen oxides ( $\text{NO}_3^-$ ,  $\text{NO}_2^{2-}$ ) and filterable reactive phosphorus ( $\text{PO}_4^{3-}$ ) were analysed colorimetrically using a scalar autoanalyser by Queensland Health (Brisbane, Australia).

#### *2. Salinity*

Salinity (as well as pH, temperature and conductivity) was measured using a HORIBA UD-10 concomitant with water and benthic sample collection.

#### *3. Filterable Suspended Solids*

Surface water (2 L) was collected at each station in 2.5 L polycarbonate bottles. Volumes of between 0.2 to 2 L seawater were filtered through anhydrous pre-weighed Whatman GF/F filters, followed by repeated rinses with milliQ water to remove salts from retained solids. Filters were then dried at  $60^{\circ}\text{C}$  overnight and re-weighed. The difference in filter weight with filtrate is equivalent to suspended solids greater than  $0.7 \mu\text{m}$  in diameter (pore size of filters used).

## ***Virus Production***

One litre samples of surface water were collected at stations (B18, B21, S22, S28 and S26) in acid rinsed Nalgene darkened polycarbonate bottles. Virus free (ie containing no virus particles) seawater was obtained by passing station water through a 0.02  $\mu\text{m}$  filter (Figure 2). Viruses in a whole seawater sample were removed by filtering 800 mL of a 1 L sample through a Durapore (type GV; low protein binding) filter. Virus-free seawater was added to the retentate and bacteria and phytoplankton resuspended by gently pumping water over the filter surface using a sterile 2 mL transfer pipette. The resuspension was then divided and placed into six 60 mL clear polypropylene bottles. Three bottles served as controls and nutrients ( $30 \mu\text{M NH}_4^+$ ,  $200 \mu\text{M NO}_3^{2-}$ ,  $20 \mu\text{M PO}_4^{3-}$ ,  $66 \mu\text{M SiO}_3$ ) were added to the remainder. Subsamples (5 mL) were removed from each bottle at 6 h intervals over 24 h and analysed by SYBR Green I staining and epifluorescence microscopy (Noble & Fuhrman, 1998). It was assumed that the rate of increase of viruslike particles over time is linear when calculating turnover time and that viral decay does not remove viruslike particles over the course of the experiment.

## ***Virus Concentrate Experiments***

### *1. Preparation of High Molecular Weight Concentrate*

Viruses were concentrated in several steps. All potential hosts were removed by passing 5 L of seawater (collected < 0.5m from the sediment/water interface at station) through Whatman GF/F (nominal pore size 0.7  $\mu\text{m}$ ) filters and subsequently through a 0.22  $\mu\text{m}$  Durapore filter. Filtrate was then placed in a 5 L conical flask and concentrated by Tangential Flow ultrafiltration using a peristaltic-pump driven Vivascience Vivaflow 200 unit (molecular weight cut off >30,000) using the manufacturer's recommendations. One half of the concentrated volume was denatured

by microwaving briefly (3 min) and placement under an intense ultraviolet (UV) light lamp for 5 min.

## 2. *In Situ Benthic Microalgae Experiments*

Plastic collars constructed of PVC pipe (diameter 110 mm, height 100 mm) were pushed into the sediment surface to approximately 90 mm depth and enclosed initially at the top (10 mm above sediment surface) with a UV light-permeable plastic lid secured with a rubber band. High molecular weight concentrate was injected (in 3 cores) into sediment surface to a depth of approximately 2 mm through a sterile syringe and metal needle. After 24 h of incubation, the plastic cover was removed and the cores were then subjected to ambient conditions. Denatured concentrate and unconcentrated seawater were also added to three replicate cores each. Cores were sampled at random positions at 0 and 7 d from each plot for enumeration of microorganisms, and at 0, 1, 3 and 7 d for chlorophyll-*a* analysis.

## 3. *Aquaria Benthic Microalgae Experiments*

Sediment cores (110 mm diameter, depth 60 mm) were taken using a pipe corer, placed in 250 mL polystyrene vials with the caps removed and transported intact to aquaria containing native seawater and subjected to continuous aeration. High molecular weight concentrate, denatured concentrate and seawater were injected through plastic lids as described previously and the plastic lid removed after 24 h. Cores were subject to ambient light and temperature during the experiment.

Photosynthesis and fluorescence of sediment surface microalgae was measured in aquaria using a pulse-amplitude modulated (PAM) fluorometer (Walz Mess- and Regeltechnik) (Hartig *et al.*, 1998; White & Critchley, 1999). Cores were dark adapted for 15 min under a light-impermeable tarpaulin, before initial fluorescence ( $F_0$ ) and photochemical efficiency ( $F_v/F_m$  - where  $F_m$  is the maximum excitable fluorescence and  $F_v$  is the maximum minus initial fluorescence) were measured.

Instantaneous rapid-light curves were generated at ambient light (which was consistently  $300 \mu\text{mol quanta m}^{-2} \text{ s}^{-2}$  each day sampled) at random positions in the cores. Three replicate measurements of fluorescence and photosynthesis were generated in each core at 1, 3 and 7 d after virus addition.

#### *4. Phytoplankton Experiments*

Seawater samples were placed in 50 mL polypropylene centrifuge tubes and inoculated with high molecular weight concentrate, denatured concentrate and seawater as described above. These were incubated in aquaria with ambient light. *In vivo* fluorescence of assayed phytoplankton was measured on three replicates of each treatment at 0, 1, 3 and 5 d after high molecular weight concentrate addition using a Turner Designs TD-700 fluorometer after dark adaptation for 30 min.

Phytoplankton productivity was measured by  $^{14}\text{C}$ -bicarbonate incorporation (Parsons *et al.* 1985) after 7 d of incubation. Samples (50 mL) of seawater were placed in polypropylene centrifuge tubes to which 50  $\mu\text{L}$  of 5 mCi  $^{14}\text{CO}_3^{2-}$  per replicate was added. Samples were incubated for 2 h at ambient light intensity (approximately  $200 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ) with one darkened replicate from each station. Samples were then filtered through 0.45  $\mu\text{m}$  polycarbonate filters (Osmonics) and rinsed twice with 1 N HCl to remove unincorporated radioactive carbon. Filters were then placed in 5 mL polycarbonate scintillation vials and 4 mL scintillation fluid (Beckman Ready Scint) added. Disintegrations per minute (dpm) were measured using a Packard 1600TR scintillation counter and these were converted to carbon fixation rate (Parsons *et al.*, 1985). Two replicate measurements of each treatment were made and the third replicate was used for microorganism enumeration as previously described.

### *Statistical Analysis*

Parameters comparing two replicated, independent parameters (e.g. virus abundance, bacterial abundance) were analysed by one-way analyses of variance (ANOVA) using the statistical software package on Microsoft Excel 98. This included analysis of photosynthetic parameters at different sampling times in high molecular weight concentrate addition experiments.

Virus abundance (both benthic and in the water column) was compared with salinity, suspended solids, summed ambient nutrient concentrations, bacterial and microalgal abundance by multiple- regression analysis using a statistical software package (Statistica).

## RESULTS

### *Characteristics of Nutrient and Salinity Gradients*

The transect formed between stations B15 and S26 in the Brisbane River / Moreton Bay estuary (Figure 1b) exhibits decreasing inorganic nutrient concentration from the most upriver station to the most oceanic station, however large nutrient concentrations (particularly  $\text{NH}_4^+$ ) are present at the river mouth, which is at the same location as a sewage treatment facility (Figure 3). Salinity increases from 0 to 36 from upriver to the open ocean.

The Noosa River estuary (Figure 1c) has comparatively low ambient inorganic nutrient concentrations (0 to 4  $\mu\text{M}$ ) which decrease marginally from the furthest upriver station (N01) to the river mouth (N12). In contrast, salinity increases from 0 (approximately 20 km from the river mouth) to 36 at the river mouth (Figure 3).

### *Distribution of Viruses, Bacteria and Microalgae*

The abundance of benthic viruses in the Brisbane River/Moreton Bay Estuary and Noosa River was significantly ( $p < 0.001$ ,  $n = 26$ ) higher than the abundance of pelagic viruses per millilitre. In addition, the ratio of viruses to bacterium (VBR) was significantly ( $p < 0.01$ ,  $n = 26$ ) higher in sediments than in the overlying water column.

Water column viral and bacterial abundances and the ratio of viruses to bacteria (VBR) were significantly ( $p < 0.05$ ,  $n = 5$ ) lower at oligotrophic stations than eutrophic stations in the Brisbane River/Moreton Bay Estuary (Figure 4). This decreasing trend was also observed in sediments. Benthic viral and bacterial

abundances and VBR were elevated at mid-river stations (N02, N03, N04) compared with the furthest extents in the Noosa River estuary (Figure 5). However, water column viruses were not elevated at these stations.

Microalgal community composition in the water column varied in different geographic locations. In all portions of the Brisbane River/ Moreton Bay estuary, phytoplankton was dominated by centric (29.3% of eutrophic and 66.3% of oligotrophic communities) and pennate (35.9% eutrophic, 26.4% oligotrophic) diatoms. Benthic microalgal communities in all geographic locations were dominated by pennate diatoms (55.1% eutrophic, 91.0% oligotrophic), however green algae (*Euglena* sp.) were also present only in eutrophic sediments. The remainder of benthic microalgal and phytoplankton communities comprised centric diatoms and dinoflagellates.

In both eutrophic (station B18) and oligotrophic (station S28) sediments, there were clear subsurface maxima of both viruses and heterotrophic bacteria, which declined with sediment depth (Figure 6). VBR was substantially greater in the eutrophic sediments compared to oligotrophic sediments, and at the oligotrophic station bacterial abundance exceeded virus abundance at approximately 50 mm beneath the sediment surface.

Virus-like particles with capsid diameters of 20-50 nm dominated the Brisbane River / Moreton Bay viroplankton (Table 1). Virus-like particles included tailed and untailed morphologies, although the majority observed were *Styloviridae*-type viruslike particles which indicates that they may be bacteriophage (Figure 7) (Prescott *et al.*, 1993).

### ***Correlations Between Virus Abundance and Biotic / Abiotic Parameters***

Abundance of benthic viruses showed significant correlation to pelagic virus abundance in the Brisbane River/Moreton Bay estuary ( $r^2 = 0.80$ ,  $n = 32$ ,  $p < 0.001$ ) (Figure 7). Upriver sections of the estuary exhibited large abundances of both benthic and pelagic viruses, while in Moreton Bay and outside the bay there is lower abundance of both pelagic and benthic viruses. There was large variation in the abundance of benthic (but not pelagic) viruses in oligotrophic areas. The lack of correlation between benthic and pelagic viruses in the oligotrophic Noosa River appears similar to that in oligotrophic portions of the Brisbane River/Moreton Bay estuary ( $r^2 = 0.17$ ,  $n = 11$ ,  $p > 0.50$ ).

No significant correlation was found between viruses and bacteria, cyanobacteria and microalgae in sediments (Table 2), however water column virus abundance correlated significantly ( $r^2 = 0.83$ ,  $n = 44$ ,  $p < 0.001$ ) with the abundance of bacteria. The strong correlation ( $r^2 = 0.71$ ,  $n = 32$ ,  $p < 0.001$ ) between viruses and VBR in sediments suggests independence of viral and bacterial abundances. The lack of correlation between viruses and microalgal direct count in sediments was also evident in correlations between virus abundance and water column ( $r^2 = 0.35$ ,  $n = 24$ ,  $p < 0.10$ ) and benthic ( $r^2 = 0.19$ ,  $n = 20$ ,  $p > 0.50$ ) chlorophyll-*a*.

Pelagic viruses significantly correlated with abiotic parameters including salinity ( $r^2 = 0.60$ ,  $n = 44$ ,  $p < 0.001$ ) and suspended matter ( $r^2 = 0.33$ ,  $n = 44$ ,  $p < 0.05$ ), however there were not significant correlations with inorganic nutrients ( $r^2 = 0.24$ ,  $n = 44$ ,  $p > 0.05$  for inorganic nitrogen;  $r^2 = 0.36$ ,  $n = 44$ ,  $p < 0.05$  for inorganic phosphorus) (Table 1). Benthic virus abundance correlated significantly with suspended solids in the water column ( $r^2 = 0.73$ ,  $n = 26$ ,  $p < 0.001$ ), however as in the water column, no significant correlation with inorganic nutrients was found (Table 2). Multiple regression analysis of virus abundance with component variables: bacterial abundance; microalgal abundance; total inorganic nutrient concentration; salinity; and suspended solids, indicated that abiotic and host parameters are poor predictors of

benthic virus abundance. Salinity is the best predictor of these ( $\beta=-0.56$ ,  $n=21$ ) followed by VBR ( $\beta=0.600$ ,  $n=21$ ) and bacterial abundance ( $\beta=0.236$ ,  $n=21$ ). In the water column, bacterial abundance best explained virus abundance ( $\beta=0.892$ ,  $n=32$ ), followed by VBR ( $\beta=0.356$ ,  $n=32$ ) and inorganic nutrient concentration ( $\beta=-0.126$ ,  $n=32$ ).

### ***Virus Production***

Virus production was significantly ( $p < 0.05$ ,  $n = 3$ ) higher in eutrophic portions of the Brisbane River/Moreton Bay estuary than oligotrophic areas (Figure 9). The theoretical ambient viral turnover time increases by 2 fold between the most eutrophic (B18) to oligotrophic (S26) stations. The specific rate of production (virus per bacterium; which compensates for bacterial growth) shows no clear trend with the highest production occurring at eutrophic stations B18 and B21 as well as oligotrophic station S28. Nutrient addition elevated the production of viruses by hosts at three stations where nutrients were added (Figure 9). The most probable hosts observed during this experiment were heterotrophic bacteria.

### ***Effects of Virus Enrichment on Benthic Microalgae along Eutrophication Gradient***

Direct counts showed an absence of bacteria in high molecular weight concentrates and final virus titres were approximately 3000% above ambient seawater abundances. The efficiency of the concentration procedure is estimated to be 30%, which is similar to previous efficiencies found by Fuhrman & Hewson (unpub data) using this method of ultrafiltration. High molecular weight concentrate addition is estimated to have elevated benthic virus abundance in the top 2 mm of sediment by approximately 20%.

Treatments with additions of seawater, denatured concentrate and high molecular weight concentrates all had negative effects on the microalgae present in eutrophic

sediments both *in situ* and in aquaria, which may be attributable to substantial resuspension of substrate. Oligotrophic sediments did not show this negative effect, probably due to relatively low settling time of large-grained sands. Data is not reported for the first 24 h of the experiment at either station due to resuspension of sediments.

The effects of virus enrichment were observed within the first 3 d of the experiment in eutrophic sediment, while at the oligotrophic station there was no evidence of virus infection at 7 d. Evidence of viral infection in eutrophic sediments was decreased electron transport rate ( $ETR_{max}$ ), photoinhibition (Figure 10) and significant increase ( $p < 0.05$ ) in initial fluorescence ( $F_o$ ) (Figure 11). There was no significant effect of high molecular weight concentrate addition on photochemical efficiency ( $F_v/F_m$ ) or the concentration of sediment chlorophyll-*a* at either station. Nonphotochemical quenching (NPQ) increased in both eutrophic and oligotrophic sediments at 3 d and 7 d, respectively with high molecular weight concentrate addition. However, this was only coupled to saturation of photochemical quenching (qP) at the eutrophic station.

Microbial community composition changed at both stations with the addition of high molecular weight concentrates (Table 3). The abundance of bacteria at both the oligotrophic and eutrophic stations decreased ( $p < 0.05$ ) significantly with virus enrichment. The abundance of the dinoflagellate *Gymnodinium* sp. increased significantly ( $p < 0.05$ ) at the oligotrophic station with high molecular weight concentrate and denatured concentrate additions. However, the abundance of bacteria did not change significantly in denatured concentrate additions. Diatom abundance showed no change during the experiment at either station.

Virus enrichment significantly ( $p < 0.05$ ) decreased the abundance of the green alga *Euglena* sp., however denatured concentrate additions also exhibited lower abundances of this alga.

## ***Effects of virus enrichment on Phytoplankton along Eutrophication Gradient***

High molecular weight concentrate addition depressed *in vivo* chlorophyll fluorescence of phytoplankton significantly ( $p < 0.05$ ) at the eutrophic station after 3 d, while chlorophyll fluorescence at the oligotrophic station was not significantly different between treatments (Figure 12). After 7 d high molecular weight concentrate additions in eutrophic water showed no significant effects on fluorescence compared to controls. Although photosynthesis in high molecular weight concentrate-added samples were significantly ( $p < 0.05$ ) elevated, the productivity (as measured by  $^{14}\text{C}$  incorporation) of oligotrophic phytoplankton to which high molecular weight concentrate was added was not significantly different to controls after 7 d (Table 4).

There was no difference in community composition at either station with high molecular weight concentrate addition after 7 d. Containment of phytoplankton communities from eutrophic and oligotrophic stations in both controls and high molecular weight concentrate additions significantly ( $p < 0.05$ ) stimulated the abundance of the chain-forming centric diatoms *Skeletonema costatum*, *Melosira sulcata* and the solitary centric diatom *Coscinodiscus argus*, while in oligotrophic incubations, the abundance of *Chaetoceros* spp was significantly ( $p < 0.05$ ) stimulated in all treatments. Large bacterial aggregates (which were rich in viruses) formed at both stations with high molecular weight concentrate addition (not present in controls) which could not be enumerated by the methods used.

## DISCUSSION

### *Distribution of Microbial Communities along Eutrophication Gradients*

#### *1. Benthic Virus Abundance and Distribution*

The abundances of benthic viruses in the present study are 10 to 1000 fold higher than viral abundances in the overlying water column. This is consistent with previous studies of benthic virus abundance (Paul *et al.*, 1993; Maranger & Bird, 1996; Steward *et al.*, 1996) (Table 5). The higher abundance of viruses in sediments is a reflection of the higher abundance of suitable hosts in sediments compared with overlying waters. However, there are several biotic and abiotic factors that potentially contribute to the high abundance of virobenthos in the Brisbane River / Moreton Bay and Noosa River estuaries which may in part, account for the poor correlation between the abundances of viruslike particles and their hosts (Table 2).

The greater virus-to-bacteria ratios observed in sediments may be the result of greater nutrient availability in sediments, as increased nutrient availability has been previously been linked with elevated virus to bacteria ratios (Tuomi *et al.*, 1995; Wilson *et al.*, 1996). Estuarine sediments are a sink for settled and adsorbed materials from the water column and are therefore enriched with organic material (Valiela, 1995). Decomposition of this organic material results in remineralisation of inorganic nutrients. The greater availability of nutrients in sediments may result in greater microalgal (Nilsson & Sundbaeck, 1991) and bacterial abundances (Tufail, 1987) than the overlying water column where nutrient availability is related to allochthonous inputs (Valiela, 1995) or flux from the sediments (LundHansen *et al.*, 1999).

The distribution of benthic viruses and hosts in vertical profile indicates that viruses, along with their potential hosts, may be photoinhibited at the sediment surface.

Destruction of viruses results from predation, natural decay, or ultraviolet (UV) light irradiation (reviewed in Suttle & Chen, 1992 and Noble & Fuhrman, 1997). In sediments, UV light is attenuated quickly at the sediment surface (Fenchel & Staarup, 1971) and therefore is less likely to impact virus abundance compared to the water column. The large subsurface maxima of virus and bacterial abundances resemble pelagic profiles of microbial distributions (Figure 6) (Bird *et al.*, 1993; Cochlan *et al.*, 1993; Marie *et al.*, 1999) which are attributable to processes of mixing, nutrient availability and photoinhibition. Vertical mixing and nutrient availability are unlikely to play a role in sediments as porewaters are not as well mixed as the water column and organism distribution is probably not limited by nutrient availability. The greater virus to bacteria ratio (VBR) and deeper maximum of virus abundance in eutrophic compared with oligotrophic sediments is likely due to either sediment type, differences in organic nutrient loading or a combination of both factors.

## 2. Pelagic Virus Distribution and Productivity

Nutrient availability may play a key role in the distribution and abundance of pelagic viruses and the ratio of viruses to bacteria. There is a clear trend of decreasing virus-to-bacteria ratios and virus abundances in the Brisbane River/ Moreton Bay estuary from the most eutrophic and freshwater station (B15) to the East Australian Current station (S26). There is no correlation between virus abundance and salinity in the Noosa River, which lacks a distinct nutrient gradient. Therefore, it can be argued that salinity does not play a role in the distribution of pelagic viruses in the Brisbane River/ Moreton Bay estuary, despite their strong correlation. Inorganic nutrient concentrations decrease concurrently with virus abundance and virus to bacteria ratio between stations B15 and S26. The increased burst size (ie number of progeny virus released from cells on lysis) in infected phytoplankton noted by Wilson *et al.* (1996) with phosphorus additions and by Tuomi *et al.* (1995) with additions of the amino acids threonine (*thr*) and serine (*ser*) supports the hypothesis that nutrient availability plays a key role in determining the ambient abundance of pelagic viruses with respect to hosts.

Production rates in eutrophic waters of the Brisbane River / Moreton Bay estuary ranges from 0 to  $2.3 \times 10^6$  VLP mL<sup>-1</sup> h<sup>-1</sup>, which is consistent with those previously observed in neritic waters (0 to  $58.33 \times 10^6$  VLP mL<sup>-1</sup> h<sup>-1</sup> (Table 6)). The production of viruses along the eutrophication gradient shows that in addition to their high abundance in eutrophic portions of the Brisbane River/Moreton Bay estuary, lytic viruses have relatively short turnover times and replicate rapidly within hosts compared with oligotrophic virus communities.

The absence of virus production in the East Australian Current (S26), and lower virus production rates in oligotrophic portions of Moreton Bay, suggest that non-lytic infection (which may be lysogeny or chronic infection) is more common in these areas than in eutrophic waters. It has been proposed previously that lysogeny (infection cycle in which the viral genome is incorporated into the genetic material of the hosts and lysis does not occur until induced by environmental stimuli) may be an important survival mechanism for viruses where host densities or resources are low (Wilson *et al.*, 1998), however Cochran & Paul (1998) found a large abundance of lysogenic bacteria in eutrophic waters with high host abundance and nutrient availability.

The observed increase in virus production at three stations with nutrient addition could potentially be due to the switch between lysogeny and lysis of temperate viruses, however it is impossible to differentiate infection type stimulated by this method. It has been previously suggested that nutrient addition to nutrient-limited environments causes the switch between lysogenic and lytic infection (Wilson *et al.*, 1998). The stimulation of virus production by inorganic nutrient addition may also demonstrate that burst size is responsive to host nutritional status.

### *3. Interaction between Pelagic and Benthic Virus Communities*

The present study did not distinguish between viruses produced in interstitial waters and those produced in the water column which may have settled after adsorption to

particles. Viruses readily adsorb to particles in the water column (Suttle & Chen, 1992; Bird *et al.*, 1993; Maranger & Bird, 1996). Bird *et al.* (1993) determined that approximately 4% of viruses  $d^{-1}$  were lost as a result of adsorption to particles in the oligotrophic Southern Ocean which has relatively low concentrations of suspended solids. Furthermore, Noble & Fuhrman (1997) showed that heat-labile suspended matter was responsible for removal of 20% of viruses  $d^{-1}$  in neritic waters near the coast of Southern California (Santa Monica Bay) which are also relatively low in suspended matter. Settling of unadsorbed viruses into sediments is unlikely as they have little mass and therefore are largely unaffected by gravity.

In the estuaries studied, benthic virus abundance was positively correlated with particulate material that was  $0.7\mu\text{m}$  or larger. The significant correlation between benthic virus abundance and virus to bacteria ratio suggests that the relationship between virus abundance and host abundance is obscured by other factors. Hence the import of viruses to sediments from overlying waters potentially contributes to high benthic virus abundances, particularly in upper sections of the estuaries which have high levels of suspended solids in the water column. This may also help explain the significant correlation between benthic and pelagic virus abundance in eutrophic areas (which have high levels of suspended solids) and lack of correlation in oligotrophic areas (which have low levels of suspended solids).

#### *4. Potential Hosts of Viruses*

Direct observation of *Syloviridae*, *Myoviridae* and *Podoviridae*-type viruslike particles by transmission electron microscopy (TEM) suggests that the majority of viruses in the Brisbane River/Moreton Bay estuary are bacteriophage (Figure 7). This is consistent with previous reports which have shown that the majority of hosts of viruses in marine waters are bacteria (Torella & Morita, 1979; Bergh *et al.*, 1989; Cochran *et al.*, 1998; Cochran & Paul, 1998). However, the slightly elevated abundance of *Parvoviridae*-type untailed viruslike particles at oligotrophic stations may indicate the presence of hosts other than bacteria. This trend is reflected in the

lower bacterium to phytoplankton cell ratio in oligotrophic (10,000:1) compared to eutrophic (300,000:1) waters of the Brisbane River and Moreton Bay estuary.

The potential for terrestrial hosts of benthic marine viruses has been alluded to in previous studies (Lewis *et al.* 1986; Bosch *et al.* 1988) which found high abundance of human enteric viruses (i.e. viruses originating from human intestines) in sediments downstream of sewage outfalls. Human viruses may contribute to the high viral abundance found in sediments near sewage outfalls in the Brisbane and Bremer Rivers (Figure 1b). Transmission electron microscopy (TEM) of virus communities in the water column did not suggest the presence of human viruses, despite the proximity of stations B18 (< 1 km) and B21 (< 0.5 km) to sewage treatment plant outfalls. Enteric viruses have been suggested through numerical modelling to be removed quickly from the water column by sedimentation processes (Murray & Jackson, 1992) and enteric viruses do not persist in marine sediments (Lewis *et al.*, 1986). Therefore they may not contribute substantially to overall viral abundances.

The higher virus to bacteria ratio observed in sediments compared with overlying waters may be the result of the abundance of hosts other than bacteria, which increase the abundance of viruses observed in relation to bacterial hosts. The ratio of bacterium per microalgal cell in sediments (approximately 2000:1) is lower than in the water column (approximately 150000:1), therefore it is conceivable that a larger proportion of the viruses observed in sediments are specific to microalgae than in the water column.

##### *5. Potential Underestimation of Virus Abundance*

The abundance of viruses, particularly in the benthos is underestimated using SYBR Green I staining as it is a double stranded (ds) DNA and RNA fluorochrome and therefore single-stranded (ss) DNA viruses (which may be common in the marine environment (Fuhrman & Suttle, 1993; Fuhrman, 1999)) are not counted using this method. The centrifugation of samples in the present study may have contributed to

the underestimate of benthic virus abundance, as the assumption that this process does not remove viruses may be invalid.

### ***Effects of Viruses on Hosts in the Brisbane River and Moreton Bay***

The effects of high molecular weight concentrate additions to estuarine benthic microalgae and phytoplankton indicates that viruses may potentially exert a large influence on the photosynthesis, biomass and community composition of microalgae and bacteria living in both the water column and on surficial sediments in Moreton Bay. The effects may vary depending on the nutritional history of bacterial hosts, as well as with the ambient composition of microalgal host communities.

The addition of high molecular weight concentrates to microalgae and bacteria both in culture and to seawater has been the subject of recent controversy. Ultrafiltration to 30,000 MW concentrates viruses with a capsid diameter of greater than 3 nm (Suttle, 1992), but may also concentrate other bioactive high-molecular weight compounds. Hence observed effects of high molecular weight concentrate addition on microalgae and bacteria may simply be the result of interactions with abiotic compounds in the concentrates. However, autoclaved (denatured) controls used by Milligan & Cospér (1994) and Suttle *et al.* (1990) also contain large quantities of non-heat labile substances from dissociated virus particles (e.g. amino acids and organic and inorganic nitrogen and phosphorus), which are not present in high molecular weight concentrates. Denatured concentrate controls may stimulate microalgal communities above ambient abundances due to increased nutrient availability. For this reason, controls reported in the present study refer to unautoclaved seawater which was added in a similar manner to ambient host communities.

#### ***1. Physiological Effects of Viruses on Benthic Microalgae***

There is little effect of high molecular weight concentrate addition to sediments on the biomass of benthic microalgae at either the eutrophic or oligotrophic stations.

However, significant impacts on photosynthesis (e.g. change in maximum photosynthetic rate, photoinhibition and fluorescence, as well as saturation of photochemical quenching) at the eutrophic station suggest viral infection of benthic microalgae. 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 cause damage to photosynthetic membranes unless eliminated by photochemical or nonphotochemical quenching (Demmig-Adams & Adams, 1992). Viral infection of algae results in an increase in nonphotochemical quenching and consequent reduction of  $F_o$ . When photoinhibition occurs (due to strain on photoprotection systems), an increase in  $F_o$  is observed due to saturation of both photochemical and nonphotochemical quenching. This is observed in benthic microalgae to which high molecular weight concentrate is added at the eutrophic station after 7 d.

## *2. Ecological Effects of Viruses on Benthic Microalgae and Bacteria*

High molecular weight concentrate addition decreased bacterial abundance by approximately 20-42% compared to additions of untreated seawater and denatured concentrate after 7 days. This indicates that the majority of viruses in near-benthic seawater are bacteriophage (which is consistent with electron microscope observations of virus morphology) specific to hosts on and within the sediments. It has been established previously that high molecular weight concentrate additions initially result in a surge of bacterial productivity as resistant and uninfected bacteria take up lysis products of lysed bacteria (Bratbak *et al.*, 1998). Over longer periods (up to 7 d), the abundance of bacteria has been observed to be suppressed in samples which have had high molecular weight concentrates added (Peduzzi & Weinbauer, 1993b). The observation of viral lysis of bacteria with high molecular weight concentrate additions in oligotrophic sediments is interesting as it has previously been proposed that infection of pelagic bacteria in these areas is largely lysogenic due to relatively low host contact rates and low resource availability (Wilcox & Fuhrman, 1994). In oligotrophic sediments, lysis may be more pronounced than lysogeny due to comparatively high virus and bacterial host abundances.

High molecular weight concentrate addition to eutrophic sediments decreased the abundance of *Euglena* sp. (Euglenophyceae, Chlorophyta), however other microalgae were unaffected by this treatment. Although there are no previous reports of viruses specific to *Euglena* sp. viruses of several related species of green algae including *Platymonas* sp., and *Pyramimonas orientalis* have been observed (Moestrup & Thomsen, 1974; Pearson & Norris, 1974). It has been suggested previously by mathematical modelling that microalgae which exude mucilage are less susceptible to virus infection than organisms that do not exude mucilage. Exudate supports the growth of bacteria which in turn reduce virus contact rates with microalgae (Murray, 1995). *Euglena* sp., when compared to other microalgae at the same station (primarily diatoms), are more susceptible to viral attack as they do not exude mucus. In addition, *Euglena* sp. does not have thecal plates or thick cell walls, and therefore may be less mechanically resistant to potential infection as diatoms.

High molecular weight concentrate addition did not significantly decrease the abundance of oligotrophic benthic microalgae, which are dominated by diatoms (which have siliceous exoskeletons) and thecate dinoflagellates. Oligotrophic benthic microalgae are therefore more mechanically resistant to potential infection than eutrophic benthic microalgae.

The abundance of the unarmoured dinoflagellate *Gymnodinium sanguineum* is elevated in samples which have had both high molecular weight concentrate and denatured concentrates added, concurrent with a slight increase in photosynthetic rate. Dinoflagellates have a high affinity for dissolved organic matter, and are responsive to additions of dissolved organic nitrogen (Qin *et al.*, 1995; Heil, 1996; Doblin *et al.*, 1999). The increased abundance of *G. sanguineum* may be due to uptake of dissolved organic material from bacterial lysis products (Middelboe *et al.*, 1996) in high molecular weight concentrate additions, or uptake of dissociated genetic material and proteins in denatured concentrate additions. Additionally, it is speculated that marine dinoflagellates are largely resistant to virus infection due to complex genetic structure (Spector, 1984; Minguez *et al.*, 1994) or exudation (Murray, 1995). This is supported

by few reported observations of viruses specific to dinoflagellates (Franca, 1976; Soyer, 1978; Sicko-Goad & Walker, 1979).

### *3. Effects of Viruses on Phytoplankton*

High molecular weight concentrate addition depressed *in vivo* fluorescence ( $F_0$ ) of eutrophic phytoplankton communities after only 3 d of incubation, although addition of high molecular weight concentrate to oligotrophic seawater increased *in vivo* fluorescence at the same time. After 7 d of incubation photosynthetic rates were either stimulated or not significantly different between controls and high molecular weight concentrate additions. This is probably due to uptake of lysis products (of both microalgae and bacteria) by resistant phytoplankton, as observed in previous studies (Suttle, 1992). Aggregation of bacteria at both stations with high molecular weight concentrate addition (which has been observed previously (Peduzzi & Weinbauer, 1993a)), may be due to growth of uninfected bacteria on lysis products of infected bacteria.

### *4. Comparison of Virus Infection in Eutrophic and Oligotrophic Microalgae*

It has been shown previously in higher terrestrial plants that the severity or susceptibility to infection is dependent on host nutrition. Tobacco (*Nicotinia tabacum*) plants grown in low nutrient soils exhibit greater symptoms of virus infection than plants grown in nutrient replete soils (Balanchandran & Osmond, 1994). However, there is no evidence from the present study that microalgal nutrition plays a role in susceptibility to virus infection as community composition of microalgae is different in the two stations assayed.

Despite this, in low nutrient environments lysogeny (or latent infection) may be more common than lytic infection, hence addition of lytic viruses would have little effect on the abundance of microalgae. This is consistent with the lack of change in hosts other than bacteria in oligotrophic environments. In addition, the turnover time of organisms in oligotrophic waters is slower than in eutrophic waters (Valiela, 1995),

hence latent period (i.e. the time between infection and lysis) of both bacteria and microalgae may help explain the lack of infection observed in oligotrophic waters in comparison to eutrophic waters.

##### *5. Ecological Significance of Viral Infection in Sediments and Water Column*

Infection and subsequent decrease in benthic microalgal photosynthesis in eutrophic sediments suggests that viral lysis of algae in these environments may be an important source of microalgal mortality. In addition, the stimulation of dinoflagellate populations in oligotrophic sediments by viral lysis products from bacteria, suggests that lysis products are a large source of organic nutrients for uninfected or resistant auto- and heterotrophs. Viruses may play a large role in organic nutrient cycling in low nutrient benthos. Depressed phytoplankton biomass in seawater with elevated virus abundances and subsequent aggregation of uninfected bacteria on lysis products from bacteria suggests that viruses also have significant impacts on nutrient cycles in the water column in both eutrophic and oligotrophic waters of the Brisbane River and Moreton Bay.

The present study emphasises the necessity of considering benthic viruses when studying estuarine microbial communities.

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## **TABLES**

- TABLE 1:** Morphology of viruslike particles in the Brisbane River and Moreton Bay. Capsid diameter is the mean of 50 measurements ( $\pm$  Standard Error)
- TABLE 2:** Simple linear correlation coefficients ( $r^2$ ) between abundance of viruses, potential hosts and abiotic parameters at all stations in the Brisbane River, Moreton Bay and Noosa River (collected in Spring 1999- February 2000). Below diagonal line indicates relationships between benthic organisms and water column abiotic parameters; those above are relationships between pelagic organisms ( $n = 44$  for water column and  $n = 40$  for benthos;  $p < 0.05^*$ ,  $p < 0.005^{**}$ ,  $p < 0.001^{***}$ )  
VBR = virus-to-bacteria
- TABLE 3:** Changes in abundance ( $\pm$  Standard Error) of major microbial components in sediments from stations B18 (Eutrophic) and S28 (Oligotrophic) with high molecular weight concentrate (HMWC) addition in March 2000. Statistically significant ( $p < 0.05$ ) differences upon enrichment are indicated by \*.
- TABLE 4:** Effects of High-Molecular Weight Concentrate (HMWC) additions upon mean abundance ( $\pm$  Standard Error) of pelagic diatoms and bacteria and mean carbon fixation rate ( $\pm$  Standard Error) at oligotrophic (S28) and eutrophic (B18) stations in April 2000. Statistically significant ( $p < 0.05$ ) differences upon enrichment are indicated by \*
- TABLE 5:** Comparison of literature values of benthic viral abundance.
- TABLE 6:** Comparison of literature virus production rates.

**TABLE 1:** Morphology of viruslike particles in the Brisbane River and Moreton Bay. Capsid diameter is the mean of 50 measurements ( $\pm$  Standard Error)

| <b>Sediment Trophic</b> | <b>Station</b> | <b>Capsid Diameter<br/>(nm)</b> | <b>% Tailed Viruses</b> |
|-------------------------|----------------|---------------------------------|-------------------------|
| Eutrophic               | B18            | 30.5 ( $\pm$ 3.7)               | 69.2                    |
|                         | B21            | 40.8 ( $\pm$ 7.4)               | 64.3                    |
| Oligotrophic            | S22            | 95.0 ( $\pm$ 10.1)              | 26.7                    |
|                         | S28            | 51.7 ( $\pm$ 3.4)               | 33.3                    |

**TABLE 2:** Simple linear correlation coefficients ( $r^2$ ) between abundance of viruses, potential hosts and abiotic parameters at all stations in the Brisbane River, Moreton Bay and Noosa River (collected in Spring 1999- February 2000). Below diagonal line indicates relationships between benthic organisms and water column abiotic parameters; those above are relationships between pelagic organisms (n = 44 for water column and n = 40 for benthos; p < 0.05\*, p < 0.005\*\*, p < 0.001\*\*\*)

|   | <b>Virus<br/>Abundance</b> | <b>Bacterial<br/>Abundance</b> | <b>Virus to<br/>Bacteria<br/>ratio</b> | <b>Unicellular<br/>Cyano-<br/>bacteria</b> | <b>Microalgae</b> | <b>Salinity</b> | <b>Dissolved<br/>Inorganic N<br/>(NO<sub>3</sub>, NH<sub>4</sub>)</b> | <b>Dissolved<br/>Inorganic P<br/>(PO<sub>4</sub>)</b> | <b>Suspended<br/>Solids</b> |
|---|----------------------------|--------------------------------|--|--|-------------------|-----------------|---|---|-----------------------------|
| <b>Virus<br/>Abundance</b>  |                            | 0.83***                        | 0.40*                                  | 0.22                                       | 0.04              | -0.60***        | 0.24  | 0.36*   | 0.33*                       |
| <b>Bacterial<br/>Abundance</b>  | 0.03                       |                                | 0.12                                   | 0.33*                                      | 0.04              | -0.48***        | 0.40  | 0.49***   | 0.40*                       |
| <b>Virus to<br/>Bacteria Ratio</b>  | 0.71***                    | 0.06                           |  | 0.04                                       | 0.05              | -0.44**         | 0.06  | 0.14  | 0.23                        |
| <b>Unicellular<br/>Cyanobacteria</b>  | 0.08                       | 0.14                           | 0.14                                   |  | 0                 | -0.06           | 0.12  | 0.17  | 0.06                        |
| <b>Microalgae</b>   | 0.08                       | 0                              | 0.07                                   | 0.06                                       |                   | 0.15            | -0.09   | -0.09   | 0.16                        |
| <b>Salinity</b>   | -0.81***                   | -0.06                          | -0.43**                                | 0.09                                       | 0.08              |                 | -0.39*  | -0.52***  | -0.64***                    |
| <b>Dissolved<br/>Inorganic N<br/>(NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>)</b> | 0.28                       | 0                              | 0.29*                                  | -0.04                                      | -0.06             |                 |   | 0.95***   | 0.12                        |
| <b>Dissolved<br/>Inorganic P<br/>(PO<sub>4</sub><sup>3-</sup>)</b>                            | 0.28                       | 0.02                           | 0.28                                   | -0.05                                      | -0.06             |                 |   |   | 0.13                        |
| <b>Suspended<br/>Solids<sup>a</sup></b>   | 0.73***                    | 0.27                           | -0.58***                               | -0.07                                      | -0.05             |                 |   |   |                             |

<sup>a</sup> Suspended solids >0.7µm

**TABLE 3:** Changes in abundance ( $\pm$  Standard Error) of major microbial components in sediments from stations B18 (Eutrophic) and S28 (Oligotrophic) with high molecular weight concentrate (HMWC) addition in March 2000. Statistically significant ( $p < 0.05$ ) differences upon enrichment are indicated by \*.

| Station      | Treatment        | Heterotrophic<br>Bacteria<br>( $\times 10^6$ cells $m^{-2}$ ) | Total Diatoms<br>( $\times 10^5$ cells $m^{-2}$ ) | <i>Euglena</i> sp.<br>( $\times 10^5$ cells $m^{-2}$ ) | Dinoflagellates<br>( $\times 10^5$ cells $m^{-2}$ ) |
|--------------|------------------|---|---|--|---|
| Eutrophic    | Seawater Control | 2.6 ( $\pm 0.3$ )   | 3.6 ( $\pm 0.3$ )                                 | 2.5 ( $\pm 1.0$ )                                      | -   |
|              | HMWC             | 1.5 ( $\pm 0.2$ )*  | 3.4 ( $\pm 0.9$ )                                 | 1.1 ( $\pm 0.1$ )*                                     | -   |
| Oligotrophic | Seawater Control | 3.9 ( $\pm 0.2$ )   | 28.6 ( $\pm 2.6$ )                                | -  | 0.8 ( $\pm 0.3$ )                                   |
|              | HMWC             | 2.5 ( $\pm 0.1$ )*  | 28.5 ( $\pm 2.7$ )                                | -  | 10.5 ( $\pm 7.5$ )*                                 |

n = 3 replicates for all samples

**TABLE 4:** Effects of High-Molecular Weight Concentrate (HMWC) additions upon mean abundance ( $\pm$  Standard Error) of pelagic diatoms and bacteria and mean carbon fixation rate ( $\pm$  Standard Error) at oligotrophic (S28) and eutrophic (B18) stations in April 2000. Statistically significant ( $p < 0.05$ ) differences upon enrichment are indicated by \*

| Station      | Treatment        | Heterotrophic<br>Bacteria<br>( $\times 10^5$ cells L <sup>-1</sup> ) | Total Diatoms<br>( $\times 10^4$ cells L <sup>-1</sup> ) | Carbon Fixation<br>Rate<br>(mg C m <sup>-3</sup> hr <sup>-1</sup> ) |
|--------------|------------------|--|--|---|
| Eutrophic    | Seawater Control | 5.3 ( $\pm$ 0.5)   | 2.1 ( $\pm$ 1.2)   | 0.0214 ( $\pm$ 0.0004)  |
|              | HMWC Addition    | nd <sup>a</sup>  | 1.1 ( $\pm$ 0.5)   | 0.0202 ( $\pm$ 0.0097)  |
| Oligotrophic | Seawater Control | 4.5 ( $\pm$ 0.7)   | 28.4 ( $\pm$ 1.1)  | 1.6263 ( $\pm$ 0.2401)  |
|              | HMWC Addition    | nd <sup>a</sup>  | 39.1 ( $\pm$ 0.6)*                                       | 3.0849 ( $\pm$ 0.1580)*   |

n = 3 replicates per treatment

<sup>a</sup> nd (no data) due to bacterial aggregation

**TABLE 5:** Comparison of literature values of benthic viral abundance. (VLP = Virus-Like Particle; VBR = Virus-to-Bacteria Ratio)

| Location                                  | Virus Abundance<br>(VLP cm <sup>-3</sup> ) | Range of VBR | Reference                      |
|---|--|--------------|--------------------------------|
| Moreton Bay and<br>Noosa River, Australia | $1.7 \times 10^7 - 2.4 \times 10^9$        | 21-56        | This study                     |
| Lac Gilbert, Quebec,<br>Canada            | $0.8 - 4 \times 10^9$                      | 1-32         | (Maranger & Bird,<br>1996)     |
| Florida Bay, USA                          | $1 - 5 \times 10^8$                        | nd           | (Paul <i>et al.</i> , 1993)    |
| Bering Sea, Alaska,<br>USA                | $2.7 \times 10^7$                          | nd           | (Steward <i>et al.</i> , 1996) |

**TABLE 6:** Comparison of literature virus production rates. (VLP = Virus-Like Particle)

| <b>Location</b>                                  | <b>Virus Production Rate<br/>(VLP x10<sup>6</sup> mL<sup>-1</sup> h<sup>-1</sup>)</b> | <b>References</b>              |
|--|---|--------------------------------|
| <b>Moreton Bay, Australia</b>                    | <b>0 – 2.25</b>   | <b>This Study</b>              |
| Coastal waters, Southern California, USA         | 1.02 – 1.78   | (Fuhrman & Noble, 1995)        |
| Coastal waters, Raujenfjord, Norway              | 1.67-58.33  | (Heldal & Bratbak, 1991)       |
| Coastal Seawater, Southern California Bight, USA | 0 - 9.58  | (Steward <i>et al.</i> , 1992) |
| Offshore Seawater from Southern California, USA  | 0-0.58  | (Steward <i>et al.</i> , 1992) |

## FIGURES

**FIGURE 1:** Location of estuaries (a) and sampling stations in the Brisbane River / Moreton Bay (b) and Noosa River (c) estuaries.

**FIGURE 2:** Method of virus production estimation a) Viruses removed by filtering through 0.22 $\mu$ m Durapore filter, b) virus free filtrate (seawater filtered through 0.02 $\mu$ m anodisc), c) Retentate is mixed with virus-free seawater in a ratio of 1:5 and virus abundance observed over 24 h.

**FIGURE 3:** Inorganic nutrient concentrations and salinity along eutrophication gradient from the Brisbane River to Coral Sea in November 1999 (a) and along Noosa River in August 1999 (b).

**FIGURE 4:** Viruslike particle and bacterial abundance and change in the ratio of viruses to bacteria along a gradient from the Brisbane River to Moreton Bay estuary in water column (a) and sediments (b).

**FIGURE 5:** Viruslike particle and bacterial abundance and change in the ratio of viruses to bacteria along salinity gradient in Noosa River in water column (a) and sediments (b).

**FIGURE 6:** Vertical distribution of viruslike particles and hosts in eutrophic (left) and oligotrophic (right) sediments.

**FIGURE 7** Transmission electron micrographs of viruslike particles from the Brisbane River / Moreton Bay estuary: a) *Styloviridae*-type viruslike particle from station B18 in the Brisbane River; b) *Myoviridae*-type viruslike particle from station B21 in the Brisbane River; c) *Parvoviridae*-type viruslike particle from station B28 d) *Styloviridae*-type viruslike particle with visible tail-end processes from station B18 in the Brisbane River; e) *Styloviridae*-type viruslike particle attached to bacterium from station B21 in the Brisbane River; f) Unidentified viruslike particle from station B21 in the Brisbane River. All scale bars = 50nm.

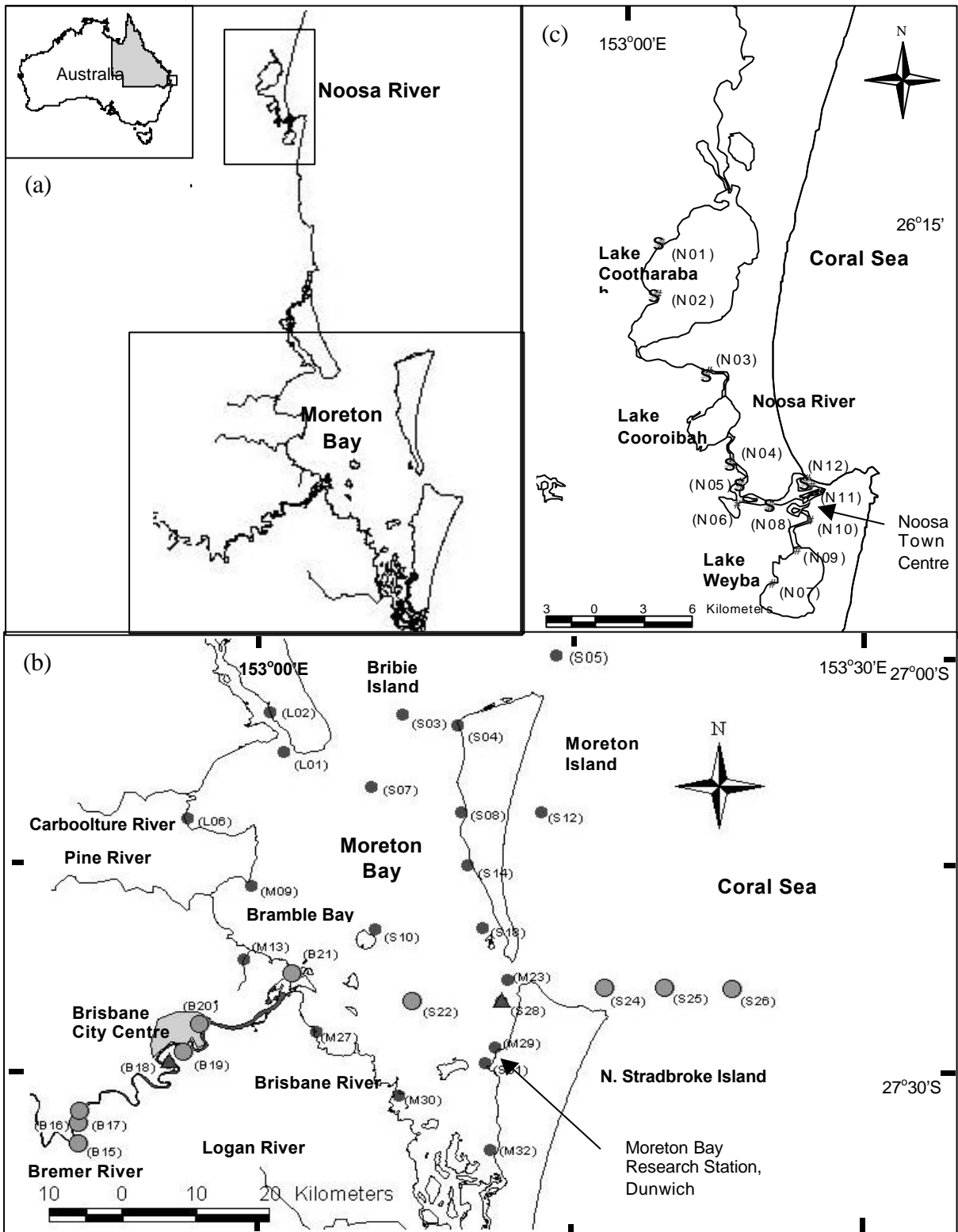
**FIGURE 8:** Correlation between benthic and pelagic virus abundance in the Brisbane River / Moreton Bay and the Noosa River estuaries.

**FIGURE 9:** Virus production along eutrophication gradient (a) and virus production per bacterium (b) nd – indicates below limits of detection.

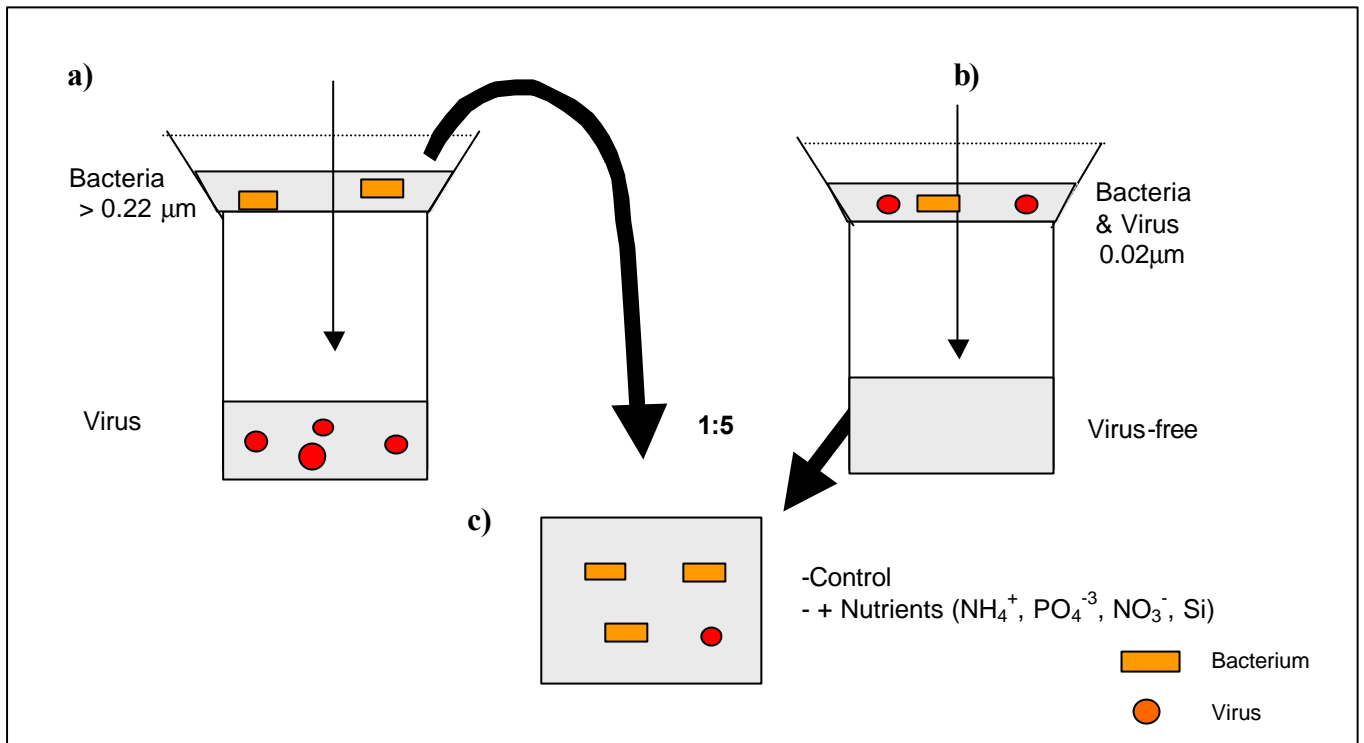
**FIGURE 10:** Effects of high molecular weight concentrate (HMWC) on photosynthesis in benthic microalgae of oligotrophic (right) and eutrophic (left) sediments over time

**FIGURE 11:** Effects of high molecular weight concentrate (HMWC) addition on photosynthetic parameters and biomass of benthic microalgae in eutrophic (a) and oligotrophic sediments (b) after 3 d.

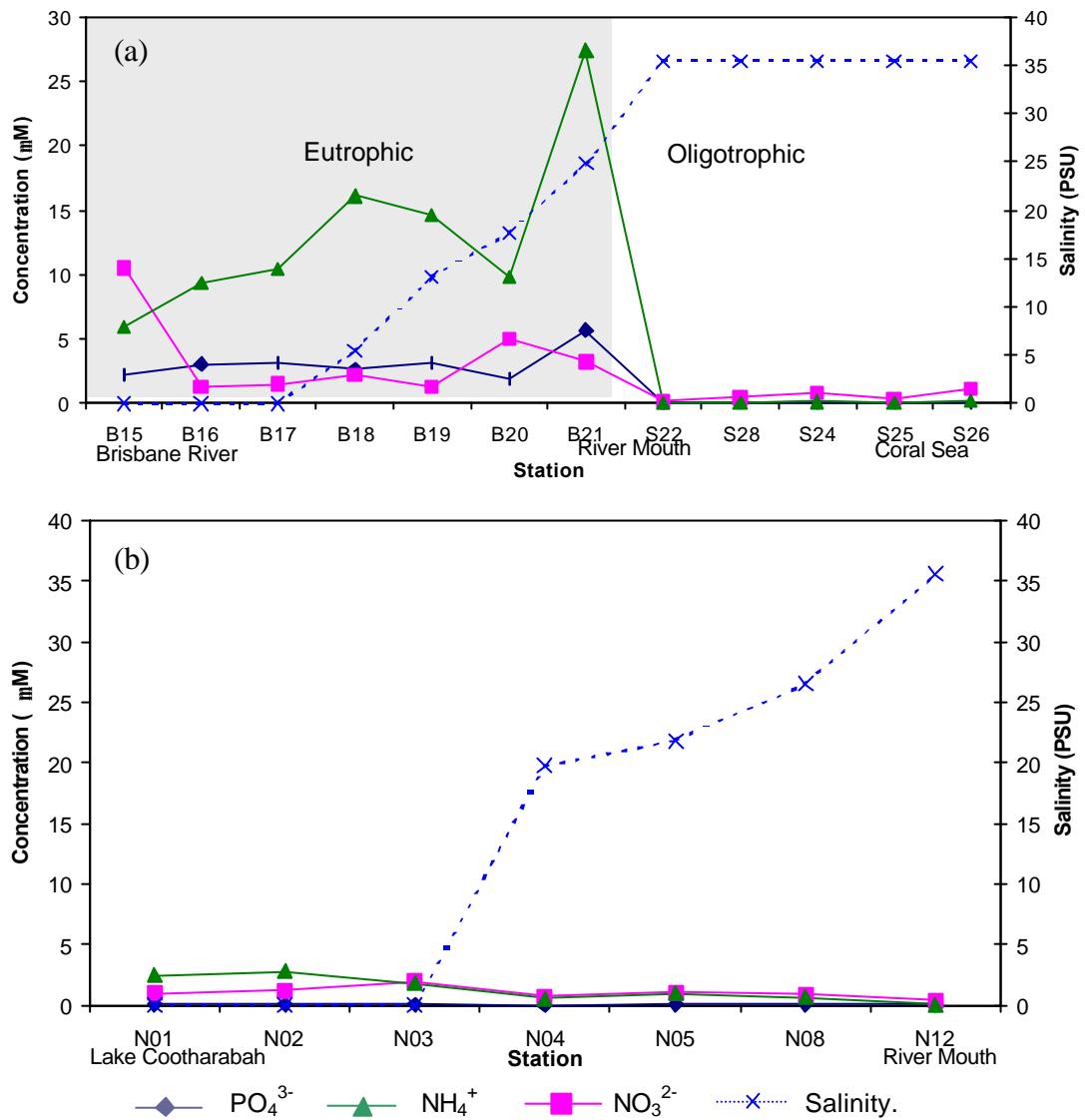
**FIGURE 12:** Effect of high molecular weight concentrate (HMWC) addition to eutrophic (station B18)(a) and oligotrophic (station S28) (b) phytoplankton *in vivo* chlorophyll fluorescence.



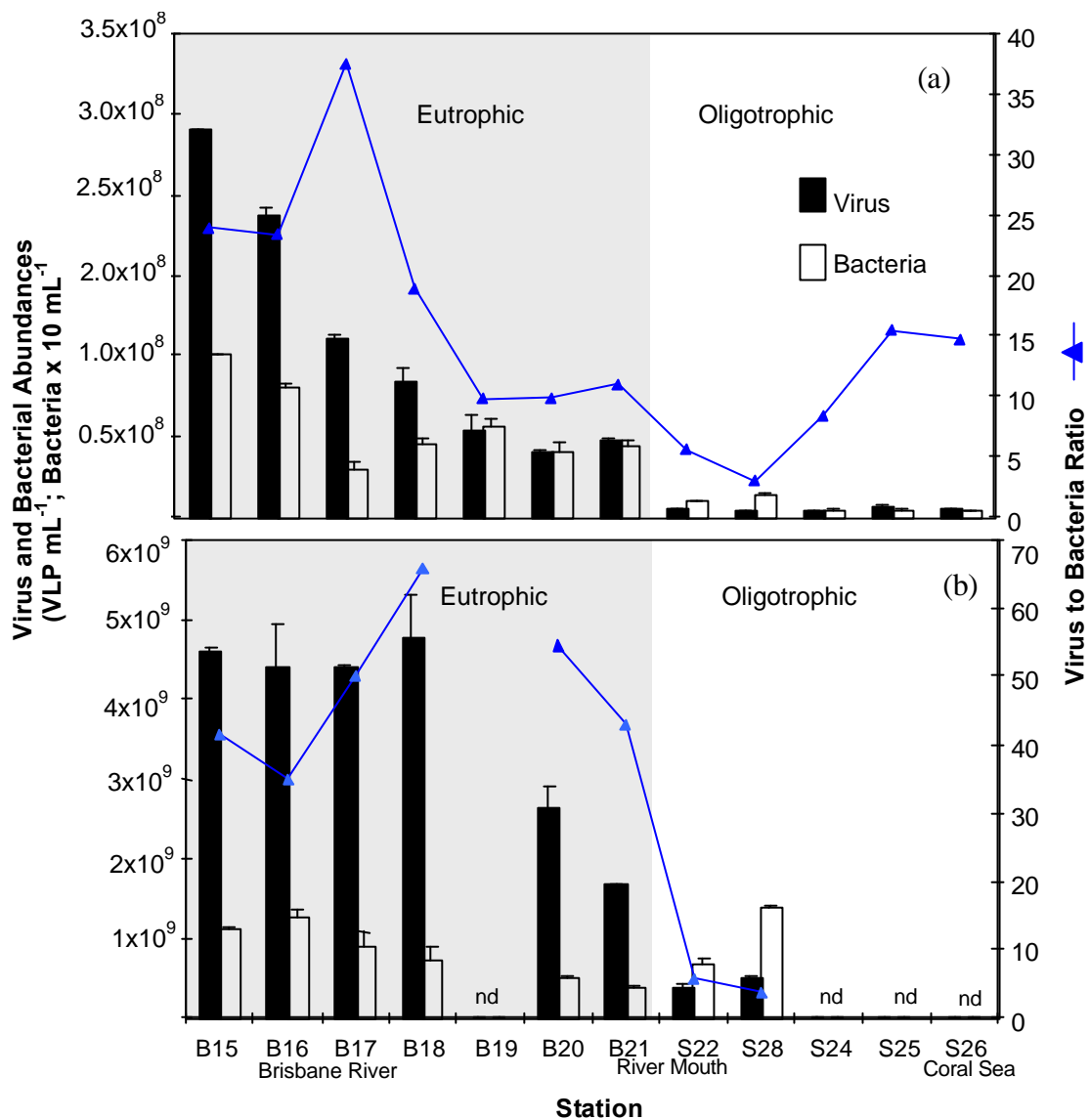
**FIGURE 1:** Location of estuaries (a) and sampling stations in the Brisbane River / Moreton Bay (b) and Noosa River (c) and estuaries. Station numbers indicated in brackets. ○ eutrophication/ salinity gradient stations  
 ▲ stations for benthic vertical distribution and high molecular weight concentrate (HMWC) addition experiments



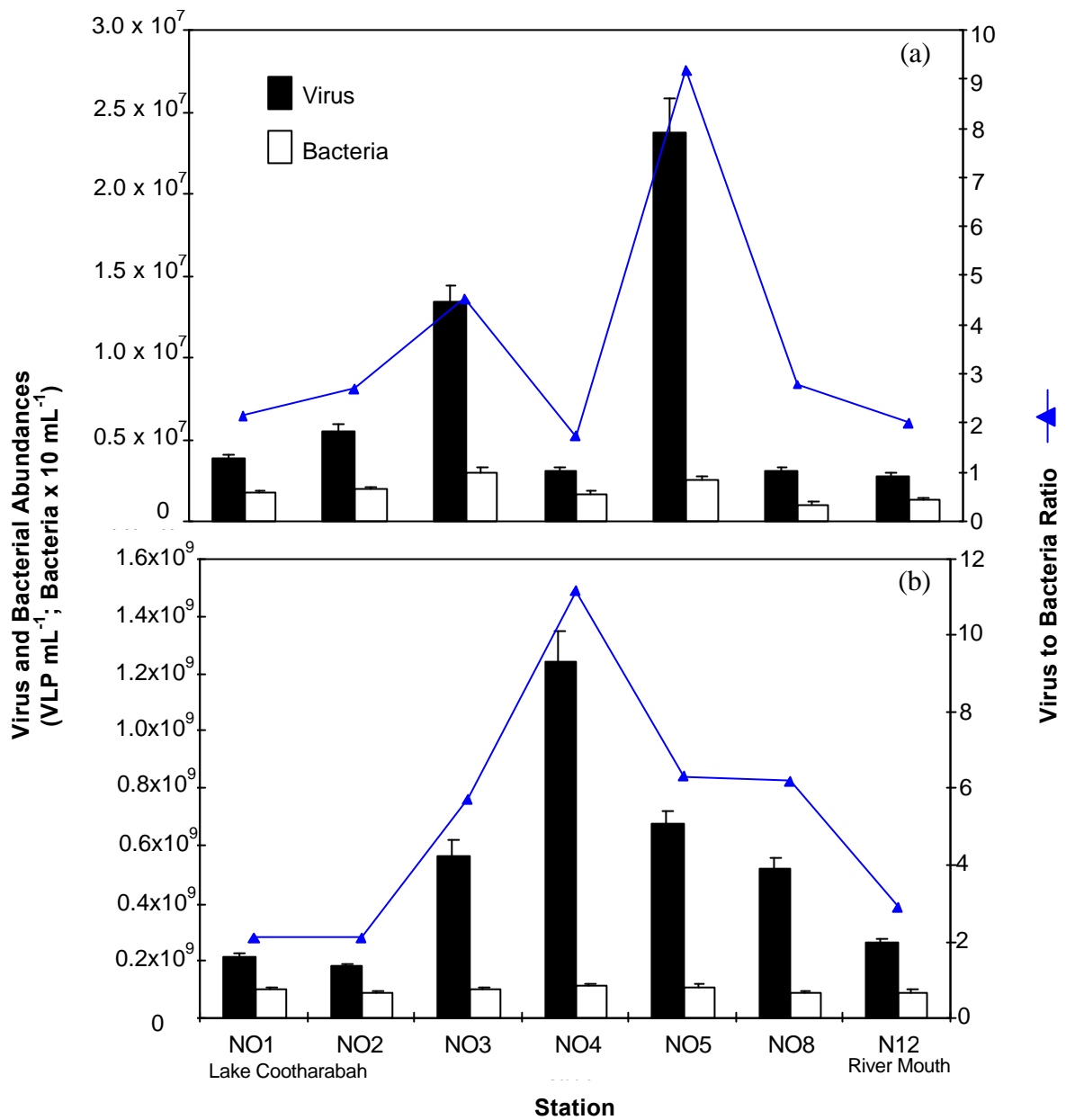
**FIGURE 2:** Method of virus production measurement a) Viruses removed by filtering through  $0.22\mu\text{m}$  Durapore filter, b) virus free filtrate (seawater filtered through  $0.02\mu\text{m}$  anodisc), c) Retentate is mixed with virus-free seawater in the ratio of 1:5 and virus abundance observed over 24 h.



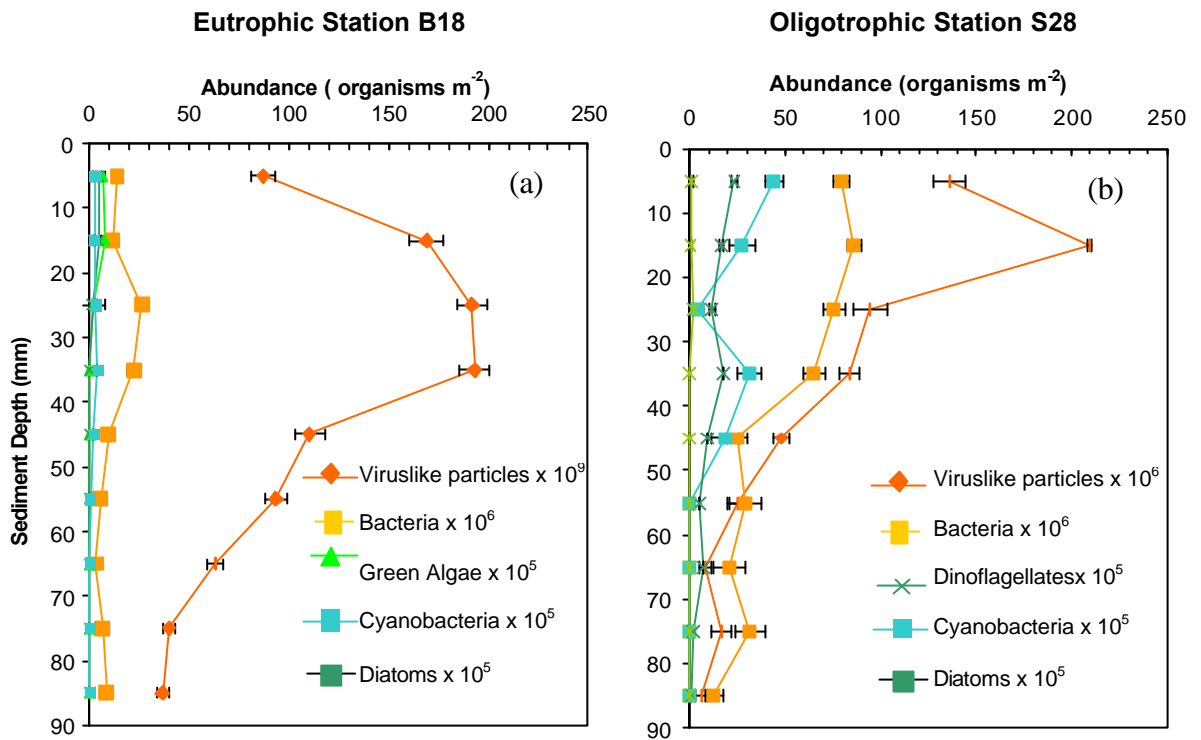
**FIGURE 3:** Inorganic nutrient concentrations and salinity along eutrophication gradient from the Brisbane River to Coral Sea in November 1999 (a) and along Noosa River in August 1999 (b) .



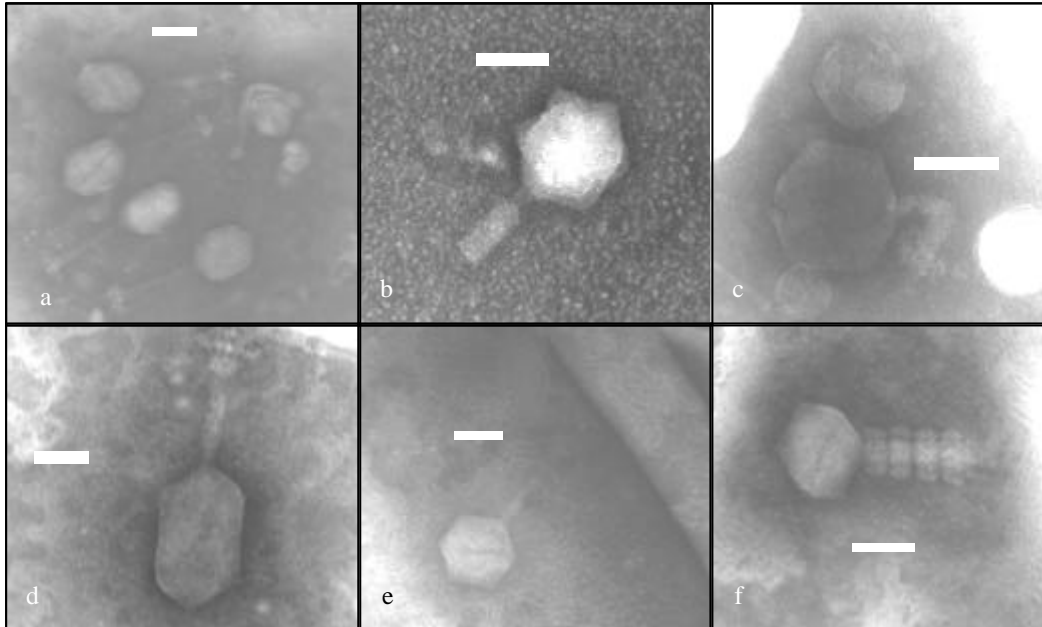
**FIGURE 4:** Viruslike particle and bacterial abundance and change in the ratio of viruses to bacteria along a gradient from the Brisbane River to Moreton Bay estuary in water column (a) and sediments (b) nd = no sediment was collected. Error bars indicate standard errors. (VLP = Virus-Like Particle).



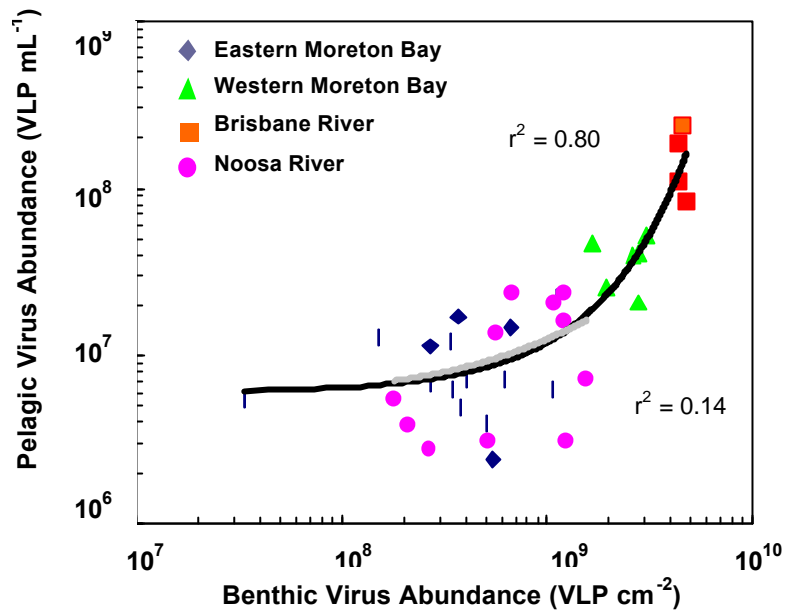
**FIGURE 5:** Viruslike particle and bacterial abundance and change in the ratio of viruses to bacteria along salinity gradient in Noosa River in water column (a) and sediments (b). Error bars indicate standard errors. (VLP = Virus-Like Particle)



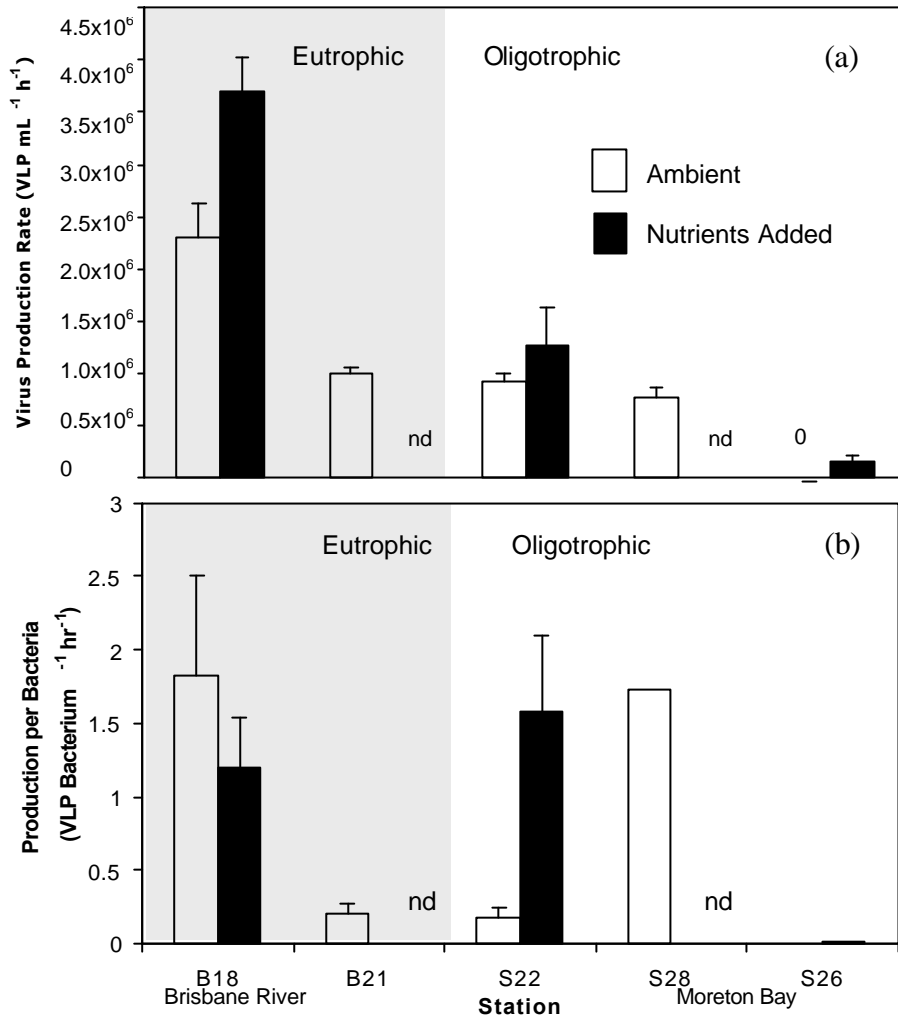
**FIGURE 6:** Vertical distribution of viruslike particles and hosts in eutrophic (a) and oligotrophic (b) sediments.



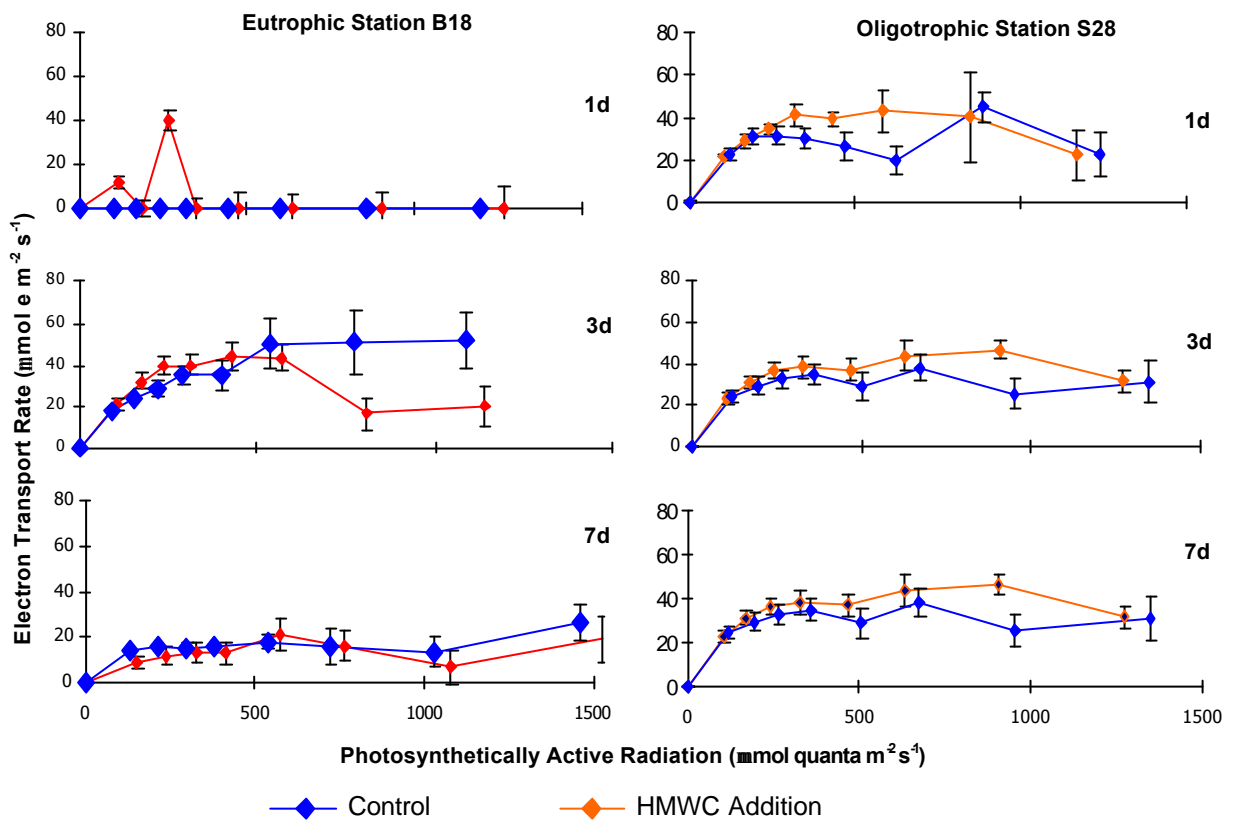
**FIGURE 7** Transmission electron micrographs of viruslike particles from the Brisbane River / Moreton Bay estuary: a) *Styloviridae*-type viruslike particle from station B18 in the Brisbane River; b) *Myoviridae*-type viruslike particle from station B21 in the Brisbane River; c) *Parvoviridae*-type viruslike particle from station B28 d) *Styloviridae*-type viruslike particle with visible tail-end processes from station B18 in the Brisbane River; e) *Styloviridae*-type viruslike particle attached to bacterium from station B21 in the Brisbane River; f) Unidentified viruslike particle from station B21 in the Brisbane River. All scale bars = 50nm



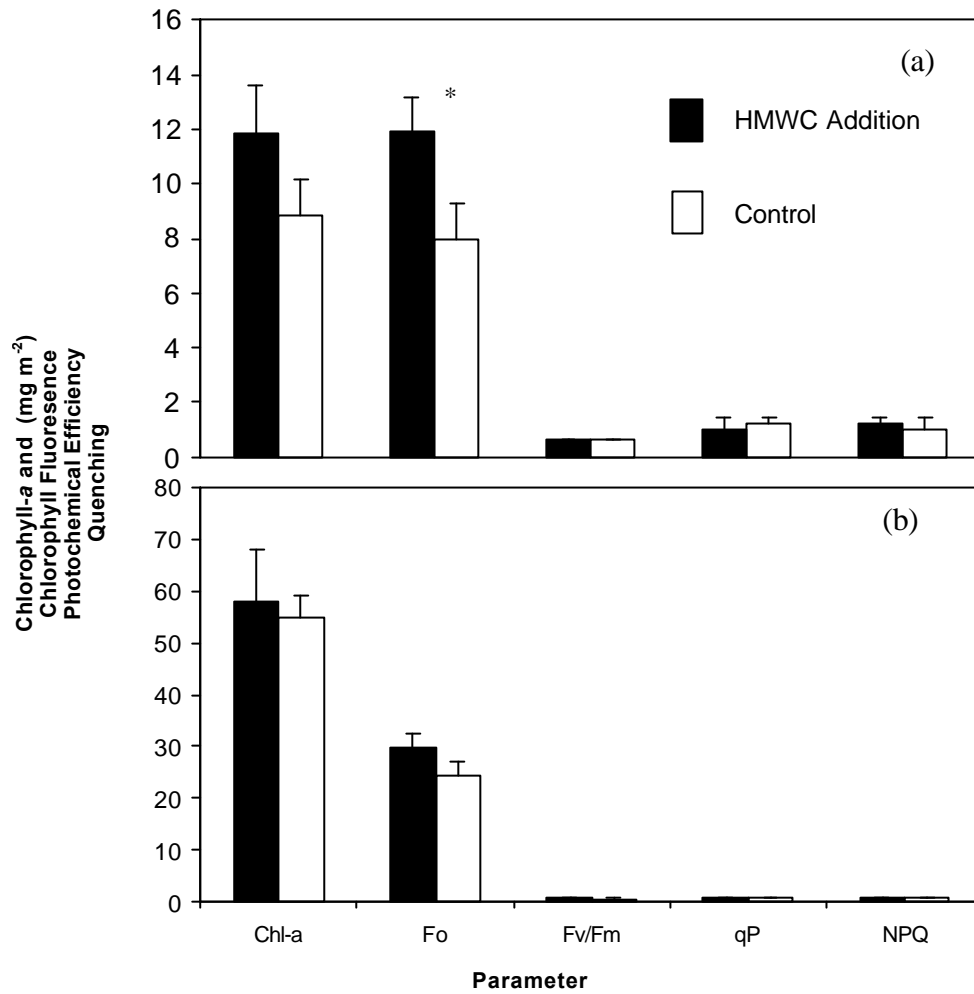
**FIGURE 8:** Correlation between benthic and pelagic virus abundance in the Brisbane River / Moreton Bay and the Noosa River estuaries. Solid line indicates linear regression for Brisbane River / Moreton Bay estuary and shorter grey line indicates linear regression for Noosa River estuary. (VLP = Virus-Like Particles)



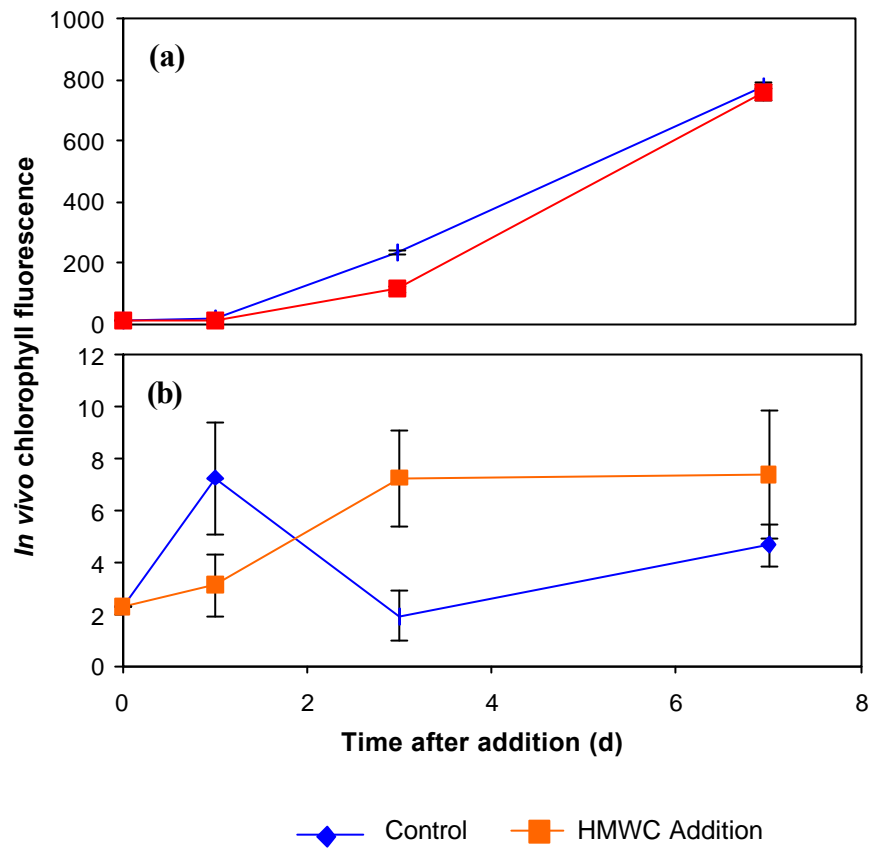
**FIGURE 9:** Virus production along eutrophication gradient (a) and virus production per bacterium (b) nd – indicates below limits of detection (VLP =Virus-Like Particles).



**FIGURE 10:** Effects of high molecular weight concentrate (HMWC) on photosynthesis in benthic microalgae of oligotrophic (right) and eutrophic (left) sediments over time



**FIGURE 11:** Effects of high molecular weight concentrate (HMWC) addition on photosynthetic parameters and biomass of benthic microalgae in eutrophic (a) and oligotrophic sediments (b) after 3 d. Error bars indicate standard errors. (Chl-a = Chlorophyll- $\alpha$ ; Fo= chlorophyll fluorescence; Fv/Fm= photochemical efficiency; qP= photochemical quenching quotient; NPQ= non-photochemical quenching).\* indicates significant ( $p < 0.05$ ) difference.



**FIGURE 12:** Effect of high molecular weight concentrate (HMWC) addition to eutrophic (station B18)(a) and oligotrophic (station S28) (b) phytoplankton *in vivo* chlorophyll fluorescence.

## APPENDICES

**APPENDIX 1:** Virus, bacteria and algal abundances along eutrophication and salinity gradient between stations B15 and S26 in the Brisbane River and Moreton Bay during Summer 1999/2000 (November to February) (VBR= virus-to-bacteria ratio)

**APPENDIX 2:** Virus, bacteria and algal abundances along salinity gradient between stations N01 and N12 in Noosa River in Spring 1999 (August). (VBR= virus-to-bacteria ratio)

**APPENDIX 3:** Ambient and nutrient enriched (30  $\mu\text{M}$   $\text{NH}_4$ , 200  $\mu\text{M}$   $\text{NO}_3$ , 20  $\mu\text{M}$   $\text{PO}_4$ , 66  $\mu\text{M}$   $\text{SiO}_3$ ) virus production along eutrophication gradient in the Brisbane River and Moreton Bay in February 2000.

**APPENDIX 4:** Microalgal species observed in phytoplankton at eutrophic (B16, B15, B17, B18, B19, B20, B21, M13 and M09) and oligotrophic (S11, S14, S08, S04, S28, S05, S31, S22) stations.

**APPENDIX 5:** Microalgal species observed in benthos at eutrophic (B16, B15, B17, B18, B19, B20, B21, M13 and M09) and oligotrophic (S11, S14, S08, S04, S28, S05, S31, S22) stations.

**APPENDIX 6: A.** Common phytoplankton in eutrophic portions of Brisbane River and Moreton Bay: a) *Coscinodiscus argus*, b) *Synura* sp., c) *Euglena* sp. and in oligotrophic portions d) *Ceratium fusus*, e) *Gymnodinium sanguineum*, f) *Trichodesmium erythreum*, g) *Thalassionema kariana* h) SYBR Green I-stained *Trichodesmium* trichome showing associated heterotrophic bacteria (indicated by arrow) **B.** Common benthic microalgae in eutrophic portions of the Brisbane River and Moreton Bay; a) *Navicula* sp., b) *Pleurosigma* sp. c) *Euglena* sp.; in oligotrophic sediments d) *Acanthnes* sp., e) *Amphidinium carterae*; and in the Noosa River f) *Amphidiniopsis kofoidii* and g) *Amphiprora* sp. Scale bars = 20 $\mu\text{m}$



**APPENDIX 1:** Virus, bacteria and algal abundances along eutrophication and salinity gradient between stations B15 and S26 in the Brisbane River and Moreton Bay during Summer 1999/2000 (November to February) (VBR= virus-to-bacteria ratio)

| Station <sup>a</sup>    | Latitude  | Longitude  | Plankton   |  |  |              | Benthos  |  |  |              |
|-------------------------|-----------|------------|--|--|--|--------------|--|--|--|--------------|
|                         |           |            | Virus  | Bacterial  | Microalgal   | VBR          | Virus  | Bacterial  | Microalgal   | VBR          |
|                         |           |            | Abundance<br>(x10 <sup>6</sup> VLP L <sup>-1</sup> ) | Abundance<br>(x10 <sup>6</sup> cells L <sup>-1</sup> ) | Abundance<br>(x10 <sup>5</sup> cells L <sup>-1</sup> ) |              | Abundance<br>(x10 <sup>8</sup> VLP m <sup>-2</sup> ) | Abundance<br>(x10 <sup>7</sup> cells m <sup>-2</sup> ) | Abundance<br>(x10 <sup>4</sup> cells m <sup>-2</sup> ) |              |
| B15                     | 27°34.2'S | 152°54.0'E | 239.90   | 10.02  | 9.24   | 23.93        | 46.09  | 11.11  | 1.55   | 41.47        |
| B16                     | 27°33.5'S | 152°54.2'E | 110.26   | 2.93   | 11.18  | 37.62        | 44.08  | 8.81   | 2.00   | 50.05        |
| B17                     | 27°29.5'S | 153°00.6'E | 83.67  | 4.44   | 5.36   | 18.85        | 47.69  | 7.23   | 0.71   | 65.91        |
| B18                     | 27°19.4'S | 153°23.6'E | 17.16  | 0.98   | 32.38  | 17.57        | 3.69   | 10.28  | 14.06  | 3.59         |
| B19                     | 27°28.9'S | 153°01.6'E | 53.74  | 5.55   | 2.79   | 9.68         | nd <sup>c</sup>                                      | nd   | Nd   | nd           |
| B20                     | 27°26.6'S | 153°03.1'E | 39.30  | 3.98   | 2.42   | 9.88         | 26.30  | 4.82   | 1.29   | 54.52        |
| B21                     | 27°22.7'S | 153°09.5'E | 47.29  | 4.35   | 12.31  | 10.88        | 16.69  | 3.88   | 3.37   | 43.03        |
| S22                     | 27°25.0'S | 153°18.6'E | 4.93   | 0.89   | 24.14  | 5.52         | 3.77   | 6.71   | 16.15  | 5.63         |
| S28                     | 27°25.0'S | 153°25.0'E | 3.91   | 1.32   | 2.21   | 2.97         | 5.03   | 13.63  | 21.80  | 3.69         |
| S24                     | 27°24.0'S | 153°32.5'E | 3.57   | 0.42   | 0.00   | 8.40         | nd   | nd   | nd   | nd           |
| S25                     | 27°24.0'S | 153°37.0'E | 6.57   | 0.42   | 0.14   | 15.47        | nd   | nd   | nd   | nd           |
| S26                     | 27°24.0'S | 153°42.0'E | 4.98   | 0.34   | 0.08   | 14.67        | nd   | nd   | nd   | nd           |
| <b>Mean<sup>b</sup></b> |           |            | <b>34.50</b>   | <b>2.15</b>  | <b>1.31</b>  | <b>13.20</b> | <b>16.00</b>   | <b>7.06</b>  | <b>5.70</b>  | <b>24.60</b> |
| <b>Standard Error</b>   |           |            | <b>9.94</b>  | <b>0.43</b>  | <b>0.25</b>  | <b>1.27</b>  | <b>3.19</b>  | <b>0.84</b>  | <b>1.13</b>  | <b>4.27</b>  |

<sup>a</sup> Station positions are indicated on Figure 1.

<sup>b</sup> Mean for all stations in Moreton Bay (n=32 for water column and n=28 for benthos)

<sup>c</sup> Sediment was not collected at stations where nd is indicated

**APPENDIX 2:** Virus, bacteria and algal abundances along salinity gradient between stations N01 and N12 in Noosa River in Spring 1999 (August). (VBR= virus-to-bacteria ratio)

| <i>Station<sup>a</sup></i>     | Latitude  | Longitude  | Plankton   |  |   | Benthos     |  |  |   |              |
|--------------------------------|-----------|------------|--|--|---|-------------|--|--|---|--------------|
|                                |           |            | Virus Abundance<br>(x10 <sup>6</sup> VLP L <sup>-1</sup> ) | Bacterial Abundance<br>(x10 <sup>6</sup> cells L <sup>-1</sup> ) | Microalgal Abundance<br>(x10 <sup>5</sup> cells L <sup>-1</sup> ) | VBR         | Virus Abundance<br>(x10 <sup>8</sup> VLP m <sup>-2</sup> ) | Bacterial Abundance<br>(x10 <sup>7</sup> cells m <sup>-2</sup> ) | Microalgal Abundance<br>(x10 <sup>4</sup> cells m <sup>-2</sup> ) | VBR          |
| N01                            | 26°15.4'S | 152°59.9'E | 3.87   | 1.78   | 0.85  | 2.17        | 2.12   | 10.07  | 0.52  | 2.10         |
| N02                            | 26°17.1'S | 152°59.8'E | 5.52   | 2.04   | 1.10  | 2.71        | 1.80   | 8.60   | 0.30  | 2.10         |
| N03                            | 26°19.6'S | 153°01.5'E | 13.46  | 2.97   | 0.55  | 4.53        | 5.64   | 9.86   | 0.02  | 5.72         |
| N04                            | 26°22.5'S | 153°02.2'E | 3.06   | 1.74   | 1.87  | 1.76        | 12.41  | 11.11  | 0.04  | 11.17        |
| N05                            | 26°23.2'S | 153°02.5'E | 23.83  | 2.59   | 0.97  | 9.20        | 6.77   | 10.69  | 0.04  | 6.33         |
| N08                            | 26°23.9'S | 153°03.5'E | 3.09   | 1.10   | 0.91  | 2.79        | 5.20   | 8.39   | 12.87   | 6.20         |
| N12                            | 26°23.1'S | 153°04.7'E | 2.77   | 1.39   | 1.13  | 2.00        | 2.66   | 9.23   | 1.98  | 2.89         |
| <b>Mean (n=12)<sup>b</sup></b> |           |            | <b>11.00</b>   | <b>1.84</b>  | <b>1.24</b>   | <b>6.01</b> | <b>7.93</b>  | <b>8.25</b>  | <b>1.12</b>   | <b>11.70</b> |
| <b>Std Error</b>               |           |            | <b>2.37</b>  | <b>0.16</b>  | <b>0.22</b>   | <b>1.24</b> | <b>1.46</b>  | <b>0.70</b>  | <b>0.45</b>   | <b>2.89</b>  |

<sup>a</sup> Only microalgae enumerated by SYBR Green I staining included

<sup>b</sup> Mean for all stations in Noosa River

**APPENDIX 3:** Ambient and nutrient enriched (30  $\mu\text{M}$   $\text{NH}_4$  , 200  $\mu\text{M}$   $\text{NO}_3$ , 20  $\mu\text{M}$   $\text{PO}_4$ , 66  $\mu\text{M}$   $\text{SiO}_3$ ) virus production along eutrophication gradient in the Brisbane River and Moreton Bay in February 2000. (VLP = Virus-Like Particles; VBR = Virus to Bacteria Ratio)

| Trophic Condition | Station | Mean <sup>a</sup> Rate<br>(VLP x 10 <sup>5</sup> mL <sup>-1</sup> hr <sup>-1</sup> ) |                  | Mean Specific Rate<br>(VBR mL <sup>-1</sup> hr <sup>-1</sup> ) |                  | Turnover Time (h) |
|-------------------|---------|--|------------------|--|------------------|-------------------|
|                   |         | <i>Ambient</i>   | <i>Nutrients</i> | <i>Ambient</i>   | <i>Nutrients</i> |                   |
|                   |         | Eutrophic  | B18              | 23.0   | 37.0             |                   |
|                   | B21     | 9.2  | nd               | 0.21   | nd               | 47.3              |
| Oligotrophic      | S22     | 9.3  | 12.8             | 1.58   | 0.17             | 15.7              |
|                   | S28     | 7.8  | Nd               | 1.73   | nd               | 49.9              |
|                   | S26     | 0  | 2.5              | 0  | 0.01             | n/a               |

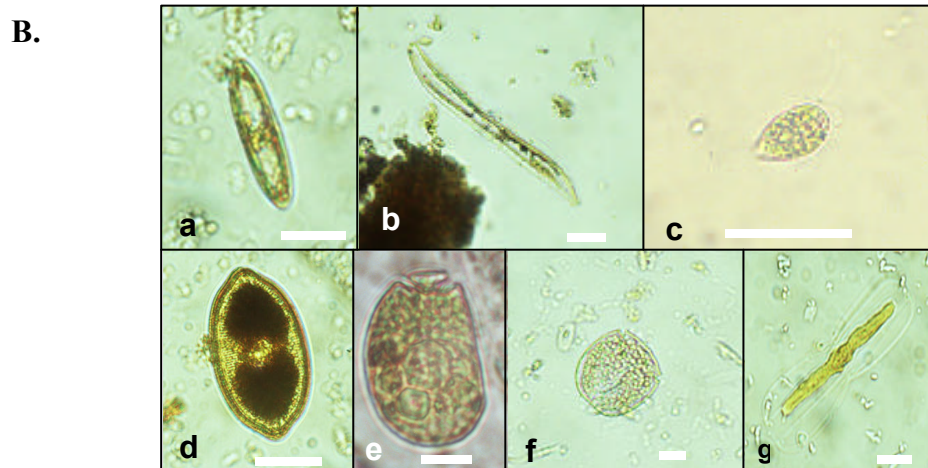
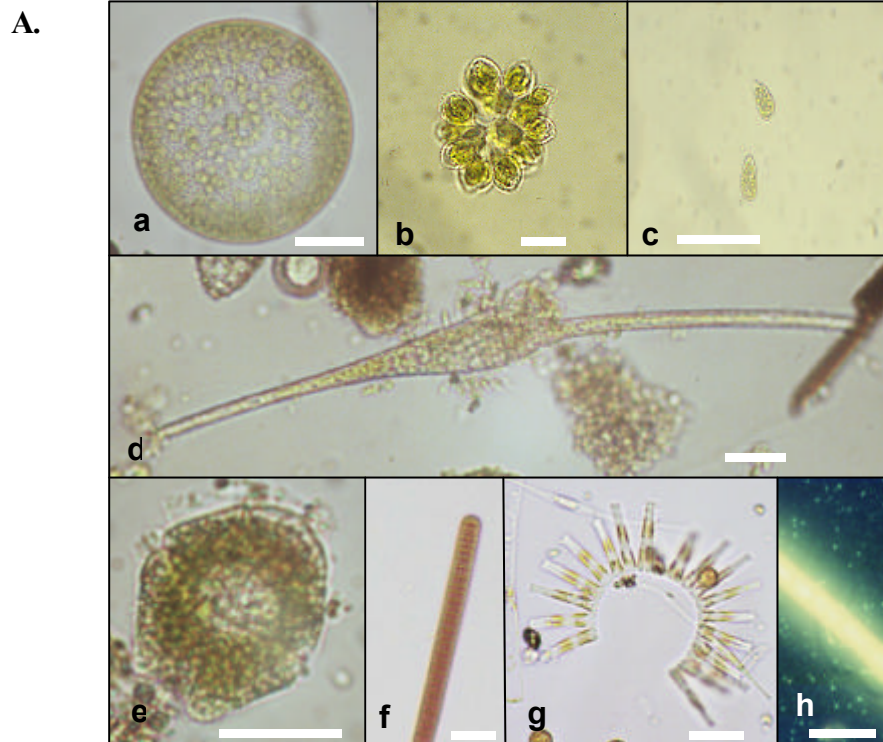
<sup>a</sup>n=3 replicates per station

**APPENDIX 4:** Microalgal species observed in phytoplankton at eutrophic (B16 , B15 , B17 , B18 , B19 , B20 , B21 , M13 and M09) and oligotrophic (S11 , S14 , S08 , S04 , S28 , S05 , S31 , S22) stations

| Stations                     | Division                              | Species                               | Mean Abundance x 10 <sup>2</sup> L <sup>-1</sup> |       |      |       |
|------------------------------|---------------------------------------|---------------------------------------|--|-------|------|-------|
| Eutrophic                    | <b>Bacillariophyta</b><br>(Centrales) | <i>Cyclotella littoralis</i>          | 75.5   | ±     | 57.0 |       |
|                              |                                       | <i>Coscinodiscus argus</i>            | 15.0   | ±     | 5.8  |       |
|                              |                                       | <i>Skeletonema costatum</i>           | 13.3   | ±     | 12.6 |       |
|                              |                                       | <i>Thalassiosira rotula</i>           | 6.7  | ±     | 6.3  |       |
|                              |                                       | (Pennales)                            | <i>Pleurosigma spp.</i>                          | 33.3  | ±    | 20.5  |
|                              |                                       |                                       | <i>Navicula spp.</i>                             | 20.0  | ±    | 5.9   |
|                              |                                       |                                       | <i>Acanthnes sp.</i>                             | 9.4   | ±    | 4.8   |
|                              |                                       |                                       | <i>Cylindrotheca closterium</i>                  | 7.8   | ±    | 4.9   |
|                              |                                       |                                       | <i>Thalassionema spp.</i>                        | 5.6   | ±    | 3.6   |
|                              |                                       | <i>Plagiotropis sp.</i>               | 4.4  | ±     | 1.8  |       |
|                              | <b>Dinophyta</b>                      | <i>Gonyaulax spp.</i>                 | 14.4   | ±     | 13.7 |       |
|                              |                                       | <i>Gymnodinium sanguineum</i>         | 9.4  | ±     | 5.7  |       |
|                              |                                       | <i>Protoperidinium divergens</i>      | 3.9  | ±     | 3.7  |       |
|                              | <b>Cyanophyta</b>                     | <i>Oscillatoria spp.</i>              | 1.7  | ±     | 1.6  |       |
|                              | <b>Chlorophyta</b>                    | <i>Botrycoccus spp.</i>               | 3.3  | ±     | 1.4  |       |
|                              |                                       | <i>Scenedesmus</i>                    | 0.6  | ±     | 0.5  |       |
|                              | Oligotrophic                          | <b>Bacillariophyta</b><br>(Centrales) | <i>Leptocylindrus sp.</i>                        | 465.0 | ±    | 170.4 |
|                              |                                       |                                       | <i>Coscinodiscus argus</i>                       | 262.5 | ±    | 70.8  |
|                              |                                       |                                       | <i>Rhizosolenia spp.</i>                         | 247.5 | ±    | 122.4 |
| <i>Guinardia sp.</i>         |                                       |                                       | 195.0  | ±     | 99.3 |       |
| <i>Cyclotella littoralis</i> |                                       |                                       | 142.5  | ±     | 75.2 |       |
| <i>Thalassiosira rotula</i>  |                                       |                                       | 45.0   | ±     | 31.6 |       |
| (Pennales)                   |                                       |                                       | <i>Navicula spp.</i>                             | 217.5 | ±    | 80.1  |
|                              |                                       |                                       | <i>Acanthnes sp.</i>                             | 135.0 | ±    | 97.5  |
|                              |                                       |                                       | <i>Thalassionema spp.</i>                        | 120.0 | ±    | 61.0  |
|                              |                                       | <i>Pleurosigma spp.</i>               | 37.5   | ±     | 22.5 |       |
|                              |                                       | <i>Plagiotropis sp.</i>               | 22.5   | ±     | 15.8 |       |
|                              |                                       | <i>Cylindrotheca closterium</i>       | 7.5  | ±     | 7.5  |       |
| <b>Dinophyta</b>             |                                       | <i>Gymnodinium sanguineum</i>         | 75.0   | ±     | 29.5 |       |
|                              |                                       | <i>Ceratium fusus</i>                 | 22.5   | ±     | 22.5 |       |
|                              |                                       | <i>Protoperidinium divergens</i>      | 22.5   | ±     | 11.0 |       |
|                              |                                       | <i>Prorocentrum micans</i>            | 15.0   | ±     | 9.8  |       |
|                              |                                       | <i>Gonyaulax sp.</i>                  | 15.0   | ±     | 9.8  |       |

**APPENDIX 5:** Microalgal species observed in benthos at eutrophic (B16 , B15 , B17 , B18 , B19 , B20 , B21 , M13 and M09) and oligotrophic (S11 , S14 , S08 , S04 , S28 , S05 , S31 , S22) stations

| Station                      | Division                              | Species                               | Average Abundance<br>$\times 10^3 \text{ cm}^{-2}$ |      |      |      |
|------------------------------|---------------------------------------|---------------------------------------|--|------|------|------|
| Eutrophic                    | <b>Bacillariophyta</b><br>(Centrales) | <i>Cyclotella littoralis</i>          | 3.3  | ±    | 1.5  |      |
|                              |                                       | <i>Coscinodiscus argus</i>            | 1.6  | ±    | 0.5  |      |
|                              |                                       | <i>Melosira sulcata</i>               | 1.6  | ±    | 0.7  |      |
|                              |                                       | <i>Odontella</i>                      | 0.1  | ±    | 0.0  |      |
|                              | (Pennales)                            | <i>Navicula spp.</i>                  | 4.8  | ±    | 1.7  |      |
|                              |                                       | <i>Pleurosigma spp.</i>               | 1.8  | ±    | 0.4  |      |
|                              |                                       | <i>Plagiotropis spp.</i>              | 1.6  | ±    | 1.4  |      |
|                              |                                       | <i>Cylindrotheca closterium</i>       | 0.3  | ±    | 0.3  |      |
|                              |                                       | <i>Gymnodinium sp.</i>                | 0.1  | ±    | 0.1  |      |
|                              | <b>Dinophyta</b>                      | <i>Prorocentrum lima</i>              | 0.04   | ±    | 0.03 |      |
|                              |                                       | <b>Cyanophyta</b>                     | <i>Oscillatoria spp.</i>                           | 0.2  | ±    | 0.2  |
|                              |                                       | <b>Chlorophyta</b>                    | <i>Euglena sp.</i>                                 | 0.03 | ±    | 0.03 |
|                              | Oligotrophic                          | <b>Bacillariophyta</b><br>(Centrales) | <i>Skeletonema costatum</i>                        | 3.8  | ±    | 3.8  |
| <i>Thalssiosira rotula</i>   |                                       |                                       | 2.6  | ±    | 1.8  |      |
| <i>Coscinodiscus argus</i>   |                                       |                                       | 2.6  | ±    | 2.1  |      |
| <i>Cyclotella littoralis</i> |                                       |                                       | 1.3  | ±    | 0.9  |      |
| (Pennales)                   |                                       | <i>Odontella sp.</i>                  | 0.4  | ±    | 0.4  |      |
|                              |                                       | <i>Navicula spp.</i>                  | 128.9  | ±    | 64.8 |      |
|                              |                                       | <i>Bacillaria paxillifera</i>         | 84.9   | ±    | 78.4 |      |
|                              |                                       | <i>Plagiotropis spp.</i>              | 39.3   | ±    | 21.0 |      |
|                              |                                       | <i>Pleurosigma spp.</i>               | 15.4   | ±    | 8.5  |      |
|                              |                                       | <i>Amphora sp.</i>                    | 14.9   | ±    | 12.7 |      |
|                              |                                       | <i>Acanthnes sp.</i>                  | 8.9  | ±    | 8.7  |      |
|                              |                                       | <i>Cylindrotheca closterium</i>       | 3.8  |      | 1.5  |      |
| <b>Dinophyta</b>             |                                       | <i>Prorocentrum lima</i>              | 5.6  | ±    | 2.9  |      |
|                              |                                       | <i>Gymnodinium sp.</i>                | 2.7  | ±    | 1.1  |      |
|                              |                                       | <i>Amphidinium carterae</i>           | 1.7  | ±    | 1.3  |      |
|                              |                                       | <i>Amphidiniopsis kofoidii</i>        | 0.4  | ±    | 0.4  |      |
| <b>Cyanophyta</b>            | <i>Oscillatoria sp.</i>               | 8.1                                   | ±  | 5.0  |      |      |



**APPENDIX 6: A.** Common phytoplankton in eutrophic portions of Brisbane River and Moreton Bay: a) *Coscinodiscus argus*, b) *Synura* sp., c) *Euglena* sp. and in oligotrophic portions d) *Ceratium fusus*, e) *Gymnodinium sanguineum*, f) *Trichodesmium erythreum*, g) *Thalassionema kariana* h) SYBR Green I-stained *Trichodesmium* trichome showing associated heterotrophic bacteria **B.** Common benthic microalgae in eutrophic portions of the Brisbane River and Moreton Bay; a) *Navicula* sp., b) *Pleurosigma* sp. c) *Euglena* sp.; in oligotrophic sediments d) *Acanthnes* sp., e) *Amphidinium carterae*; and in the Noosa River f) *Amphidiniopsis kofoidii* and g) *Amphiprora* sp. Scale bars = 20µm

## REFERENCES

- Balanchandran, S. & Osmond, C. 1994. Susceptibility of tobacco leaves to photoinhibition following infection with two strains of tobacco mosaic virus under different light and nutrition regimes. *Plant Physiology* **104**, 1051-1057.
- Bergh, O., Borsheim, K. Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. *Nature* **340**, 467-468.
- Bird, D., Maranger, R. & Karl, D. 1993. Palmer LTER: aquatic virus abundances near the Antarctic Peninsula. *Antarctic Journal of the United States* **28**, 234-235.
- Blanchard, G. F. 1990. Overlapping microscale dispersion patterns of meiofauna and microphytobenthos. *Marine Ecology Progress Series* **68**, 101-111.
- Bosch, A., Lucena, F., Girones, R. & Jofre, J. 1988. Occurrence of enteroviruses in marine sediment along the coast of Barcelona, Spain. *Canadian Journal of Microbiology* **34**, 921-924.
- Bowen, B., Ward, T., Butler, A., Cosser, P., Holmes, N., Staples, D., Zann, L. & Haines, A. 1996. Estuaries and the sea. In *State of the Environment Australia 1996* (Lowe, I., eds). CSIRO Publishing, Melbourne, pp. .
- 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., 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.
- 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.
- Cochran, P. K., Kellogg, C. A. & Paul, J. H. 1998. Prophage induction of indigenous marine lysogenic bacteria by environmental pollutants. *Marine Ecology Progress Series* **164**, 125-133.
- Cochran, P. K. & Paul, J. H. 1998. Seasonal abundance of lysogenic bacteria in a subtropical estuary. *Applied and Environmental Microbiology* **64**, 2308-2312.
- Coles, R. G. & Greenwood, J. G. 1983. Seasonal movement and size distribution of three commercially important Australian prawn species (Crustacea: Penaeidae) within an estuarine system. *Australian Journal of Marine and Freshwater Research* **34**, 727-743.

- 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.
- Doblin, M. A., Blackburn, S. I. & Hallegraeff, G. M. 1999. Growth and biomass stimulation of the toxic dinoflagellate *Gymnodinium catenatum* (Graham) by dissolved organic substances. *Journal of Experimental Marine Biology and Ecology* **236**, 33-47.
- Fenchel, T. & Staarup, B. J. 1971. Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. *Oikos* **22**, 172-182.
- 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. & Noble, R. T. 1995. Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnology and Oceanography* **40**, 1236-1242.
- Fuhrman, J. A. & Suttle, C. A. 1993. Viruses in marine planktonic systems. *Oceanography* **6**, 51-63.
- Gonzales, J. M. & Suttle, C. A. 1993. Grazing by marine nanoflagellates on viruses and virus-sized particles: Ingestion and digestion. *Marine Ecology Progress Series* **94**, 1-10.
- Hartig, P., Wolfstein, K., Lippemeier, S. & Colijn, F. 1998. Photosynthetic activity of natural microphytobentos populations measured by fluorescence (PAM) and <sup>14</sup>C-tracer methods: A comparison. *Marine Ecology Progress Series* **166**, 53-62.
- Heil, C. 1996. *The influence of dissolved humic material (humic, fulvic and hydrophilic acids) on marine phytoplankton*. PhD Dissertation, University of Queensland, Brisbane.
- Heil, C. A., Chaston, K. A., Jones, A., Bird, P., Costanzo, S., Longstaff, B. & Dennison, W. C. submitted. Benthic Microalgae in coral reef sediments. *Submitted to Coral Reefs In prep* .
- Heldal, M. & Bratbak, G. 1991. Production and decay of viruses in aquatic environments. *Marine Ecology Progress Series* **72**, 205-212.
- Jiang, S. C. & Paul, J. H. 1998. Gene transfer by transduction in the marine environment. *Applied and Environmental Microbiology* **64**, 2780-2787.

- Kendrick, G. A., Jacoby, C. A. & Heinemann, D. 1996. Benthic microalgae: comparisons of chlorophyll *a* in mesocosms and field sites. In *Fifteenth International Seaweed Symposium* (Lindstrom, S. C. & Chapman, D. J., eds). Kluwer, Belgium, pp. 283-289.
- Kendrick, G. A., Langtry, S., Fitzpatrick, J., Griffiths, R. & Jacoby, C. A. 1998. Benthic microalgae and nutrient dynamics in wave-disturbed environments in Marmion Lagoon, Western Australia, compared with less disturbed mesocosms. *Journal of Experimental Marine Biology and Ecology* **228**, 83-105.
- Kepner, R. L., Wharton, R. A. & Suttle, C. A. 1998. Viruses in Antarctic lakes. *Limnology and Oceanography* **43**, 1754-1761.
- Klut, M. E. & Stockner, J. G. 1990. Virus-like particles in an ultra-oligotrophic lake on Vancouver Island, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 725-730.
- Lewis, G., Loutit, M. W. & Austin, F. J. 1986. Enteroviruses in mussels and marine sediments and depuration of naturally accumulated viruses by green lipped mussels (*Perna canaliculus*). *New Zealand Journal of Marine and Freshwater Research* **20**, 431-437.
- Light, B. R. & Beardall, J. 1998. Distribution and spatial variation of benthic microalgal biomass in a temperate, shallow-water marine system. *Aquatic Botany* **61**, 39-54.
- LundHansen, L. C., Petersson, M. & Nurjaya, W. 1999. Vertical sediment fluxes and wave-induced sediment resuspension in a shallow-water coastal lagoon. *Estuaries* **22**, 39-46.
- 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, P. & Bird, D. F. 1996. High concentrations of viruses in the sediments of Lake Gilbert, Quebec. *Microbial Ecology* **31**, 141-151.
- 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.
- Mathias, C. B., Kirschner, A. K. T. & Velimirov, B. 1995. Seasonal variations of virus abundance and viral control of the bacterial production in a backwater system of the Danube River. *Applied and Environmental Microbiology* **61**, 3734-3740.

- Middelboe, M., Jorgensen, N. O. G. & Kroer, N. 1996. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. *Applied and Environmental Microbiology* **62**, 1991-1997.
- Milligan, K. L. D. & Coper, E. M. 1994. Isolation of virus capable of lysing the Brown Tide microalga, *Aureococcus anophagefferens*. *Science* **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.
- 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.
- Nilsson, C. & Sundbaeck, K. 1991. Growth and nutrient uptake studied in sand-agar microphytobenthic communities. *Journal of Experimental Marine Biology and Ecology* **153**, 207-226.
- Noble, R. T. & Fuhrman, J. A. 1997. Virus decay and its causes in coastal waters. *Applied and Environmental Microbiology* **63**, 77-83.
- Noble, R. T. & Fuhrman, J. A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology* **14**, 113-118.
- Parsons, T. R., Maita, Y. & Lalli, C. M. 1985. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, Oxford.
- 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. 1974. Intranuclear virus-like particles in the marine alga *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.
- Prescott, L. M., Harley, J. P. & Klein, D. A. 1993. *Microbiology*. Wm. C. Brown Publishers, Dubuque.
- Proctor, L. M. & Fuhrman, J. A. 1992. Mortality of marine bacteria in response to enrichments of the virus size fraction from seawater. *Marine Ecology Progress Series* **87**, 283-293.
- Qin, J., Culver, D. A. & Yu, N. 1995. Effect of organic fertiliser on heterotrophs and autotrophs: implications for water quality. *Aquaculture Research* **26**, 911-920.
- Rybarczyk, H., Elkaim, B., Wilson, J. G. & Loquet, N. 1996. L'eutrophisation en Baie de Somme: mortalités des peuplements benthiques par anoxie. *Oceanologica Acta* **19**, 131-140.
- Sicko-Goad, L. & Walker, G. 1979. Viroplasm and large virus-like particles in the dinoflagellate *Gymnodinium uberrimum*. *Protoplasma* **99**, 203-210.
- Soyer, M. O. 1978. Particules de type viral et filaments trichocystoïdes chez les dinoflagelles. *Protistologica* **14**, 53-58.
- Spector, D. L. 1984. *Dinoflagellates*. Academic Press, Orlando.
- Steward, G. F., Smith, D. C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. *Marine Ecology Progress Series* **131**, 287-300.
- Steward, G. F., Wikner, J., Cochlan, W. P., Smith, D. C. & Azam, F. 1992. Estimation of Virus Production in the sea, II: Field results. *Marine Microbial Food Webs* **6**, 79-90.
- 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. 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. 1991. Use of ultrafiltration to isolate viruses from seawater which are pathogens of marine phytoplankton. *Applied and Environmental Microbiology* **57**, 721-726.
- Suttle, C. A. & Chen, F. 1992. Mechanisms and rates of decay of marine viruses in seawater. *Applied and Environmental Microbiology* **58**, 3721-3729.
- Tapper, M. A. & Hicks, R. E. 1998. Temperate viruses and lysogeny in Lake Superior bacterioplankton. *Limnology and Oceanography* **43**, 95-103.
- Thingstad, T. F., Heldal, M., Bratbak, G. & Dundas, I. 1993. Are viruses important partners in pelagic food webs? *Trends in Evolutionary Ecology* **8**, 209-213.
- Tomas, C. R. 1997. *Identifying Marine Phytoplankton*. Academic Press, San Diego.
- Torella, F. & Morita, R. Y. 1979. Evidence by electron micrographs for a high incidence of bacteriophage in the waters of Yaquina Bay, Oregon: Ecological and taxonomical implications. *Applied and environmental microbiology* **37**, 774-778.
- Tufail, A. 1987. Microbial communities colonising nutrient-enriched marine sediment. *Hydrobiologia* **148**, 245-255.
- Tuomi, P., Fagerbakke, K. M., Bratbak, G. & Heldal, M. 1995. Nutritional enrichment of a microbial community: The effects on activity, elemental composition, community structure and virus production. *FEMS Microbiology Ecology* **16**, 123-134.
- Underwood, G. J., Phillips, J. & Saunders, K. 1998. Distribution of estuarine benthic diatom species along salinity and nutrient gradients. *European Journal of Phycology* **33**, 173-183.
- Valiela, I. 1995. *Marine Ecological Processes*. Springer, New York.
- 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.
- Wilcox, R. M. & Fuhrman, J. A. 1994. Bacterial viruses in coastal seawater: Lytic rather than lysogenic production. *Marine Ecology Progress Series* **114**, 35-45.
- 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.