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Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries

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Abstract

Viruses are recognized as ubiquitous components of marine ecosystems; however, there has been limited study of viral abundance and its ecological role in sediments. Viral abundance was determined in both the water column and sediments of a eutrophic (Brisbane River/Moreton Bay; 27°25′S, 153°5′E) and oligotrophic (Noosa River; 26°15′S, 153°0′E) estuary in subtropical Queensland, Australia. Viruses, bacteria, and microalgae from both water column and extracted sediment samples were enumerated using SYBR Green I staining and epifluorescence microscopy. Sediment viral abundance ranged from 10^7 to 10^9 particles cm^{-3} of sediment, bacterial abundance ranged from 10^7 to 10^8 cells cm^{-3} of sediment, and microalgal abundance ranged from 10^4 to 10^5 cells cm^{-3} sediment. Pelagic abundances for all microorganisms were 10–1,000-fold lower than sediment abundances. Correlations between viral abundances and suspended solids suggest that viruses sorbed to suspended material in the water column may settle out and contribute to the benthic viral population. Virus production was measured by a time course increase of viral abundance in seawater using a dilution technique. Virus production was highest in eutrophic waters of the Brisbane River, and addition of inorganic nutrients ($NO_3^- + NH_4^+ + PO_4^{3-} + SiO_3$) stimulated viral production rates at all stations by 14–52% above ambient, suggesting that inorganic nutrient availability may play a key role in aquatic viral abundance.

The importance of estuaries in the flux of matter from terrestrial to marine environments and their proximity to major urban centers have 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 that are diluted by oceanic exchange, particularly in temperate regions. Australian estuaries differ from those in many other geographic locations in that they are characterized by comparatively low flow rates (because of low average rainfall) and low nutrient concentrations (Bowen et al. 1996). However, several estuaries on the East coast of Australia are eutrophic as a result of urban runoff and sewage outfalls (Dennison and Abal 1999).

Marine viruses are now recognized as ubiquitous and abundant components of aquatic ecosystems (reviewed in Fuhrman 1999). Virus-like particles (VLP; viruses that have not been cultured to identify hosts) typically have abundances between 10^5 and 10^8 particles ml^{-1} in surface waters of the marine environment. Viral infection has been shown to affect photosynthesis and biomass in phytoplankton (Suttle et al. 1990; Suttle 1992) and production and abundance of bacteria (Proctor and Fuhrman 1992; Middelboe et al. 1996) and has been implicated in genetic exchange in prokaryotic organisms (Jiang and Paul 1998). Viruses have also been implicated in the decline of phytoplankton blooms (Bratbak et al. 1993) and may be a source of nutrition for heterotrophic nanoflagellates (Gonzales and Suttle 1993).

Previous studies of estuarine and neritic viroplankton (pelagic virus) communities (Bergh et al. 1989; Cochlan et al. 1993; Cochran and Paul 1998) estimated viral abundance in surface waters between 10^5 and 10^8 particles ml^{-1}. The abundance of viruses has been largely correlated to the distribu-

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tion of bacteria, which are the most numerous hosts. Strong correlations between viral abundance and chlorophyll *a* (Chl *a*) have also been observed in neritic waters (Cochlan et al. 1993).

Viruses previously have been shown to have high abundances in marine sediments (Paul et al. 1993; Steward et al. 1996; Drake et al. 1998; Danovaro and Serresi 2000). 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.

Despite several previous reports of viral abundance in marine (e.g., Maranger and Bird 1995; Fuhrman 1999) and freshwater environments (e.g., Kepner et al. 1998; Tapper and Hicks 1998), there have been few 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.

Abundances of viruses, bacteria, and microalgae were studied in the eutrophic Brisbane River/Moreton Bay estuary and the oligotrophic Noosa River estuary, which are located in southeast Queensland on the East coast of Australia, a region of rapid population growth (Skinner et al. 1998). The aims of the present study were to determine the distribution of benthic and pelagic marine viruses in these estuaries and to determine their production in the water column along two eutrophication gradients.

Methods

Description of study sites—Moreton Bay (27°25′S, 153°5′E) is a shallow embayment (Fig. 1A) characterized by long residence time of seawater (>70 d in the western bay) and distinct nutrient gradients emanating from four rivers draining into the system (Dennison and Abal 1999). The Brisbane River (Fig. 1B), which is the major river discharging into the bay, receives treated sewage effluent from 13 treatment facilities at locations along the tidal portions of the river estuary (Dennison and Abal 1999). Moreton Bay and the Brisbane River are generally well-mixed by wind and tide (tidal range ~1.7 m). The eutrophication transect extended beyond Moreton Bay into the Coral Sea, separated from coastal waters by the East Australian Current.

In contrast, the Noosa River (26°S, 153°E) (Fig. 1C) is a well-flushed, oligotrophic estuarine system. The river seasonally receives organic matter loading from humic runoff. There is no sewage effluent and the nutrient inputs are low and diffuse. Mixing in the Noosa River is primarily driven by tidal currents; in addition, the Noosa River estuary has a series of large, shallow (<1 m) embayments in which wind mixing is effective. A shallow sand bar (<2 m deep) at the river mouth restricts oceanic exchange.

Enumeration of microorganisms—Viruses and bacteria: Seawater was collected at 44 stations in the Brisbane River/Moreton Bay estuary and at 11 stations in the Noosa River estuary in 50-ml 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 10 d of sample collection. Viruses and bacteria were enumerated by nucleic acid staining (SYBR Green I) and epifluorescence microscopy (Noble and Fuhrman 1998). Sample volumes from 0.5 to 3.0 ml were filtered through Anodisc Al_2O_3 membrane filters (pore size 0.02 μm), backed by 0.8-μm Millipore type AA filter, and dried immediately on the surface of tissue paper. These were then placed on drops of SYBR Green I (Molecular Probes, Inc.; in 0.02-μm filtered distilled water) diluted 1 : 10,000 and stained in darkness for 15 min. After drying, filters were placed on glass slides and drops of phosphate-buffered saline (PBS) diluted 1 : 1 (120 mM NaCl, 10 mM NaH_2PO_4, 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 ×100 magnification on an Olympus BX-60 epifluorescence microscope, whereas green light excitation was used to count unicellular cyanobacteria and small photosynthetic protists. A minimum of 200 cells of each organism category was counted in independent duplicate samples.

Duplicate benthic samples were collected at 32 stations throughout the Brisbane River/Moreton Bay estuary and at 11 stations in the Noosa River estuary using 50-ml cut-off syringes to form sediment corers. Corers were pushed into sediment to a depth of 2 cm with the syringe plunger flat against the sediment surface. The cores were then withdrawn and stoppered using large rubber stoppers to exclude water column virus and prevent loss of sediment pore water. Samples were extruded in air into 50-ml sterile centrifuge tubes. Samples for vertical profiles were collected using syringe cores that were kept vertical until sectioning with a steel razor blade in the laboratory. Benthic samples (15 ml) were extracted by placing sediments in 35-ml 0.02-μm filtered PBS and agitated on a shaker table at 120 rpm for 30 min. At some stations where sediments were composed of fine silty muds, the extract was centrifuged at 1000 × g for 10 min to remove suspended sediment. It was assumed that centrifugation did not remove substantial numbers of viruses or bacteria. The supernatant was processed and counted as described previously with SYBR Green I staining.

The centrifugation of samples in the present study may have contributed to the underestimate of benthic viral abundance because the assumption that this process does not remove viruses may be invalid. In addition, it has been noted previously that there is no satisfactory method at present to fully desorb viruses from sediment particles (Drake et al. 1998). Therefore, the abundance of viruses presented here may only account for a small percentage of the total viral community present in estuarine sediments. Aldehyde preservation of samples has been shown to lead to 25–50% fewer bacteria after only 7 d (Gundersen et al. 1996), hence it is conceivable that viral counts decrease at a similar rate in formaldehyde-preserved samples. SYBR Green I has unstable fluorescence; hence, it fades quickly (Bettarel et al. 2000). Additionally, the abundance of viruses, particularly in the benthos, is underestimated using SYBR Green I staining because it is a double-stranded (ds) DNA and RNA fluorochrome; therefore, single-stranded (ss) DNA viruses, which may be common in the marine environment (Fuhrman 1999), are not counted using this method.

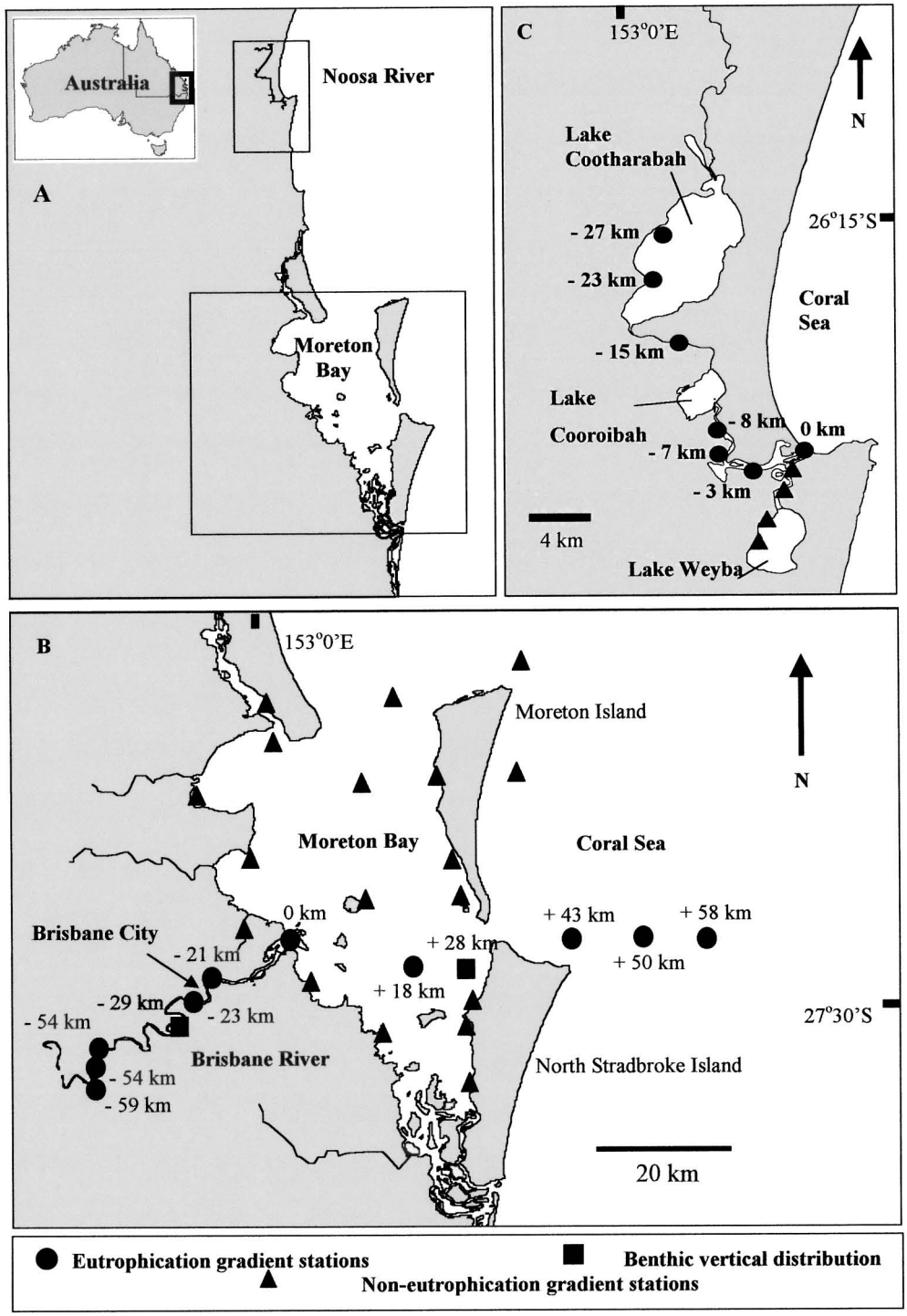

Fig. 1. Location of (A) estuaries and sampling stations in the (B) Brisbane River/Moreton Bay and (C) Noosa River estuaries.

Microalgae: Whole-water phytoplankton samples (50 ml) were allowed to settle for 24 h, and the top 45-ml sample was removed leaving a final volume of 5 ml. Phytoplankton were then enumerated using bright field microscopy at ×10 to ×40 magnification in a Sedgwick–Rafter slide. Benthic microalgae cores (2 cm deep) were resuspended in 0.22-μm filtered seawater, and aliquots of elution were 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.

Chl *a* samples were analyzed 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°C and were then centrifuged at 3,000 × g for 25 min to remove flocculated sediment and subcellular components. Absorbances at 630, 647, 664, and 750 nm were measured on a Pharmacia spectrophotometer, and Chl *a* was calculated using acidified absorbances at 664 and 750 nm to correct for phaeopigments (Parsons et al. 1985).

Chemical and physical parameters—Samples (100 ml) of seawater from each station were collected in polycarbonate bottles that were prerinsed in 0.1 N HCl for 2 h. Total ammonium (NH_4^+) samples and total nitrogen oxide (NO_3^-, NO_2^{2-}) samples were prefiltered through Sartorius Minisart 0.45-μm syringe filter units immediately before cooling to −80°C. Nutrient analyses for total ammonium, total nitrogen oxides, and filterable reactive phosphorus (PO_4^{3-}) were analyzed colorimetrically using a scalar autoanalyzer by Queensland Health (Brisbane, Australia). Salinity, pH, temperature, and conductivity were measured using a HORIBA UD-10 concomitant with water and benthic sample collection.

Suspended solids were determined by collecting 2 liters of surface water at each station in 2.5-liter polycarbonate bottles. Volumes of between 0.2 and 2 liters of seawater were filtered through anhydrous preweighed Whatman GF/F filters, followed by repeated rinses with milli-Q water to remove salts from retained solids. Filters were then dried at 60°C overnight and reweighed. The difference in filter weight with filtrate was equivalent to suspended solids greater than 0.7 μm in diameter (pore size of filters used).

Virus production—Virus production was measured using a dilution technique modified from Wilhelm et al. (1998). Samples of surface water (1 liter) were collected at −29, 0, +18, +28, and +58 km (Fig. 1) in acid-rinsed darkened Nalgene polycarbonate bottles. Virus-free seawater was obtained by passing station water through a 0.02-μm filter. Viruses in a whole seawater sample were removed by filtering 800 ml of a 1-liter sample through a 0.22-μm Durapore (type GV; low protein–binding) filter. Virus-free seawater was added to the retentate, and bacteria and phytoplankton were 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 polycarbonate bottles. Three bottles served as controls, and nutrients (30 μM NH_4^+, 200 μM NO_3^{2-}, 20 μM PO_4^{3-}, 66 μM SiO_3) were added to the remainder. Bottles were incubated in the dark at ambient seawater temperature (25°C). Subsamples (5 ml) were removed from each bottle at approximate intervals 3, 6, 12, and 24 h and analyzed by SYBR Green I staining and epifluorescence microscopy (Noble and Fuhrman 1998). It was assumed that the rate of increase of VLP over time was linear when calculating turnover time and that viral decay did not remove VLP over the course of the experiment.

Statistical analyses—Parameters comparing two replicated, independent parameters (e.g., viral abundance, bacterial abundance) were analyzed by one-way analyses of variance (ANOVA) using the statistical software package on Microsoft Excel 97. Stepwise multiple linear regression (forward selection procedure) was conducted using the statistical software package on Microsoft Excel 97, using largest percent change of r^2 on addition as entry order. Homogeneity of variance was assessed using Cochran's test before conducting linear regression analysis.

Results

Characteristics of nutrient and salinity gradients—The transect formed from −59 km upriver to +58 km from the river mouth in the Brisbane River/Moreton Bay estuary (Fig. 1B) exhibits decreasing inorganic nutrient concentration from the most upriver station (11 μM NO_3^-, 2 μM PO_4^{3-}, 6 μM NH_4^+) to the most oceanic station (1 μM NO_3^-, 0.01 μM PO_4^{3-}, 0.1 μM NH_4^+); however, large nutrient concentrations (28 μM NH_4^+) were present at the river mouth, which is at the same location as the largest sewage effluent outfall (Fig. 2). Salinity increased from 0 to 36‰ from upriver to the open ocean.

The Noosa River estuary had comparatively low ambient inorganic nutrient concentrations (0.1 to 0.8 μM NO_3^-, 0.01 to 0.1 μM PO_4^{3-}, 0.1 to 2.4 μM NH_4^+), which decreased marginally from the furthest upriver station (−27 km) to the river mouth. Salinity increased from 0 (approximately −20 km from the river mouth) to 36‰ at the river mouth (Fig. 2).

Distribution of viruses, bacteria, and microalgae—Abundance of benthic viruses (0.2–4.8 × 10^9 VLP cm^{-3} sediment) in the Brisbane River/Moreton Bay estuary and Noosa River was significantly higher than the abundance of pelagic viruses (0.5 × 10^7 to 3.0 × 10^8 VLP ml^{-1}; ANOVA $P < 0.001$, $n = 26$). In addition, the ratio of viruses to bacteria was significantly higher in sediments (2–65 VLP per bacterium) than in the overlying water column (3–37 VLP per bacterium; ANOVA $P < 0.01$, $n = 26$; Fig. 3).

Water column viral and bacterial abundances and the ratio of viruses to bacteria were significantly lower at oligotrophic stations than eutrophic stations in the Brisbane River/Moreton Bay Estuary (Fig. 4; $P < 0.05$, $n = 5$). This decreasing trend was also observed in sediments. Benthic viral and bacterial abundances and the ratios of viruses to bacteria were elevated at mid-river stations (−7, −8, and −15 km) compared with the furthest extents in the Noosa River estuary (Fig. 4); however, water column viruses were not elevated at these stations. There was large variation in the abundances of benthic (but not pelagic) viruses in oligotrophic areas of both the Noosa River and the Brisbane River/Moreton Bay estuaries.

In both eutrophic (−29 km) and oligotrophic (+28 km) sediments, there were clear subsurface maxima of both viruses and heterotrophic bacteria, which declined with sediment depth (Fig. 5). Ratios of viruses to bacteria were substantially greater in the eutrophic sediments compared to oligotrophic sediments, and at the oligotrophic station, bacterial abundance exceeded viral abundance at approximately 50 mm beneath the sediment surface.

Correlations between viral abundance and biotic/abiotic parameters—No significant correlation was found between

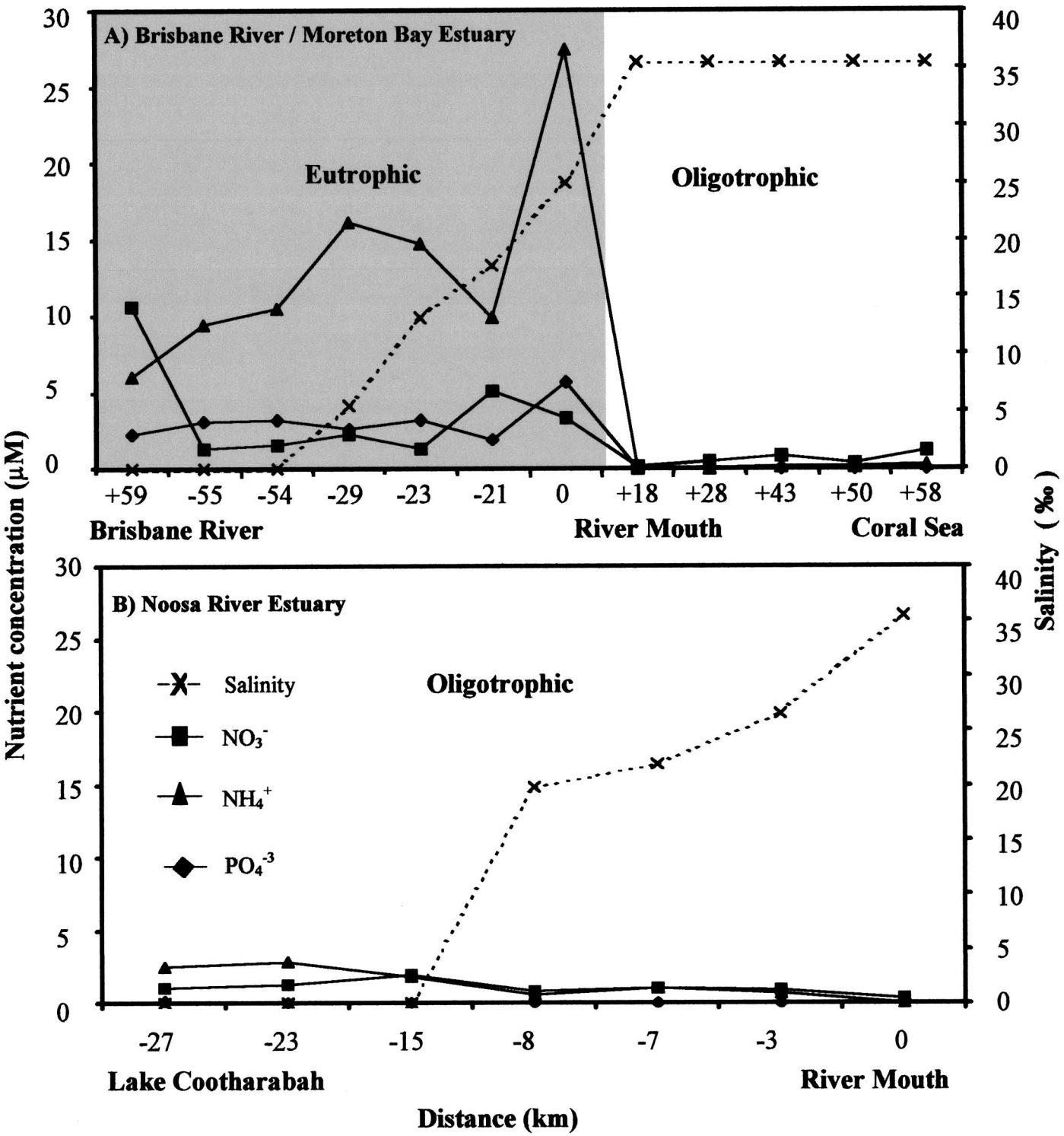

Fig. 2. Inorganic nutrient concentrations and salinity along eutrophication gradient from (A) the Brisbane River to Coral Sea in November 1999 and (B) along Noosa River estuary gradient in August 1999. Shaded area indicates eutrophic stations.

viruses and bacteria, cyanobacteria, or microalgae in sediments (Table 1); however, water column viral abundance correlated significantly ($r = 0.91$, $n = 44$, $P < 0.001$) with the abundance of bacteria (Table 2). The lack of correlation between viral and microalgal direct count in sediments was also evident in the lack of correlations between viral abundance and water column and benthic Chl *a*. Abundance of benthic viruses was significantly correlated to pelagic viral abundance in the Brisbane River/Moreton Bay estuary ($r = 0.89$, $n = 32$, $P < 0.001$) (Fig. 6).

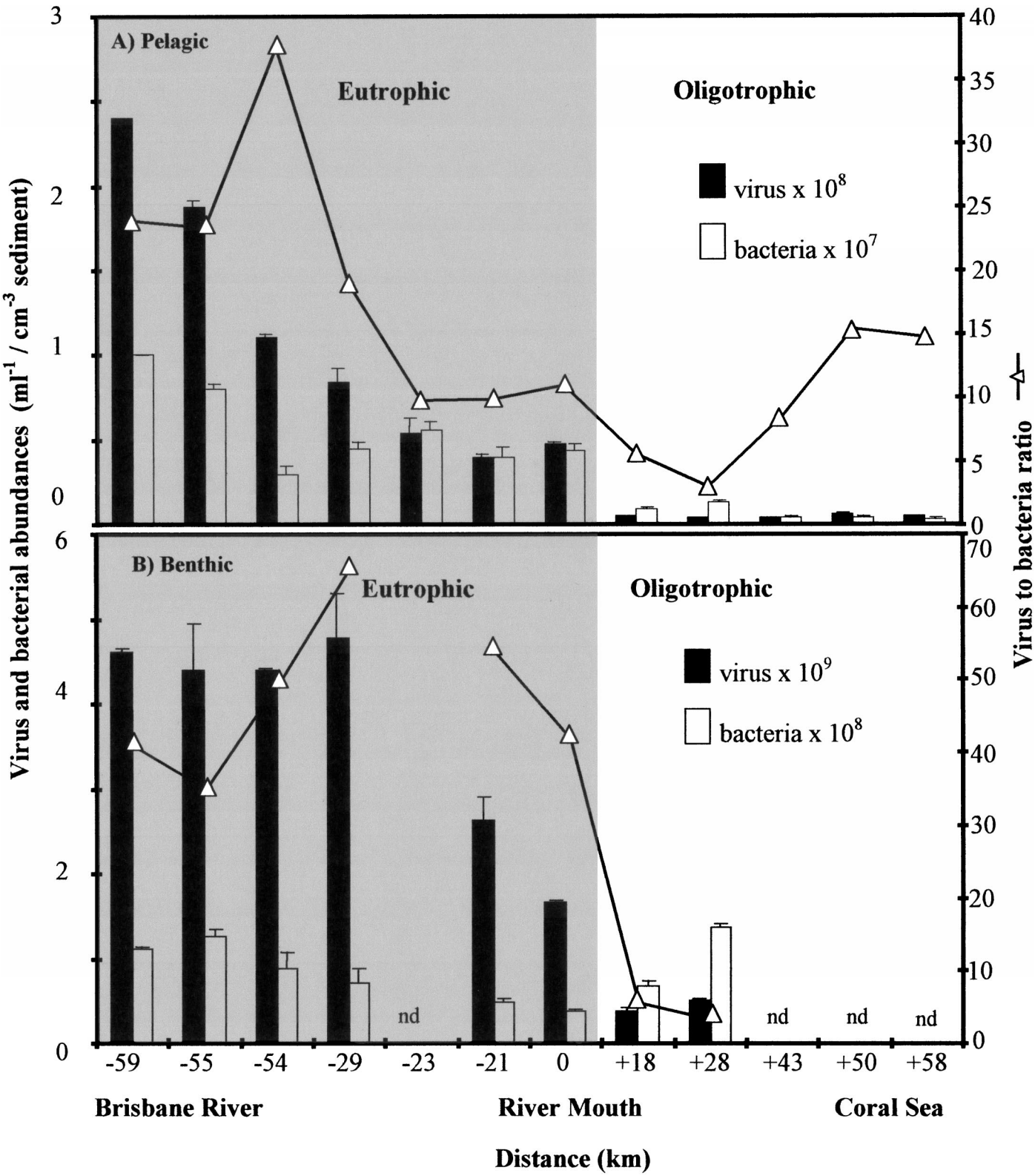

Fig. 3. VLP and bacterial abundance and change in the ratio of viruses to bacteria along a gradient from the Brisbane River to Moreton Bay estuary in (A) the water column and (B) sediments. Error bars = SE. Shaded area indicates eutrophic stations.

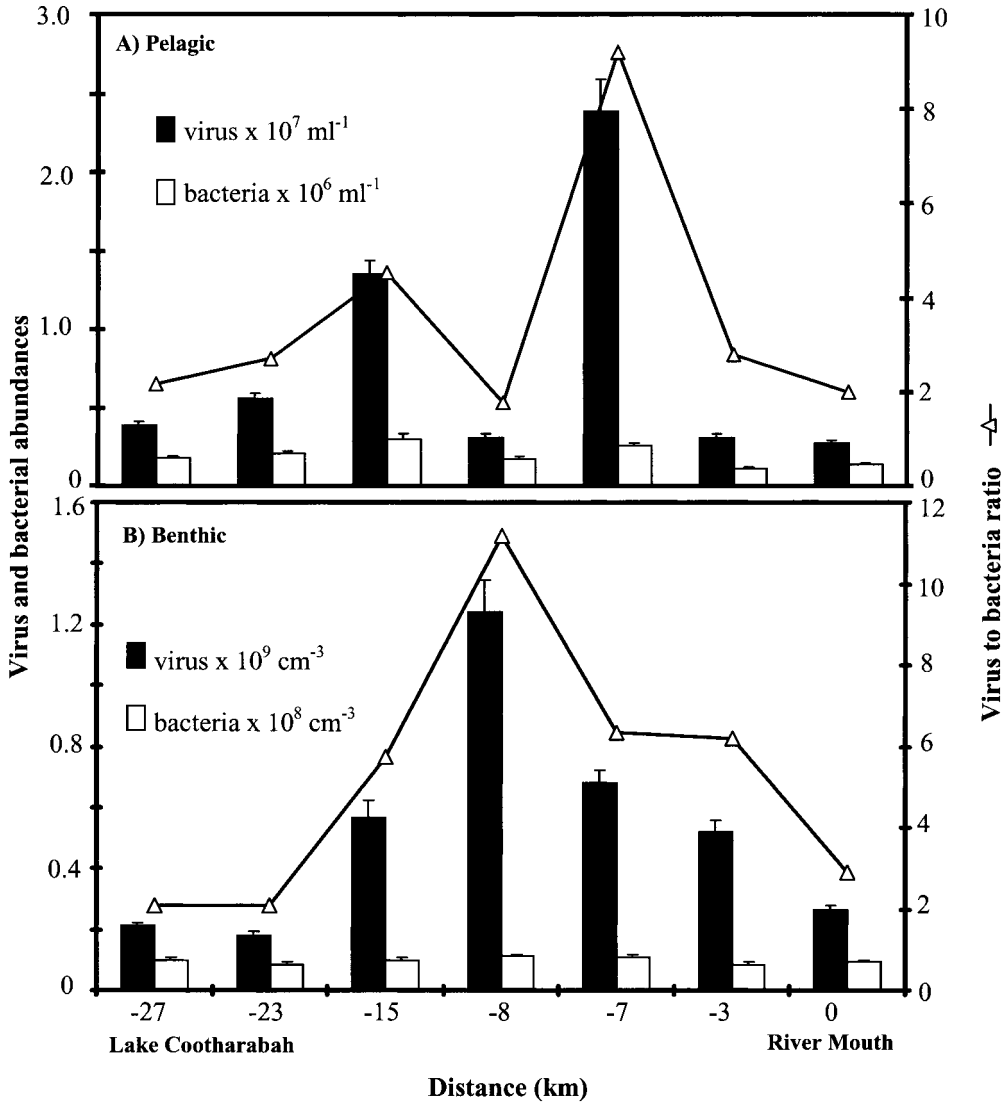

Fig. 4. VLP and bacterial abundance and change in the ratio of viruses to bacteria along salinity gradient in Noosa River in (A) the water column and (B) sediments. Error bars = SE.

Pelagic viral abundance was significantly correlated with abiotic parameters, including salinity ($r = 0.77$, $n = 44$, $P < 0.001$) and suspended matter ($r = 0.57$, $n = 44$, $P < 0.05$); however, there were not significant correlations with inorganic nutrients (Table 1). Benthic viral abundance correlated significantly with suspended solids in the water column ($r = 0.85$, $n = 26$, $P < 0.001$); however, as in the water column, no significant correlation with inorganic nutrients was found (Table 1).

Stepwise multiple regression of benthic viral abundance showed that suspended solids and salinity explained 92% of the variance in benthic viral abundance, and suspended solids could account for 68% of the variance in the ratio of viruses to bacteria (Table 2). The variation in benthic bacterial abundance could not be significantly explained by any independent parameter. Pelagic viral abundance variation was best explained by pelagic bacterial abundance and salinity (94.3%), whereas variation in pelagic bacterial abundance was best explained by dissolved inorganic phosphate (PO_4^{3-}) concentration and salinity (74.6%). Variation in ratios of pelagic viruses to bacteria could not be explained by any independent variable.

Viral production—Regressions of viral production were all positive except at +58 km, where viruses were removed from the incubations, probably through decay or sorption to the vessel walls (eutrophic and oligotrophic production regressions given in Fig. 7). Virus production was significantly higher in eutrophic portions of the Brisbane River/Moreton Bay estuary than in oligotrophic areas ($P < 0.05$, $n = 3$; Table 3, Fig. 7). The theoretical viral turnover time increased 1.5-fold from the most eutrophic (−29 km; 23 h) to most oligotrophic (+28 km; 36 h) station. The rate of production normalized per bacterium (which compensates for bacterial abundance and growth) showed no clear trend, with the highest production occurring at eutrophic stations at −29

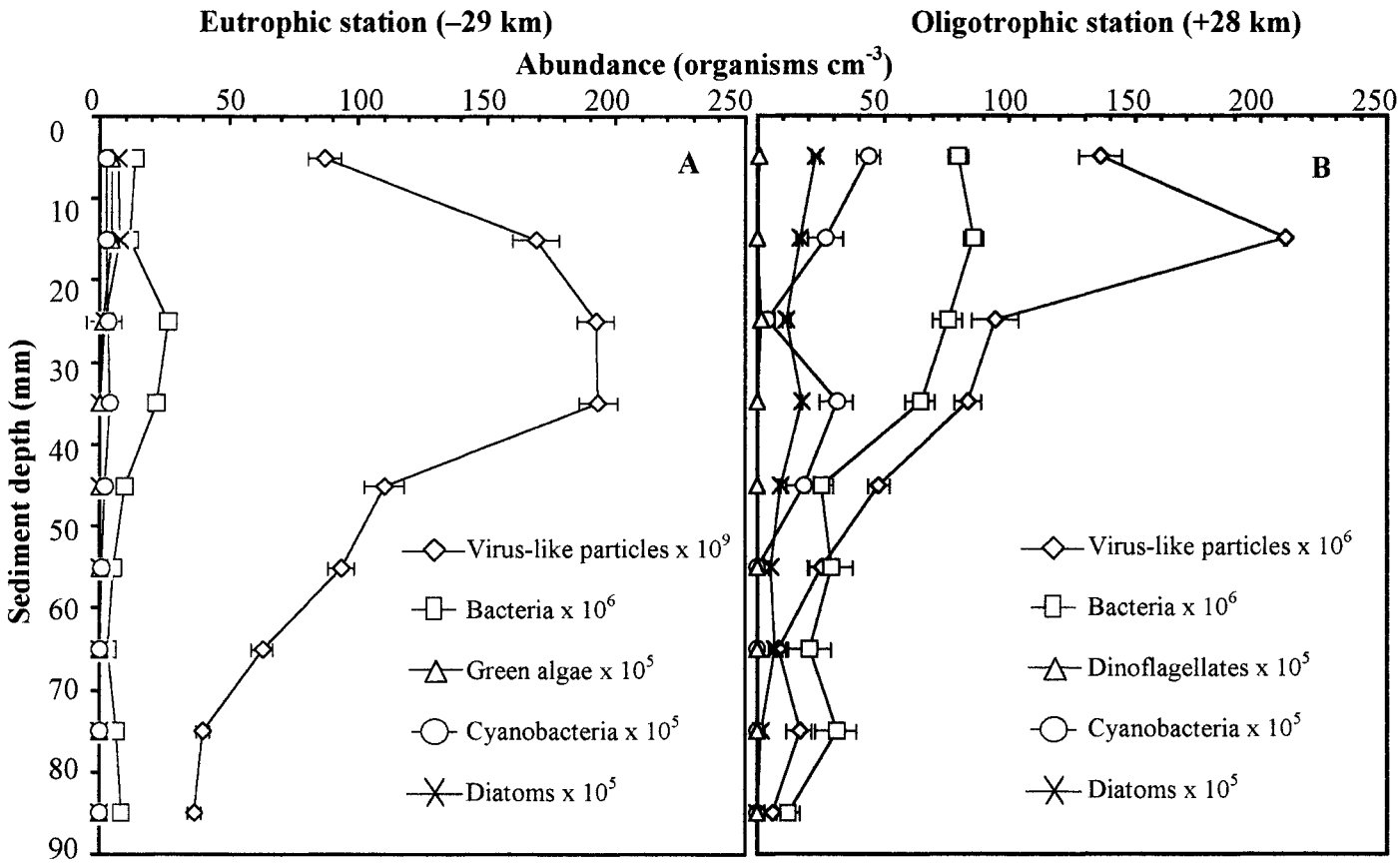

Fig. 5. Vertical distribution of VLP and hosts in (A) eutrophic and (B) oligotrophic sediments. Error bars = SE.

Table 1. Simple linear correlation coefficients (*r*) between abundance, potential hosts, and abiotic parameters for pelagic (top) and benthic (bottom) viruses at all stations in the Brisbane River, Moreton Bay, and Noosa River. Pelagic $n = 44$; benthic $n = 32$.

	Bacterial abundance	Viruses : bacteria	Unicellular cyanobacteria	Microalgae	Salinity	$NH_4^+ + NO_3^- + NO_2^-$	PO_4^{3-}	Suspended solids†
Pelagic viruses								
Viral abundance	0.91**	0.63*	0.47	0.20	−0.77**	0.49	0.60*	0.57*
Bacterial abundance		0.34	0.57*	0.20	−0.69**	0.63	0.70**	0.63*
Viruses : bacteria			0.20	0.22	−0.66**	0.24	0.37	0.48
Unicellular cyanobacteria				0	−0.24	0.34	0.41	0.24
Microalgae					0.39	−0.30	−0.30	0.40
Salinity						−0.62*	−0.72**	−0.80**
$NH_4^+ + NO_3^- + NO_2^-$							0.97**	0.34
PO_4^{3-}								0.36
Benthic viruses								
Viral abundance	0.17	0.24	0.28	0.28	−0.90**	0.53	0.53	0.85**
Bacterial abundance		0.84**	0.37	0	−0.24	0	0.14	0.52
Viruses : bacteria			0.37	0.26	−0.66*	0.54*	0.53	−0.76**
Unicellular cyanobacteria				0.24	0.30	−0.20	−0.22	−0.26
Microalgae					0.28	−0.24	−0.24	−0.22

* $P < 0.05$, ** $P < 0.001$.
† Suspended solids >0.7 μm.

Table 2. Stepwise multiple linear regression (forward selection procedure, $\alpha = 0.05$) between microorganisms and environmental parameters (benthic = pelagic virus, benthic bacteria, suspended solids, NO_3^-, PO_4^{3-}, salinity, benthic Chl *a*; pelagic = pelagic bacteria, suspended solids, NO_3^-, PO_4^{3-}, salinity, pelagic Chl *a*) fitting the model $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2$, where y is the dependent variable, x is the independent variable, and β is the coefficient. No other significant multiple regressions were found.†

	Stepwise selection			Analysis of variance for full regression						
y	*x*	β	*P*	Source	Sum of squares	df	*F*	*P*	r^2 (%)	Partial r^2
Benthic virus	β_0 intercept	2.0×10^9	—	Model	5.0×10^{19}	3	62	b	92.0	—
	β_1 TSS	3.5×10^7	b	Error	9.9×10^{18}	24				0.89
	β_2 salinity	-3.9×10^7	a							0.81
Benthic V:B	β_0 intercept	16.1	—	Model	5.1×10^3	1	22	b	67.7	—
	β_1 TSS	0.5	b	Error	6.0×10^3	26				0.68
Pelagic virus	β_0 intercept	-4.2×10^7	—	Model	7.4×10^{16}	2	113	b	94.3	—
	β_1 bacteria	26.6	b	Error	9.1×10^{15}	28				0.87
	β_2 salinity	-6.0×10^5	b							0.24
Pelagic bacteria	β_0 intercept	3.3×10^6	—	Model	8.0×10^{13}	2	18	b	74.6	—
	β_1 DIP	7.7×10^6	a	Error	6.4×10^{13}	28				0.64
	β_2 salinity	-7.1×10^4	a							0.40

a: $P < 0.05$, b: $P < 0.001$.

† V:B, ratio of viruses to bacteria; β_x, coefficient; *P*, two-tailed probability values; df, degrees of freedom; r^2 (%), percentage of variation explained. The order in which variables are added is determined by selecting the variable whose addition brings about the largest increment in r^2. TSS, total suspended solids greater than 0.7 μm in diameter; DIP dissolved inorganic phosphorus.

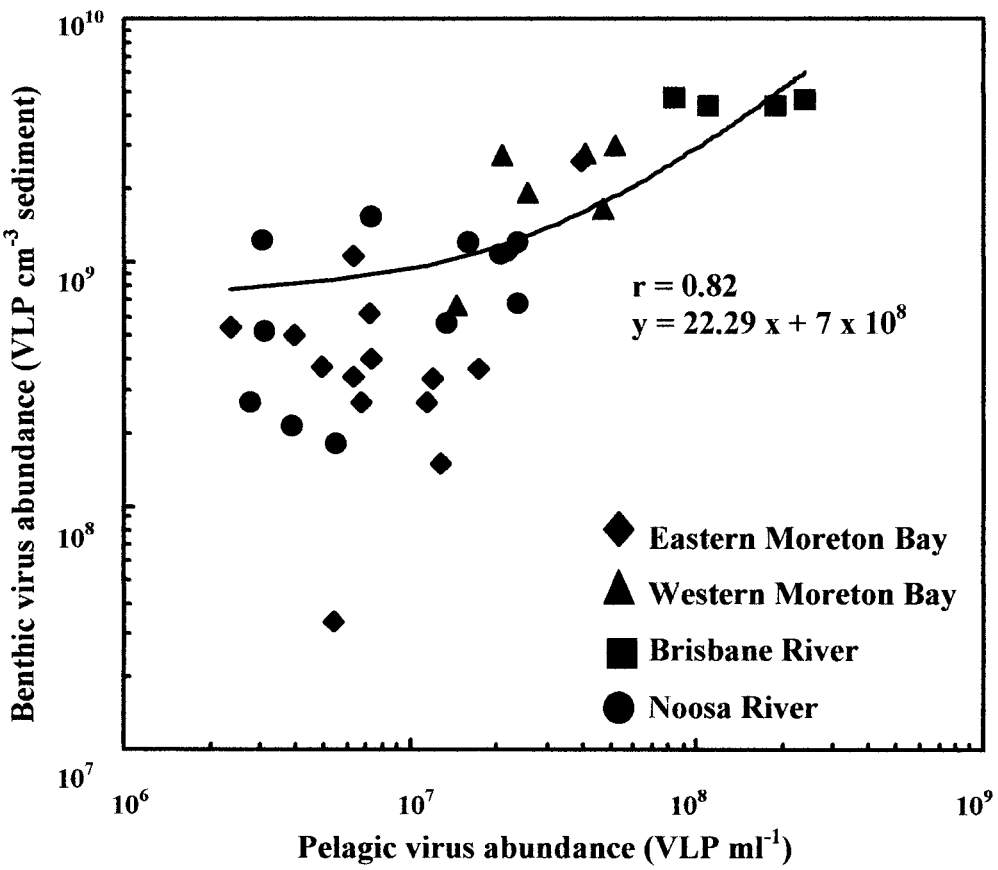

Fig. 6. Correlation of benthic to pelagic viral abundance in the Brisbane River/Moreton Bay and the Noosa River estuaries. Variance is homogeneous (Cochran's test; $C = 0.20$, df 3, k = 20 for benthos; $C = 0.25$, df 3, k = 20). Sediment viral abundance is assumed to be a function of overlying water viral abundance.

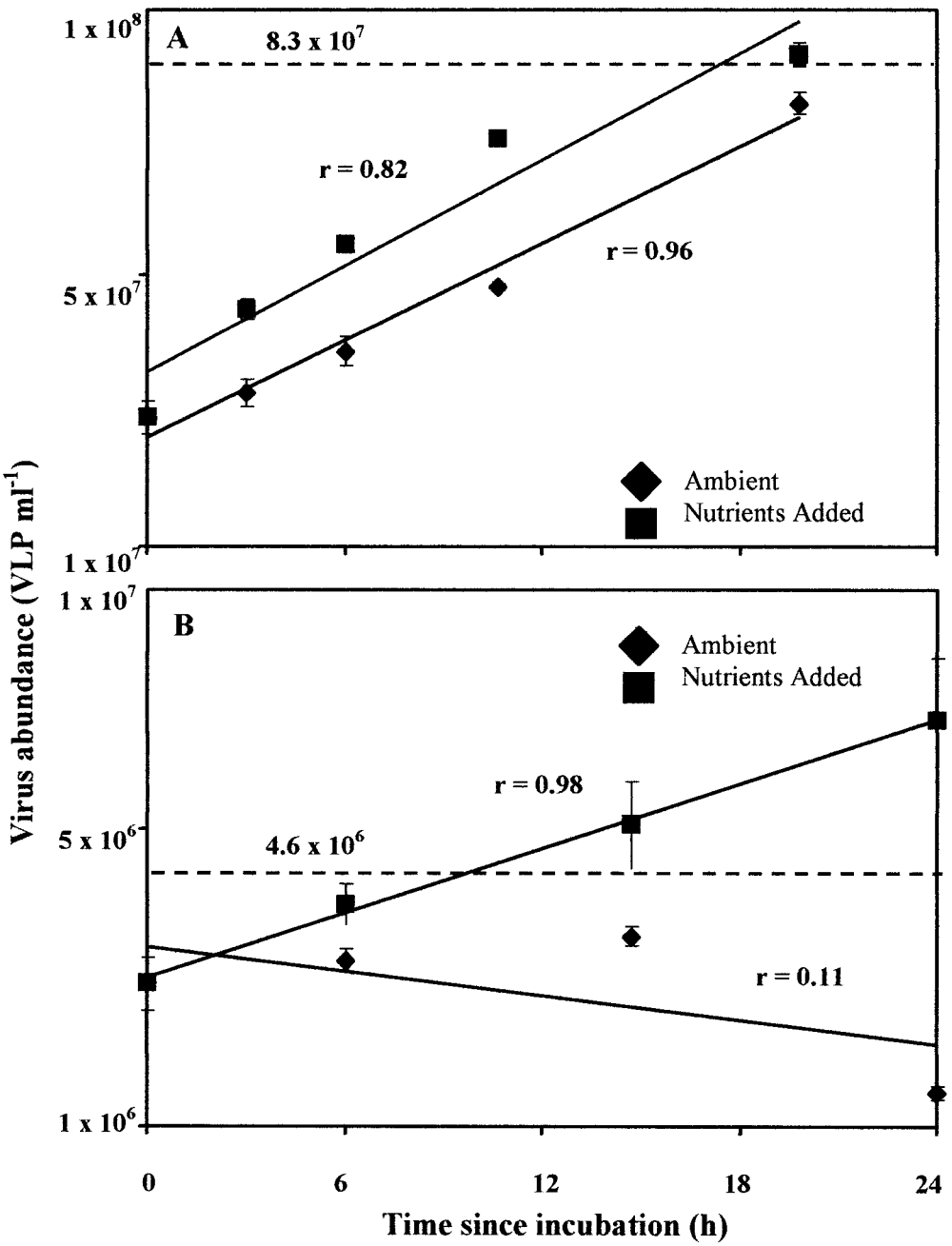

Fig. 7. Representative graphs of viral production from (A) −29 km and (B) +58 km under ambient and nutrient-enriched conditions. Dotted line indicates ambient abundance of viruses. Regressions of −29 km ambient ($y = 2.3 \times 10^6x + 2.0 \times 10^7$) and nutrient-enriched ($y = 3.3 \times 10^6x + 3.4 \times 10^7$) viral production rates were higher than +28 km ambient ($y = -6.4 \times 10^4x + 4 \times 10^6$) and nutrient-enriched ($y = 1.8 \times 10^5x + 3 \times 10^6$) viral production rates. Error bars = SE.

and 0 km, as well as the oligotrophic station at +28 km. The production of viruses by hosts, except the specific viral production rate at −29 km, was elevated at three stations where nutrients were added (Table 4).

Discussion

Distribution of microbial communities along eutrophication gradients—Benthic viral abundance and distribution: The abundances of benthic viruses in the present study were 10- to 1,000-fold higher than viral abundances in the overlying water column, consistent with previous studies of benthic viral abundance (Paul et al. 1993; Maranger and Bird 1996; Steward et al. 1996; Drake et al. 1998; Danovaro and Serresi 2000) (Table 3). The higher abundance of viruses in sediments may be a reflection of the higher abundance of suitable hosts in sediments compared with overlying waters, although other biotic and abiotic factors may in part account

Table 3. Comparison of literature values of benthic viral abundance VLP, virus-like particle; nd, not determined; V : B, ratio of viruses to bacteria.

Location	Viral abundance (VLP cm^{-3})	Range of V : B	Reference
Moreton Bay and Noosa River	1.7×10^7–2.4×10^9	21–56	Present study
Lac Gilbert, Quebec	0.8–4×10^9	1–32	Maranger and Bird 1996
Florida Bay	1–5×10^8	nd	Paul et al 1993
Bering Sea	2.7×10^7	nd	Steward et al 1996
Chesapeake Bay	3.6×10^8	29–85	Drake et al 1998
Sporades Basin and Ierapetra Trench, Mediterranean Sea	1–2×10^9	2–5	Danavaro and Serresi 2000

for the poor correlation between the abundances of viruses and their hosts (Table 1).

The greater ratios of viruses to bacteria observed in sediments may be the result of greater nutrient availability in sediments, because increased nutrient availability previously has been linked with these elevated 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 typically enriched with organic material (Fisher et al. 1982). Decomposition of this organic material results in remineralization of inorganic nutrients. The greater availability of nutrients in sediments may result in greater microalgal and bacterial abundances than the overlying water column, where nutrient availability is related to allochthonous inputs or flux from the sediments (Lund Hansen et al. 1999).

The large subsurface maxima of viral and bacterial abundances resemble pelagic profiles of microbial distributions (Fig. 5) (Bird et al. 1993; Cochlan et al. 1993) that are attributable to processes of mixing, nutrient availability, and photoinhibition. Because light is attenuated quickly at the sediment surface (Fenchel and Staarup 1971), it is not likely to affect viral abundance within the sediments. Viral abundances may also be highest near the sediment surface because of higher bacterial activity (van Duyl et al. 1999).

Pelagic viral distribution and productivity: There is a clear trend of decreasing ratios of viruses to bacteria and pelagic viral abundances, concurrent with inorganic nutrient concentrations, in the Brisbane River/Moreton Bay estuary from the most eutrophic and freshwater station (−59 km) to the most oligotrophic station (+58 km) in the East Australian Current. In contrast, there is no correlation between viral abundance and salinity in the Noosa River, which lacks a distinct nutrient gradient. Therefore, it is possible that salinity does not play a direct role in the distribution of pelagic viruses in the Brisbane River/Moreton Bay estuary, despite their strong correlation, but that salinity is covarying with nutrient concentrations. The increased burst size (i.e., number of progeny virus released from cells on lysis) in infected phytoplankton following phosphorus and amino acid additions (Tuomi et al. 1995; Wilson et al. 1996) and the observation of phosphate metabolic genes in a bacteriophage genome (Rowher et al. 2000) supports the hypothesis that nutrient availability may play a role in determining the ambient abundance of pelagic viruses with respect to hosts.

Viral production rates in eutrophic waters of the Brisbane River/Moreton Bay estuary ranged from below detection limits to 2.3×10^6 VLP ml^{-1} h^{-1}, which is consistent with rates previously observed in neritic waters (0–58.33×10^6 VLP ml^{-1} h^{-1}) (Steward et al. 1992; Heldal and Bratbak 1991; Fuhrman and Noble 1995). The high rates of production of viruses along the eutrophication gradient shows that lytic viruses in eutrophic regions may have relatively short turnover times and replicate more rapidly within hosts compared with those in oligotrophic regions.

Low viral production in the East Australian Current (+58 km) and lower viral production rates in oligotrophic portions of Moreton Bay suggest that lytic infection may be less 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 subsequent lysis does not occur until induced by environmental stimuli) may be an important survival

Table 4. Pelagic viral production and specific viral production rate (viral production normalized per bacterium) ± SE along a eutrophication gradient.*

		Ambient		Nutrients added	
Trophic state	Station (km)	Viral production rate (VLP × 10^6 ml^{-1} h^{-1})	Specific rate (VLP $bacterium^{-1}$ h^{-1})	Viral production rate (VLP × 10^6 ml^{-1} h^{-1})	Specific rate (VLP $bacterium^{-1}$ h^{-1})
Eutrophic	−29	2.3 (±0.3)	1.82 (±0.68)	3.3 (±0.3)	1.20 (±0.34)
	0	1.0 (±0.1)	0.21 (±0.07)	nd	nd
Oligotrophic	+18	0.9 (±0.1)	0.17 (±0.08)	1.3 (±0.4)	1.58 (±0.57)
	+28	0.8 (±0.1)	1.73 (±0.57)	nd	nd
	+58	bdl	bdl	0.2 (±0.1)	0.01 (±0.01)

* VLP, virus-like particle; nd, not determined; bdl, below detection limit of $<0.001 \times 10^6$ ml^{-1} h^{-1}.

mechanism for viruses where host densities or resources are low (Wilson et al. 1998).

Interaction between pelagic and benthic viral communities—The lack of correlation between benthic viral and bacterial abundance, but positive correlation between benthic viral abundance and particulate matter, suggests that the relationship between viral and host abundance in sediments is obscured by other factors. Benthic viruses observed in this study could originate either in the sediments or in overlying waters. The present study did not distinguish between viruses produced in interstitial waters and those produced in the water column on settling particles. Viruses are known to readily adsorb to particles in the water column (Suttle and Chen 1992; Bird et al. 1993; Maranger and Bird 1996). For example, Bird et al. (1993) determined that approximately 4% of viruses were lost per day as a result of adsorption to particles in the Southern Ocean, which has relatively low concentrations of suspended solids. Similarly, Noble and Fuhrman (1997) showed that heat-labile suspended matter was responsible for the removal of 20% of viruses per day in neritic waters of the Santa Monica Bight (Southern California), which are also relatively low in suspended matter. The abundance of viruses in deep-water sediments is greatest at the sediment surface (Drake et al. 1998; Danovaro and Serresi 2000); hence, it has been proposed that the primary source of viruses in these environments is from overlying waters. Import of viruses from overlying waters may also help explain the significant correlation between benthic and pelagic viral 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).

The present study emphasizes the necessity of considering benthic viruses when studying estuarine microbial communities. Benthic viruses were not only highly abundant in the Brisbane River/Moreton Bay and Noosa River estuaries, when compared to pelagic viruses, but also have strong interactions with abiotic parameters, most notably particulate matter. Virus production and consequent turnover rates, the ratio of viruses to bacteria, and the total abundance of both benthic and pelagic viruses all appear related to the trophic status of the waters they inhabit. This study highlights the need for further research into the influence benthic viruses exert over their co-occurring hosts, as well as their endogenous production within sediments along trophic gradients.

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