Journal of
Plankton Research

plankt.oxfordjournals.org

J. Plankton Res. (2013) 35(6): 1283–1293. First published online August 17, 2013 doi:10.1093/plankt/fbt074

Contribution of viral lysis and nanoflagellate grazing to bacterial mortality in the inner and outer regions of the Changjiang River plume during summer

AN-YI TSAI[1]*, GWO-CHING GONG[1,2,3], ROBERT W. SANDERS[4] AND JUN-KAI HUANG[1]

[1]INSTITUTE OF MARINE ENVIRONMENTAL CHEMISTRY AND ECOLOGY, NATIONAL TAIWAN OCEAN UNIVERSITY, KEELUNG 202-24, TAIWAN, REPUBLIC OF CHINA, [2]CENTER OF EXCELLENCE FOR THE OCEANS, NATIONAL TAIWAN OCEAN UNIVERSITY, TAIWAN, REPUBLIC OF CHINA, [3]NATIONAL APPLIED RESEARCH LABORATORIES, TAIWAN OCEAN RESEARCH INSTITUTE, TAIWAN, REPUBLIC OF CHINA AND [4]DEPARTMENT OF BIOLOGY, TEMPLE UNIVERSITY, PHILADELPHIA, PA 19122, USA

*CORRESPONDING AUTHOR: anyitsai@mail.ntou.edu.tw

Received March 6, 2013; accepted July 6, 2013

Corresponding editor: John Dolan

This study investigated the relative effect of nanoflagellate grazing and lytic mortality on bacteria. Growth and removal rates for bacteria in the inner and outer regions of the Changjiang River plume were characterized by a series of dilution experiments at four sites, two inner sites (I-1 and I-2) and two outer sites (O-1 and O-2) in the summers of 2011 and 2012. Bacterial growth rates varied between 0.058 and 0.157 h^{-1}, with higher growth rates detected in the inner plume. Grazing mortality rates ranged from 0.042 to 0.126 h−1, with highest grazing rates detected in the inner plume. Viral lysis of bacteria was not significant at three of eight stations, and accounted for >50% of bacterial mortality only once. These findings suggest that grazing nanoflagellates may play a key role in controlling bacterial biomass, and the impact of the nanoflagellates exceeds that of viral lysis during the summer period, especially in the inner region of the Changjiang River plume. It is speculated that the weakening correlation between viruses and bacteria in the inner plume, as well as the increasing virus to bacteria ratio from offshore oligotrophic waters to estuarine waters,

doi:10.1093/plankt/fbt074,available online at www.plankt.oxfordjournals.org.

may be a consequence of the increasing relative abundance of non-bacteriophage viruses with increasing environmental productivity.

KEYWORDS: bacterial mortality; Changjiang River plume; nanoflagellate; virus

INTRODUCTION

It is well established that a significant fraction (half or more) of the total carbon flux in marine food webs passes through heterotrophic bacteria (Cole *et al.*, 1988; Pace and Cole, 1994). Bacterial production (BP) is dependent on both abiotic and biotic factors. Abiotic factors (bottom-up control) include temperature, salinity and substrate availability (Almeida *et al.*, 2001; Ameryk *et al.*, 2005). The biotic factors (top–down control) that may control BP in aquatic systems include predation by protists (Pace, 1988) as well as viral infection and lysis (Wilhelm *et al.*, 2002; Taira *et al.*, 2009). The relative contribution of viruses and grazers to bacterial mortality has important consequences for the processing and transfer of organic matter in the ocean. When bacterial cells are grazed, energy is made available to higher trophic levels, whereas when they are lysed, viruses redirect matter and energy away from higher trophic levels and generate substrates for non-infected bacteria (Suttle, 2005, 2007).

Estuaries are highly dynamic and rapidly changing systems characterized by sharp gradients in salinity, temperature, turbidity and nutrient concentrations (Meire *et al.*, 2005). Therefore, bacterial abundance and production can be predicted to show a decrease along the increasing salinity gradient in estuaries, either because of conservative mixing and tidal loss or because of a parallel decrease in autochthonous or allochthonous resources along the salinity gradient. In many instances, bacterial growth and grazing appear to be nearly balanced (Christaki *et al.*, 1999; Zubkov *et al.*, 2000), suggesting a strong top–down control of bacterial abundance. Viral lysis has been measured in fewer systems, but in several studies when viruses and bacteria were compared, strong correlations between their abundances were observed (Cochlan *et al.*, 1993; Kepner *et al.*, 1998; Bettarel *et al.*, 2003), suggesting that most viruses are bacteriophages. Generally, an increase in both bacteria and viral abundance is observed with increasing trophic status (Wommack and Colwell, 2000). However, one study of an estuarine gradient of Mobile Bay did not find any environmental factor related to grazing or viral lysis (Ortmann *et al.*, 2011), suggesting that trophic status may be less effective as an indicator of the balance between bacterivory and viral lysis at a given site.

Viral-induced mortality is thought to be responsible for 5–50% of bacterial mortality and at times can cause similar or higher bacterial mortality than nanoflagellate grazing, depending on the system (Weinbauer and Höfle, 1998; Fischer and Velimirov, 2002; Choi *et al.*, 2003). It is likely that the importance of viral lysis can increase under conditions where protozoan grazing is reduced (Bettarel *et al.*, 2004). For example, Weinbauer and Höfle (Weinbauer and Höfle, 1998) reported lytic mortality to be strongly dominant in the anaerobic hypolimnion layer of Lake Plussee, Germany, where protists are scarce due to oxygen depletion. The relative contribution of viral lysis and grazing to bacterial mortality does change with depth (Weinbauer and Höfle, 1998), and viruses also are important mortality agents for prokaryotes in the sediments of bathypelagic waters (Danovaro *et al.*, 2008). Magagnini *et al.* (Magagnini *et al.*, 2007) suggested that deep waters may represent optimal environments for viral survival or proliferation. Despite these findings, the relative effects of grazing and viral lysis dynamics in relation to trophic conditions in marine systems are still largely unknown.

Biogeochemical cycles on the shelf in the East China Sea (ECS) are influenced by riverine freshwater input from the Changjiang River, which introduces DOC and inorganic nutrients as substrates for heterotrophic bacteria and phytoplankton. Previous studies in the area have largely been concerned with the temporal and spatial variations of viral, bacterial and nanoflagellate abundance in the ECS (Jiao *et al.*, 2005, 2006; Tsai *et al.*, 2010), and data showed that bacteria and nanoflagellates were more abundant in the coastal areas than in the open waters during summer. As for viruses, Jiao *et al.* (Jiao *et al.*, 2006) found viral abundance had peaks in both summer and winter, differing from previous reports of a single peak in summer. Jiao *et al.* (Jiao *et al.*, 2006) also suggested that the majority of the viruses in the Changjiang River estuary were bacteriophages. Tsai *et al.* (Tsai *et al.*, 2012a) showed that nanoflagellates largely depend on heterotrophic bacteria as an energy source in the ECS during summer. On average, heterotrophic bacteria contributed 76 and 59% of carbon consumed by nanoflagellates within the Changjiang River plume (salinity <31) and outside of it (salinity >31), respectively. To date, no study investigated the relative effect of nanoflagellate grazing and lytic mortality on BP. In the present study, we examine the relative and combined effects of these processes on bacteria in the inner and outer regions

of the Changjiang River plume in summer using a modified dilution technique to better understand the biogeochemical cycles and energy flows via the microbial loop.

METHOD

Sampling

The ECS, located on the western edge of the Northwest Pacific Ocean, is an area of dynamic interaction of several water systems, including the nutrient-enriched freshwater input from the Changjiang River (Gong *et al.*, 1996, 2003). Before 2003, fresh water discharge peaked between June and August (summer), forming what is known as Changjiang Diluted Water (CDW) (Gong *et al.*, 1996). The CDW, which has an average salinity of <31, is generally influenced by the southwest monsoon, although this zone has undergone significant changes since the filling of the reservoir that was created by the Three-Gorges Dam in June 2003 (Gong *et al.*, 2006).

Research was conducted aboard the R/V Ocean Research I in the ECS in July 2011 and 2012 (Fig. 1). To further characterize growth and removal rates for bacteria in the inner and outer regions of Changjiang River plume, we carried out a series of dilution experiments at four sites; two in the inner region (I-1 and I-2) and two in the outer region (O-1 and O-2) of the Changjiang River plume (Fig. 1). During these cruises, seawater samples were collected at a depth of 2 m using a SeaBird CTD-General Oceanic Rosette assembly with 20 L Go-Flo bottles. Continuous measurements of temperature and salinity were taken at each station from the surface to near bottom using a SeaBird CTD-General Oceanic Rosette. Water samples were filtered (25 mm GF/F) for Chl *a* analysis and measured after extraction with an *in vitro* fluorometer (Turner Design 10-AU-005) (Parsons *et al.*, 1984). Samples for enumerating viruses, bacteria and nanoflagellates were fixed immediately with glutaraldehyde at a final concentration of 1 % (v/v) and stored at 4°C until analysis, which was normally completed within 30 days of sample collection.

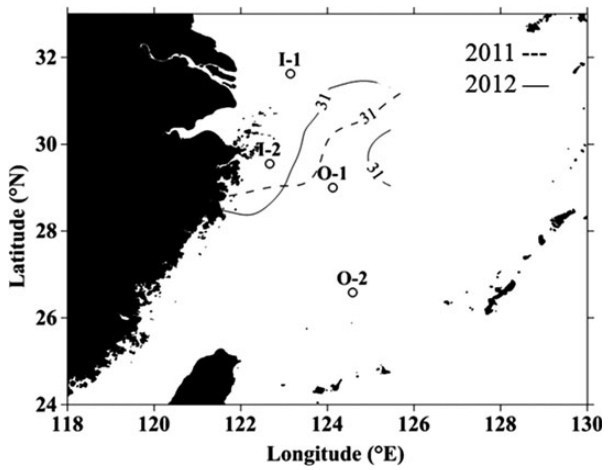

Fig. 1. Map of sampling stations (I-1, I-2, O-1 and O-2). The broken and solid lines are contours of surface salinity of 32 (isohaline) in 2011 and 2012, respectively.

Dilution experiments

We estimated viral lysis and nanoflagellate grazing rates using a modified technique of parallel dilution experiments, a "standard" set that reduces grazers and a set that reduces both grazers and viruses (Evans *et al.*, 2003). Filter holders and incubation bottles were acid-cleaned with 10% HCl and rigorously rinsed with Milli-Q water. To prepare the "standard" diluent, the natural sample was passed through a 10 μm mesh and then filtered through a 47 mm Nuclepore filter (type PC, pore size of 0.2 μm). The filtered seawater sample (<10 μm) was then diluted with the 0.2 μm filtered seawater in a 4-point dilution series: 25, 50, 75 and 100% seawater (<10 μm). The mixtures were incubated for 12 h in triplicate 50-mL polycarbonate bottles under natural light in a water bath set at the temperature of the seawater at the time of sampling (Tsai *et al.*, 2012b). The size fractionation used for grazers (<10 μm) was chosen based on previous studies at this site to eliminate most ciliates, but include nanoflagellates (Tsai *et al.*, 2011). A preliminary study found about 2% of the ciliates in our samples passed through the 10 μm filter. An additional dilution series, which used 30 kDa filtered seawater in place of 0.2 μm filtered water, modified both grazing and viral mortalities. The net growth rate of bacteria (k, h^{-1}) was calculated for each sample based on microscopic cell counts at the start and the end of the experiment (N_t and N_{t_0}), assuming exponential growth (Landry and Hassett, 1982):

$$k = \ln \frac{N_t/N_{t_0}}{t - t_0}$$

The regression coefficient of apparent growth rate versus dilution factor for the 0.2 μm dilution series is usually interpreted as nanoflagellate grazing mortality (g) (Landry and Hassett, 1982), but actually includes viral mortality (v) because almost all viruses could remain in the 0.2 μm diluents. However, when virus-free seawater (30 kDa filters) is used as a diluent, the regression reflects release from both grazing and viral mortality ($g + v$), and a direct estimate of viral mortality for bacteria can be obtained from the difference in slopes of the regression lines between the two dilution series (Evans *et al.*, 2003).

A carbon budget was determined by combining the cellular carbon content estimates and data from the modified dilution experiments. Carbon content used for heterotrophic bacteria was 20 fg C cell^{-1} (Lee and Fuhrman, 1987). BP (mg C m^{-3} day^{-1}), and losses due to grazing (G, mg C m^{-3} day^{-1}) and viral lysis (V, mg C m^{-3} day^{-1}) were calculated using the following formulae: BP $= \mu \times B_0$, $G = g \times B_0$ and $V = v \times B_0$, where μ is the dilution-based specific growth (y-intercept of the 30 kDa regression), mg and mv are the dilution-based grazing and viral lysis rates, and B_0 the heterotrophic bacterial biomass (mg C m^{-3}) at the sampling time.

Viral, bacterial and nanoflagellate abundance counts

Viruses, bacteria and nanoflagellates were counted using an epifluorescence microscope (Nikon Optiphot-2) (×1000). Viruses were processed with a slight modification of the procedure described by Nobel and Fuhrman (Nobel and Fuhrman, 1998). Briefly, 0.5–1 mL samples were filtered on Anodisc filters (0.02 μm pore size, Whatman) backed by 0.45 μm pore size Millipore filter. The samples were then placed on drops of SYBR Green I (Molecular Probes) solution diluted at 1:400 in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stained for 15 min in the dark. The membranes were placed on glass slides and added with 25 μL of 50% glycerol/50% PBS buffer (0.85% NaCl, 0.05 M NaH_2PO_4, pH 7.5) containing 0.1% *p*-phenylenediamine as antifade and mounting agents. For bacteria and nanoflagellate enumeration, subsamples of 1–2 or 20 mL were filtered onto 0.2 or 0.8 μm black Nuclepore filters, respectively. Samples were stained with DAPI at a final concentration of 1 μg mL^{-1} (Porter and Feig, 1980) to count bacteria and heterotrophic nanoflagellates (HNF). Pigmented nanoflagellates (PNF) and HNF were counted based on the presence or absence of chlorophyll autofluorescence using separate filter sets optimized for chlorophyll or DAPI under a ×1000 epifluorescence microscope (Nikon-Optiphot-2). Bacteria and HNF were identified by their blue fluorescence under UV illumination. PNF were identified by their orange and red autofluorescence under blue excitation light. To obtain reliable estimates of abundance, we counted 30, 30 and 50 fields of view for viruses, bacteria and nanoflagellates, respectively.

Statistical analysis

Least-square regression analysis was performed to analyze the relationship between bacterial growth rate and fraction of 30 kDa or 0.2 μm dilution series. Significance of the regression lines was tested using an analysis of variance. Additionally, the significance between the slopes of the 30 kDa and 0.2 μm dilution series was determined using an F-test. If the regression slopes of 30 kDa and 0.2 μm dilution series were significantly different, we calculated the magnitude of viral mortality. STATISTICA 7.0 software was used for all statistical operations. A probability value of <0.05 was considered significant.

RESULTS

Physical, chemical and biological characterization

During the study period, surface water temperatures ranged from 22.65 to 25.95°C at the inner region Changjiang River plume stations and from 27.17 to 29.13°C at the outer stations (Table I). Salinity had a minimum value of 19.53 psu at St. I-1 in 2011 due to freshwater discharge from the Changjiang River, and was always >32 psu at stations in the outer regions (Table I). At the inner stations, chl *a* concentrations ranged from 0.15 to 23.18 mg m^{-3} with much higher

Table I: Time and sampling site of the experiments as well as environmental factors (temperature and salinity) and biological factors (Chl a*, viruses, bacteria and nanoflagellate)*

	Temperature (°C)	Salinity (psu)	Chl *a* (mg m^{-3})	Viruses (×10^6 virus mL^{-1})	Bacteria (×10^5 cells mL^{-1})	VBR (virus/bacteria)	Nanoflagellate (×10^3 cells mL^{-1})
2011							
I-1	24.05	19.53	3.65	36.8	11.3	32.6	5.2
I-2	22.65	29.22	4.56	42.1	10.2	41.3	4.8
O-1	27.76	32.23	0.38	7.2	7.5	9.6	3.2
O-2	27.17	33.79	0.15	6.6	6.9	9.6	2.9
2012							
I-1	25.00	27.06	23.18	70.3	13.2	53.3	5.6
I-2	25.95	27.08	18.78	52.1	9.4	55.4	5.2
O-1	27.66	32.96	0.38	8.2	8.2	10.0	4.2
O-2	29.13	32.44	0.24	6.9	7.6	9.1	3.3

VBR, virus-to-bacteria ratio.

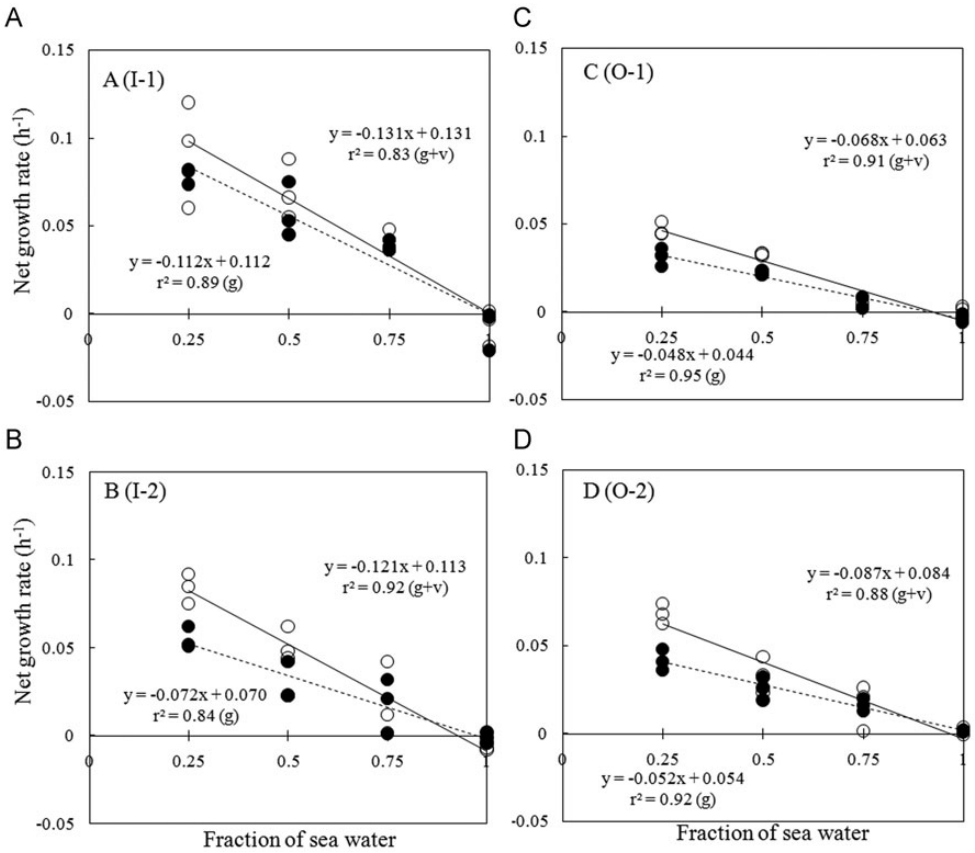

Fig. 2. Dilution plots of net bacterial growth rate (h^{-1}) versus the seawater fraction in the parallel experiments were performed in 2011. Closed and open circles represent growth rates from the 0.2 μm and 30 kDa dilution series, respectively.

values in 2011, but were always <0.5 mg m^{-3} at the outer region sites (Table I).

Viral, bacterial and nanoflagellate abundance ranged from 6.6×10^6 to 70.3×10^6 viruses mL^{-1}, 6.9×10^5 to 13.2×10^5 bacteria mL^{-1} and 2.9×10^3 to 5.6×10^3 HNF mL^{-1}, respectively (Table I). The highest abundances of all groups occurred inside the region of the plume (salinity <31) and lower abundance were found outside the plume. The virus-to-bacteria ratio (VBR) was significantly higher in the inner region (32.6–55.4) than in the outer region of the Changjiang River plume (9.1–10.0) (*t*-test, $P < 0.05$, Table I).

Bacterial growth and mortality

We used the modified dilution method to estimate rates of bacterial growth, grazing and viral lysis at four different stations during the summers of 2011 and 2012 (Figs 2 and 3). Bacterial growth rates varied between 0.058 and 0.157 h^{-1}, with high growth rates of bacteria detected in the inner plume (Table II). Inside and outside the plume, the slopes for the 0.2 μm fractionated series indicated bacterial grazing rates ranging from 0.053 to 0.126 and 0.042 to 0.052 h^{-1} (Figs 2, 3 and Table II). At Stn I-1 in 2011 and Stns I-1 and I2 in 2012, viral lysis was not detected, since the estimates were not significantly different from grazing alone (*F*-test, $P > 0.05$) (Table II). During the five other experiments, nanoflagellate grazing was responsible for most of the bacterial mortality (60–71%) relative to viral lysis on all but one occasion. Viral lysis accounted for about 51% of bacterial mortality at Stn O-2 in 2012.

Bacterial carbon production and losses

BP varied greatly between stations (27.82 to 99.47 mg C m^{-3} day^{-1}) with the highest value found at Stn I-1 in 2012 (Table II). The total bacterial carbon loss due to viral lysis and nanoflagellate grazing was approximately balanced with BP (Fig. 4). At three points when viral lysis was not determined to be significant (I-1 in 2011, I-1 and I-2 in 2012), carbon loss due to nanoflagellate grazing alone removed 81% of BP (Fig. 4).

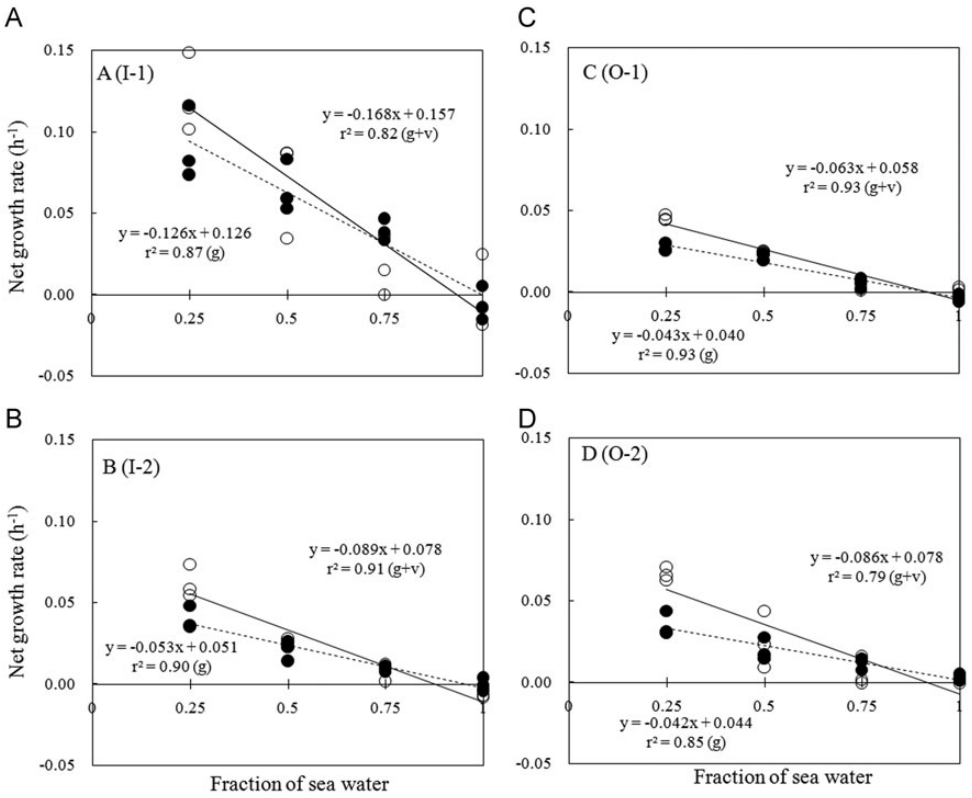

Fig. 3. Dilution plots of net bacterial growth rate (h^{-1}) versus the seawater fraction in the parallel experiments were performed in 2012. Closed and open circles represent growth rates from the 0.2 μm and 30 kDa dilution series, respectively.

Table II: Dilution-based specific growth rates in 30 kDa experiments (μ), nanoflagellate grazing (g) and viral lysis rates (v) for the heterotrophic bacteria

	Growth (30 kDa) (h^{-1})	Grazing (*g*) (h^{-1})	Viral (*v*) (h^{-1})	Ratio of grazing ($g/(g+v)$)(%)	Bacterial production (BP) (mg C m^{-3} day^{-1})	Grazing (*G*) (mg C m^{-3} day^{-1})	Viral (*V*) (mg C m^{-3} day^{-1})
2011							
I-1	0.131	0.112	<0.019>	—	71.05	60.75	—
I-2	0.113	0.072	0.049	60.0	55.32	35.25	23.99
O-1	0.063	0.048	0.020	70.6	22.68	17.28	7.2
O-2	0.084	0.052	0.035	59.8	27.82	17.22	11.59
2012							
I-1	0.157	0.126	<0.042>	—	99.47	79.83	—
I-2	0.078	0.053	<0.036>	—	39.31	26.71	—
O-1	0.058	0.043	0.020	68.3	22.83	16.92	7.87
O-2	0.078	0.042	0.044	48.8	28.45	15.32	16.05

Daily bacterial carbon production (BP) and the fraction of carbon losses by nanoflagellate grazing (*G*), viruses (*V*) and the ratios of nanoflagellate grazing (*g*) to total mortality of bacteria ($g+v$) was calculated for each experiment. < >, viral lysis estimates were not statistically significant ($P > 0.05$); —, not determined.

Grazing and viral lysis trends

Considering the whole spatial data set, there were peaks in bacterial growth, grazing and viral lysis in the inner plume that negatively corresponded with salinity (growth: $r = -0.68$, grazing: $r = -0.76$, viral: $r = -0.56$, $P < 0.05$) (Table III). Both grazing (*g*) and viral lysis (*v*) were significantly coupled to bacterial growth rates (Table III). Furthermore, the higher abundance of viruses in the inner plume may be a reflection of the higher abundance of bacteria there compared with the outer plume, although the correlation between the viral lysis and bacterial abundance was poor (Table III).

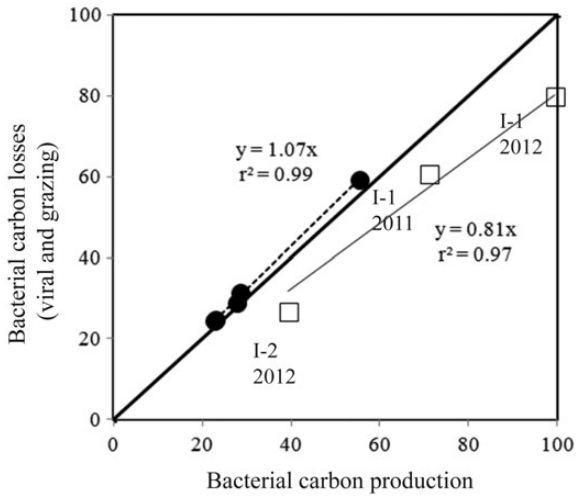

Fig. 4. The relationship between BP and total mortality losses (grazing and viral lysis) (closed black circles). Non-significant viral effects on bacteria at I-1 (2011 and 2012) and I-2 (2012) are shown in square symbols. A significant linear regression indicated a positive relationship between BP and loss for these experiments.

Table III: Spearman rank correlation coefficients for bacterial growth, bacterial grazing and viral lysis with environmental (salinity) and biological parameters (bacterial abundance and growth rate)

	Salinity	Bacterial abundance	Bacterial growth rate (μ)
Bacterial growth (μ)	−0.68	0.91	—
Bacterial grazing (g)	−0.76	0.93	0.94
Viral lysis (v)	−0.56	0.46	0.86

Bold numbers indicate significant relationship ($P < 0.05$).

DISCUSSION

A major objective of the current study was to characterize the spatial variations in bacterial mortality caused by grazing and viruses in the inner and outer regions of the Changjiang River plume. Such data add to the growing knowledge of the fate of BP in aquatic systems. Our data indicate that nanoflagellate grazing was responsible for most of the bacterial mortality within the plume in summer, whereas viral lysis predominated in oligotrophic waters on one occasion (Stn O-2 in 2012, about 51% of bacterial mortality). This shift may have major implications for the fate and cycling of organic matter along eutrophication gradients. In this study, we used the additional dilution series method (Evans *et al.*, 2003), which was successfully used to estimate the impact of viral lysis on the haptophyte *Phaeocystis globosa* (Scherffel) in temperate coastal waters (Baudoux *et al.*, 2006) and the impact of viral lysis on bacterioplankton in a mesotrophic lake (Jacquet *et al.*, 2005).

The importance of horizontal gradients in salinity and temperature in determining the distribution of estuarine organisms is now well documented (Hewson *et al.*, 2001; Froneman, 2002; 2004). In the Changjiang River estuary, the variation of intrusion of warm and relatively oligotrophic water from oceanic currents played a role alternating the distribution patterns of temperature, salinity and trophic conditions and consequently the seasonal and spatial distribution patterns of virus and bacteria (Jiao *et al.*, 2006). Such spatial trends have been observed for Chl *a* concentrations, and bacterial, nanoflagellate and viral abundance during this investigation (Table I). A similar negative correlation ($r = -0.64$) between viral abundance and salinity has been observed in the studies of viral abundance in other coastal environments (Paul *et al.*, 1993; Jiang and Paul, 1994; Jiao *et al.*, 2006). It seems unlikely that salinity directly regulates viral abundance in the Changjiang River plume. In this study, viral lysis was found to be positively related to bacterial growth rates (Table III), possibly because the input of nutrients brought in with the freshwater pulses caused an increase in microbial production resulting in higher viral production. Previous studies on viral abundance focusing on spatial distribution have shown the variation of viruses in the marine environment to be similar to that of bacteria and phytoplankton (Chl *a*) (Boehme *et al.*, 1993; Cochlan *et al.*, 1993; Jiang and Paul, 1994). In the Southern California Bight, virus abundance is generally higher in the surface and coastal waters. Viruses are more abundant in the brackish waters of the Bothnia (salinities 3.5–7 ppt) than in the oceanic environment (salinities >33 ppt) (Cochlan *et al.*, 1993). There is also clear evidence that viral abundance grows as the trophic status of lakes increases (Madan *et al.*, 2005). The previously reported abundances for viral direct counts in the Changjiang River estuary in summer, ranging from 1×10^6 to 17×10^6 viruses mL^{-1} (Jiao *et al.*, 2006), were lower than our counts here (6.6×10^6 to 70×10^6 viruses mL^{-1}). This could be a consequence of the higher bacterial abundance in the current study. Virus abundance data from our study are closer to those reported of Pan *et al.* (Pan *et al.*, 2007) in the Changjiang River estuary (viruses: 3.4×10^6 to 45.6×10^6 viruses mL^{-1}).

Positive relations between viruses and bacteria have already been reported in various freshwater (Tuomi *et al.*, 1997; Hewson *et al.*, 2001) and marine ecosystems (Steward *et al.*, 1996; Alonso *et al.*, 2001), where almost all of the viruses seem in fact to be bacteriophages (Wommack and Colwell, 2000). Moreover, VBR has been used to characterize the relationship between bacterial and viral communities (Hara *et al.*, 1991), and small variations in VBR suggest a tight coupling between bacteria and viruses (Choi *et al.*, 2003). We expected a stronger

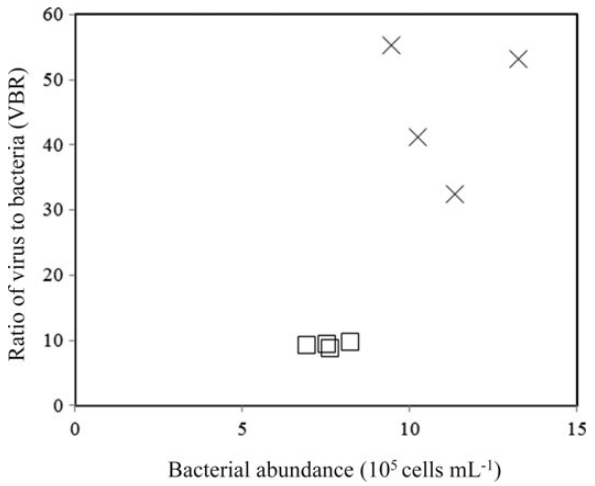

Fig. 5. The relationship between bacterial abundance and the ratio of viruses to bacteria in the inner (×) and outer (□) of the Changjiang River plume.

coupling of bacterial and viral abundance in areas with higher bacterial density, given the fact that virus-mediated mortality of cells generally increases with increasing bacterial host density (Weinbauer and Peduzzi, 1995). However, values of VBR were clearly higher at the plume stations in the inner region (32.6–55.4) than at the outer region stations (9.1–10) (Fig. 5). A previous study showed a similar result, noting a sharp drop in VBR along the increasing salinity in the Changjiang River (Jiao *et al.*, 2006). One possible explanation of this phenomenon may be that estuarine waters, which have large, rapidly growing bacterial populations, would seem ideal for phage infection and growth compared with oligotrophic offshore environments, which are characterized by slow bacterial growth and long generation times. Another possible explanation for the high variations of VBR could be that the availability of nutrients influences viral life strategies. Lysogenic infection, which does not lyse cells, is typically considered the most favorable way of bacterial infection in waters characterized by low bacterial and primary production (Weinbauer and Suttle, 1999; Williamson *et al.*, 2002). However, our results indicate a reduced impact of viral lyses on bacteria in the more eutrophic areas (Table II). Still another possibility might be that an increasing relative abundance of non-bacteriophage viruses such as cyanophages in estuarine waters where plankton communities are typically dominated by cyanobacteria. These possible reasons may explain the reason that the values and variations of VBR were significantly higher at the inner station than the outer stations. Furthermore, based on the difference of linear regression within and outside of plume stations, variations in bacterial abundance explained 85% of the variability in viral abundance and could be used as the

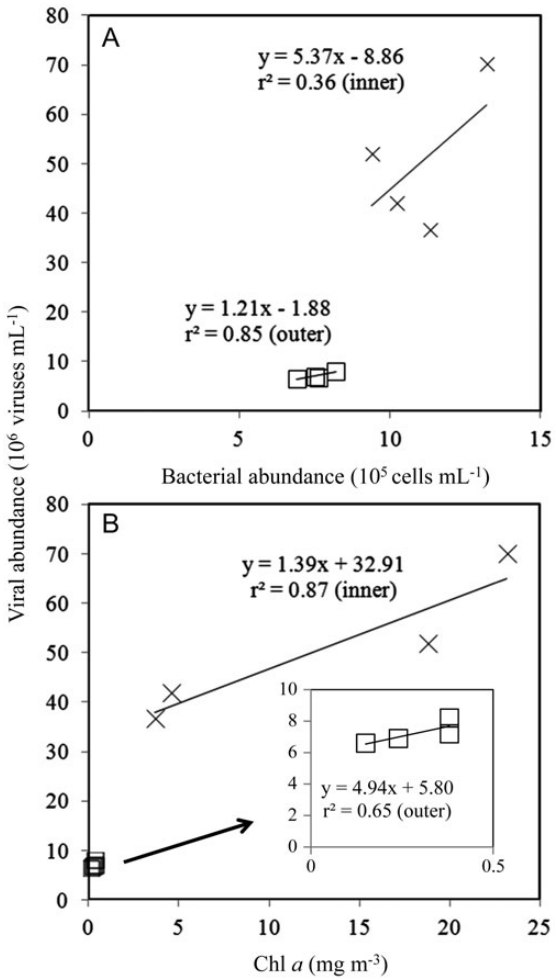

Fig. 6. The relationship between viral abundance and bacterial abundance (**A**) and Chl *a* concentrations (**B**) in the inner (crosses) and the outer (boxes) regions of Changjiang River plume.

sole predictor of changes in viral numbers outside plume stations (Fig. 6A). For stations of the inner plume, a linear regression between parameters, Chl *a* concentrations demonstrated a better degree of explanation ($r^2 = 0.87$) than bacterial abundance ($r^2 = 0.36$) (Fig. 6). This is consistent with the third hypothesis of an increase in non-bacteriophage viruses inshore.

The extent of losses due to grazing and viral lysis varied widely among geographical locations (Magagnini *et al.*, 2007; Fonda Umani *et al.*, 2010). Grazing pressure on bacteria was higher in the inner plume, but viral activity was not detected during this period (St. I-1), suggesting that nanoflagellate grazing played a key role in controlling bacterial biomass, possibly exceeding the impact of viral lysis during summer period in the inner Changjiang River plume. Our results, which are similar to those reported for the Mobile Bay estuary (Ortmann *et al.*, 2011), indicate that protist grazing was important

and bacterivorous protists could act as a food source supporting the large biomass of higher trophic levels during the study period. Personnic *et al.* (Personnic *et al.*, 2009) reported nanoflagellate grazing to be a significant cause of mortality in heterotrophic bacteria in the autumn (up to 42%) and summer (up to 76%), but in the winter and early spring, which are periods of relatively low productivity, the impact of neither viruses nor nanoflagellates could be clearly detected. The distinction and quantification of bacterial losses due to grazing or lysis, which does not directly support higher trophic levels, is essential for an optimal understanding of carbon pathway in marine environments.

Unlike grazing, bacterial mortality attributable to viruses was only detected in five of eight experiments in our study using the dilution approach (Table II). The ratio of lytic mortality to total mortality exceeded 50% only in 2012 at St. O-2. Studies investigating the contribution of grazing and viral lysis to bacterial mortality have shown that the effect of viral lysis on bacterial mortality varies widely, but it can often have as large an effect as that of grazing (Fuhrman and Noble, 1995; Weinbauer and Peduzzi, 1995; Fischer and Velimirov, 2002). Viruses are generally thought to be responsible for about 10–50% of the total bacterial mortality in surface waters (Suttle, 1994; Steward *et al.*, 1996; Weinbauer and Höfle, 1998; Wilhelm *et al.*, 2002). Evidence has suggested that viruses and protists are responsible for a similar amount of bacterial mortality in the Bering and Chukchi (Arctic) Sea, with protists dominating in some water samples and viruses in others (Steward *et al.*, 1996). Although the utility of the dilution technique for estimating viral lysis rates in microbial communities is not absolute and needs further statistical testing, viral mortality rates of $<0.1\ day^{-1}$ are unlikely to be detected (Kimmance *et al.*, 2007). For the present study, viral lysis ranged from 0.48 to 1.2 day^{-1}, but the technique may not have been sensitive enough to measure reduced viral lysis within the plume. Furthermore, mortality of bacteria due to phages during our study increased with the increase in bacterial abundance and growth rate, suggesting that the metabolic status of the host is critical for viral infection and proliferation, as it has been found in other studies (Weinbauer *et al.*, 2003; Boras *et al.*, 2009).

The interactions between viruses and grazers and their effects on picoplankton are probably very complex (Mike and Jacquet, 2008) and might include various antagonistic or synergistic effects (Sime-Ngando and Pradeep Ram, 2005). For example, nanoflagellates can directly reduce viral abundance and infectivity through direct consumption of viruses or by grazing preferentially on viral-infected cells (Bettarel *et al.*, 2005). Although our study did not directly address these complexities, it does suggest that protistan grazing and viral infections interact to modify picoplankton production in the ECS. We also found a balanced budget between BP and losses through bacterivory and viral infection (Fig. 4), suggesting strong top–down control of bacteria in the ECS during the summer. Our data indicate that in the inner of Changjiang River plume, the majority of the BP was removed by nanoflagellates and thus could be transferred to higher trophic levels of the food web. The relatively higher mortality of bacteria due to viruses in the offshore waters indicates that an important fraction of BP remains in the viral loop.

ACKNOWLEDGEMENTS

We are most grateful to Mr. James Steed for his editing and comments on the manuscript.

FUNDING

This study was supported by a grant (NSC 101-2611-M-019-009) from the National Science Council, ROC.

REFERENCES

Almeida, M. A., Cunha, M. A. and Alcântara, F. (2001) Factors influencing bacterial production in a shallow estuarine system. *Microb. Ecol.*, **42**, 416–426.

Alonso, M. C., Jimenez-Gomez, F., Rodriguez, J. *et al.* (2001) Distribution of virus-like paticles in an oligotrophic marine environment (Alboran Sea, Western Mediterranean). *Microb. Ecol.*, **42**, 407–415.

Ameryk, A., Podgórska, B. and Witek, Z. (2005) The dependence between bacterial production and environmental conditions in the Gulf of Gdaúsk. *Oceanologia.*, **47**, 27–45.

Baudoux, A. C., Noordeloos, A. A. M., Veldhuis, M. J. W. *et al.* (2006) Virally induced mortality of *Phaeocystis globosa* during two spring blooms in temperate coastal waters. *Aquat. Microb. Ecol.*, **44**, 207–217.

Bettarel, Y., Sime-Ngando, T., Amblard, C. *et al.* (2003) Virioplankton and microbial communities in aquatic systems: a seasonal study in two lakes of differing trophy. *Freshw. Biol.*, **48**, 810–822.

Bettarel, Y., Sime-Ngando, T., Amblard, C. *et al.* (2004) Viral activity in two contrasting lake ecosystems. *Appl. Environ. Microbiol.*, **70**, 2941–2951.

Bettarel, Y., Sime-Ngando, T., Bouey, M. *et al.* (2005) Low consumption of virus-sized particles by heterotrophic nanoflagellates in two lakes of French Massif Central. *Aquat. Microb. Ecol.*, **39**, 205–209.

Boehme, J., Frischer, M. E., Jiang, S. C. *et al.* (1993) Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. *Mar. Ecol. Prog. Ser.*, **97**, 1–10.

Boras, J. A., Sala, M. M., Vázquez-Domínguez, E. *et al.* (2009) Annual changes of bacterial mortality due to viruses and protists in an

oligotrophic coastal environment (NW Mediterranean). *Environ. Microbiol.*, **11**, 1181–1193.

Choi, D. H., Hwang, C. Y. and Cho, B. C. (2003) Comparison of virus- and bacterivory-induced bacterial mortality in the eutrophic Masan Bay, Korea. *Aquat. Microb. Ecol.*, **30**, 117–125.

Christaki, U., Van Wambeke, F. and Dolan, J. R. (1999) Nanoflagellates (mixotrophs, heterotrophs and autotrophs) in the oligotrophic eastern Mediterranean: standing stocks, bacterivory and relationships with bacterial production. *Mar. Ecol. Prog. Ser.*, **181**, 297–307.

Cochlan, W. P., Wikner, J., Steward, G. F. *et al.* (1993) Spatial distribution of viruses, bacteria and chlorophyll *a* in neritic, oceanic and estuarine environments. *Mar. Ecol. Prog. Ser.*, **92**, 77–87.

Cole, J. J., Findley, S. and Pace, M. L. (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, **43**, 1–10.

Danovaro, R., DellÁnno, A., Corinaldesi, C. *et al.* (2008) Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature*, **454**, 1084–1087.

Evans, C., Archer, S. D., Jacquet, S. *et al.* (2003) Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas* spp. population. *Aquat. Microb. Ecol.*, **30**, 207–219.

Fischer, U. R. and Velimirov, B. (2002) High control of bacterial production by viruses in a eutrophic oxbow lake. *Aquat. Microb. Ecol.*, **27**, 1–12.

Fonda Umani, S., Malisana, E., Focaracci, F. *et al.* (2010) Disentangling the effect of viruses and nanoflagellates on prokaryotes in bathypelagic waters of the Mediterranean Sea. *Mar. Ecol. Prog. Ser.*, **418**, 73–85.

Froneman, P. W. (2002) Response of the plankton to three different hydrological phases of the temporarity open/closed Kasouga estuary, South Africa. *Estuarine Coastal Shelf Sci.*, **55**, 535–546.

Froneman, P. W. (2004) Food web dynamics in a temperate temporarily open/closed estuary (South Africa). *Estuarine Coastal Shelf Sci.*, **59**, 87–95.

Fuhrman, J. A. and Noble, R. T. (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnol. Oceanogr.*, **40**, 1236–1242.

Gong, G. C., Chang, J., Chiang, K. P. *et al.* (2006) Reduction of primary production and changing of nutrient ratio in the East China Sea: Effect of the Three Gorges Dam? *Geophys. Res. Lett.*, **33**, doi: 10.1029/2006GL025800.

Gong, G. C., Chen, L. Y. L. and Liu, K. K. (1996) Chemical hydrography and chlorophyll a distribution in the East China Sea in summer: implications in nutrient dynamics. *Cont. Shelf Res.*, **16**, 1561–1590.

Gong, G. C., Wen, Y. H., Wang, B. W. *et al.* (2003) Seasonal variation of chlorophyll a concentration, primary production and environmental conditions in the subtropical East China Sea. *Deep-Sea Res. Pt. II*, **50**, 1219–1236.

Hara, S., Terauchi, K. and Koike, I. (1991) Abundance of viruses in marine waters: assessment by epifluorescence and transmission electron microscopy. *Appl. Environ. Microbiol.*, **57**, 2731–2734.

Hewson, I., O'Neil, J. M., Fuhrman, J. A. *et al.* (2001) Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. *Limnol. Oceanogr.*, **46**, 1734–1746.

Jacquet, S., Domaizon, I., Personnic, S. *et al.* (2005) Estimates of protozoan- and viral-mediated mortality of bacterioplankton in Lake Bourget (France). *Freshw. Biol.*, **50**, 627–645.

Jiang, S. C. and Paul, J. H. (1994) Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. *Mar. Ecol. Prog. Ser.*, **104**, 163–172.

Jiao, N., Yang, Y., Hong, N. *et al.* (2005) Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea. *Continent. Shelf Res.*, **25**, 1265–1279.

Jiao, N., Zhao, Y., Luo, T. *et al.* (2006) Natural and anthropogenic forcing on the dynamics of virioplankton in the Yangtze river estuary. *J. Mar. Biol. Assoc. UK*, **86**, 543–550.

Kepner, R. L., Wharton, R. A. and Suttle, C. A. (1998) Viruses in Antarctic Lakes. *Limnol. Oceanogr.*, **43**, 1754–1761.

Kimmance, S. A., Wilson, W. H. and Archer, S. D. (2007) Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: limitations associated with method sensitivity in natural waters. *Aquat. Microb. Ecol.*, **49**, 207–222.

Landry, M. R. and Hassett, R. P. (1982) Estimating the grazing impact of marine microzooplankton. *Mar. Biol.*, **67**, 283–288.

Lee, S. and Fuhrman, J. A. (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl. Environ. Microbiol.*, **53**, 1298–1303.

Madan, N. J., Marshall, W. A. and Laybourn-Parry, J. (2005) Virus and microbial loop dynamics over an annual cycle in three contrasting Antarctic lakes. *Freshw. Biol.*, **50**, 1291–1300.

Magagnini, M., Corinaldesi, C., Monticelli, L. S. *et al.* (2007) Viral abundance and distribution in mesopelagic and bathypelagic waters of the Mediterranean Sea. *Deep- Sea Res., I*, **54**, 1209–1220.

Meire, P., Ysebaert, T., Van Damme, S. *et al.* (2005) The Scheldt estuary: a description of a changing ecosystem. *Hydrobiologica*, **540**, 1–11.

Mike, T. and Jacquet, S. (2008) Complex interactions in the microbial world: underexplored key links between virus, bacterial and protozoan grazers in aquatic environments. *Aquatic. Microb. Ecol.*, **51**, 195–208.

Nobel, R. T. and Fuhrman, J. A. (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat. Microb. Ecol.*, **14**, 113–118.

Ortmann, A. C., Metzger, R. C., Liefer, J. D. *et al.* (2011) Grazing and viral lysis vary for different components of the microbial community across an estuarine gradient. *Aquat. Microb. Ecol.*, **65**, 143–157.

Pace, M. L. (1988) Bacterial mortality and the fate of bacterial production. *Hydrobiologica*, **159**, 41–49.

Pace, M. L. and Cole, J. J. (1994) Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. *Microb. Ecol.*, **28**, 181–193.

Pan, L. A., Zhang, J. and Zhang, L. H. (2007) Picophytoplankton, nanophytoplankton, heterotrophic bacteria and viruses in the Changjiang Estuary and adjacent coastal waters. *J. Plankton Res.*, **29**, 187–197.

Parsons, T. R., Maita, Y. and Lalli, C. M. (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, New York.

Paul, J. H., Rose, J. B., Jiang, S. C. *et al.* (1993) Distribution of viral abundance in the reef environment of Key Largo, Florida. *Appl. Environ. Microbiol.*, **59**, 718–724.

Personnic, S., Domaizon, I., Sime-Ngando, T. *et al.* (2009) Seasonal variations of microbial abundances and virus-versus flagellate-induced mortality of picoplankton in three peri-alpine lakes. *J. Plankton Res.*, **31**, 1161–1171.

Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.

Sime-Ngando, T. and Pradeep Ram, A. S. (2005) Grazer effects on prokaryotes and viruses in a freshwater microcosm experiment. *Aquat. Microb. Ecol.*, **41**, 115–124.

Steward, G. F., Smith, D. C. and Azam, F. (1996) Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. *Mar. Ecol. Prog Ser.*, **131**, 287–300.

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

Suttle, C. A. (2005) Viruses in the sea. *Nature*, **437**, 356–361.

Suttle, C. A. (2007) Marine viruses-major players in the global ecosystem. *Nat. Rev. Microbiol.*, **5**, 801–812.

Taira, Y., Uchimiya, M. and Kudo, I. (2009) Simultaneous estimation of viral lysis and protozoan grazing on bacterial mortality using a modified virus-dilution method. *Mar. Ecol. Prog Ser.*, **379**, 23–32.

Tsai, A. Y., Gong, G. C., Sanders, R. W. *et al.* (2010) The impact of the Changjiang River plume extension on the nanoflagellate community in the East China Sea. *Estuarine Coastal Shelf Sci.*, **89**, 21–30.

Tsai, A. Y., Gong, G. C., Sanders, R. W. *et al.* (2011) Importance of bacterivory by pigmented and heterotrophic nanoflagellates during the warm season in a subtropical western Pacific coastal ecosystem. *Aquat. Microb. Ecol.*, **63**, 9–18.

Tsai, A. Y., Gong, G. C., Sanders, R. W. *et al.* (2012a) Heterotrophic bacterial and *Synechococcus* spp. Growth and mortality along the inshore-offshore in the East China Sea in summer. *J. Oceanogr.*, **68**, 151–162.

Tsai, A. Y., Gong, G. C., Sanders, R. W. *et al.* (2012b) Viral lysis and nanoflagellate grazing as factors controlling diel variations of *Synechococcus* spp. summer abundance in coastal waters of Taiwan. *Aquat. Microb. Ecol.*, **66**, 159–167.

Tuomi, P., Torsvik, T., Heldal, M. *et al.* (1997) Bacterial population dynamics in a meromictic lake. *Appl. Environ. Microbiol.*, **63**, 2181–2188.

Weinbauer, M. G., Brettar, I. and Höfle, M. G. (2003) Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. *Limnol. Oceanogr.*, **48**, 1457–1465.

Weinbauer, M. G. and Höfle, M. G. (1998) Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Appl. Environ. Microbiol.*, **64**, 431–438.

Weinbauer, M. G. and Peduzzi, P. (1995) Significance of viruses versus heterotrophic nanoflagellates for controlling bacterial abundance in the northern Adriatic Sea. *J. Plankton Res.*, **17**, 1851–1856.

Weinbauer, M. G. and Suttle, C. A. (1999) Lysogeny and prophage induction in coastal and offshore bacterial communities. *Aquat. Microb. Ecol.*, **18**, 217–225.

Wilhelm, S. W., Brigden, S. M. and Suttle, C. A. (2002) A dilution technique for the direct measurement of viral production: a comparison in stratified and tidally mixed coastal waters. *Microb. Ecol.*, **43**, 168–173.

Williamson, S. J., Houchin, L. A., McDaniel, L. *et al.* (2002) Seasonal variation in lysogeny as depicted by prophage induction in Tampa Bay, Florida. *Appl. Environ. Microbiol.*, **68**, 4307–4314.

Wommack, K. E. and Colwell, R. R. (2000) Virioplankton:viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.*, **64**, 69–114.

Zubkov, M. V., Sleigh, M. A., Burkill, P. H. *et al.* (2000) Bacterial growth and grazing loss in contrasting areas of North and South Atlantic. *J. Plankton Res.*, **22**, 685–711.