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# Lytic viral infection of bacterioplankton in deep waters of the western Pacific Ocean

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**Abstract.** As the most abundant biological entities in the ocean, viruses influence host mortality and nutrient recycling mainly through lytic infection. Yet, the ecological characteristics of virioplankton and viral impacts on host mortality and biogeochemical cycling in the deep sea are largely unknown. In the present study, viral abundance and lytic infection were investigated throughout the water column in the western Pacific Ocean. Both the prokaryotic and viral abundance and production showed a significantly decreasing trend from epipelagic to meso- and bathypelagic waters. Viral abundance decreased from $0.36$–$1.05 \times 10^{10}$ particles $L^{-1}$ to $0.43$–$0.80 \times 10^9$ particles $L^{-1}$, while the virus : prokaryote ratio varied from 7.21 to 16.23 to 2.45–23.40, at the surface and 2000 m, respectively. Lytic viral production rates in surface and 2000 m waters were, on average, $1.03 \times 10^{10}\,L^{-1}\,day^{-1}$ and $5.74 \times 10^8\,L^{-1}\,day^{-1}$. Relatively high percentages of prokaryotic cells lysed by viruses at 1000 and 2000 m were observed, suggesting a significant contribution of viruses to prokaryotic mortality in the deep ocean. The carbon released by viral lysis in deep western Pacific Ocean waters was from 0.03 to 2.32 µg C $L^{-1}\,day^{-1}$. Our findings demonstrated a highly dynamic and active viral population in these deep waters and suggested that virioplankton play an important role in the microbial loop and subsequently biogeochemical cycling in deep oceans.

## 1 Introduction

Viruses are regarded as critical players in the control of marine microbial communities (Suttle, 2005; Weinbauer, 2004; Brussaard et al., 2008). They produce progeny through infection of their hosts, which include both heterotrophic and autotrophic organisms (Weinbauer, 2004a; Brussaard, 2004), and viral infection and lysis act as a major contributing factor of bacterial mortality (Fuhrman, 1999; Suttle, 2007; Wommack and Colwell, 2000; Proctor and Fuhrman, 1990). On average, 10–50 % of bacteria are estimated to be lysed daily (Wilhelm et al., 1998; Steward et al., 1996), and even higher in some specific environments where 50–100 % is estimated (Weinbauer et al., 1995; Steward et al., 1996). The ecological effects of viral lysis on prokaryotic biomass, production and community structure are demonstrated in various environments (Thingstad and Lignell, 1997; Brussaard et al., 2008; Wilhelm and Matteson, 2008; Zhang et al., 2007). Meanwhile, viral lysis of host cells releases large amounts of dissolved organic matter (DOM) into seawater, which redirects the flow of matter and energy away from higher trophic levels and influences biogeochemical cycling in the ocean. Previous studies suggest that viral lysis stimulates nutrient (such as Fe, N, and P) recycling and regeneration and modifies the efficiency of the biological pump (Gobler et al., 1997; Wilhelm and Suttle, 1999; Suttle, 2007).

Despite their ecological and biogeochemical importance, relatively little information about viral ecological characteristics in the deep sea is available compared to the surface ocean. Previous investigations, performed in the North Atlantic, Pacific and Mediterranean Sea, show high abundance

of viruses in mesopelagic, bathypelagic and abyssal waters (de Corte et al., 2012; Hara et al., 1996; Magiopoulos and Pitta, 2012; Parada et al., 2007; Umani et al., 2010; Weinbauer et al., 2003; Arístegui et al., 2009; Matteson et al., 2013; Strzepek et al., 2005; Higgins et al., 2009; Yang et al., 2014). This leads to a slower decrease or an increase of the VPR (virus : prokaryote ratio) with depth, while the general trend of other microbial parameters is a sharp decrease. The ecological and biogeochemical contribution of virioplankton in the deep sea is unknown. To answer this question, direct measurement of viral activity (e.g. production) is necessary. However, so far, most viral ecology studies focused on abundance and VPR and the data of viral production in the deep sea is scarce. Recently, direct measurement of viral production using the dilution method shows considerable viral activity in the deep regions of the north and the tropical Atlantic Ocean and the Mediterranean Sea (de Corte et al., 2012; de Corte et al., 2010; Umani et al., 2010). Nevertheless, there is still debate regarding the role of abundant viruses in the ecosystem and biogeochemical cycling in the deep ocean (Arístegui et al., 2009).

The western Pacific Ocean is characterised by low nutrient and low primary production, but strong light radiation (Barber and Chavez, 1991; Schneider and Zhu, 1998). As it is affected directly by ENSO, (the most prominent year-to-year climate variation on earth), the response of biogeochemical cycling of the western Pacific Ocean has both a local and global influence (McPhaden et al., 2006). In addition, as part of the warmest ocean waters (Warm Pool), this area is an ideal scenario for studies on the effect of rising temperatures on the marine ecosystem (Rowe et al., 2012). So far, there has been no intensive investigation of virioplankton in this area. In the present study, we surveyed viral abundance and lytic viral production throughout the water column on a basin scale in the western Pacific Ocean. Assessment of the viral lytic impact on the epipelagic, mesopelagic and bathypelagic bacterioplankton community and on carbon cycling will provide a better understanding of the microbial ecology and biogeochemistry of the western Pacific Ocean.

## 2 Materials and methods

### 2.1 Sampling and environmental data collection

Samples were collected onboard the R/V *Kexue-1* during the NSFC WPO cruise (November–December, 2010) (Wang and Li, 2012). Figure 1 shows the 10 stations selected along 2 transects (18° N and 130° E). Water temperature and salinity were measured using a CTD profiler (SBE9/11 plus, Sea-Bird Inc., USA). Water for biological and chemical analysis was collected with Niskin bottles attached to the CTD profiler and was prefiltered with a 20 μm mesh to remove large particles. The measurement of $NH_4^-$ was conducted using a shipboard fluorometric flow analyser (FIA-lab, USA). Sam-
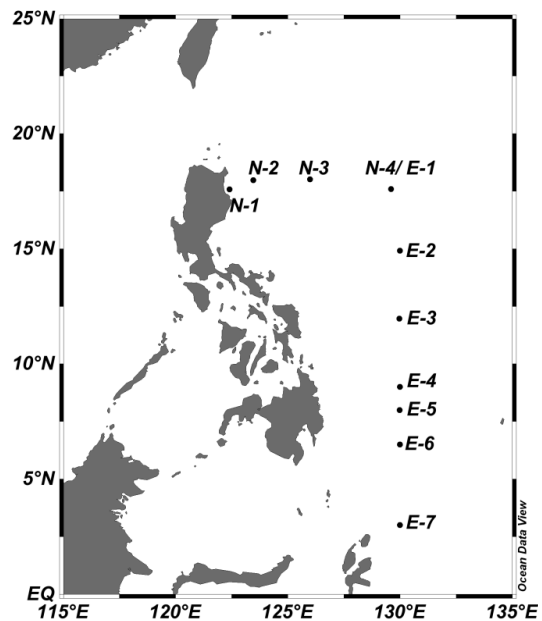


**Fig. 1.** Map of the sampling stations along two transects during the western Pacific cruise from November to December 2010. The map was generated using Ocean Data View software (Schlitzer, 2010).

ples for other nutrient analysis ($NO_3^-$, $NO_2^-$, dissolved reactive P and Si) were stored in −20 °C until measured using an Auto Analysis III, AA3 instrument (Bran-Luebbe, Germany). The monthly average MODIS Chl *a* concentrations at the surface were obtained from http://oceancolor.gsfc.nasa.gov/WIKI/OCProd.html with a resolution of 4 km.

### 2.2 Picoplankton abundance

For determination of picoplankton abundance, subsamples (2 mL) were fixed with a final concentration of 0.5 % glutaraldehyde at 4 °C for 30 min and then stored at −80 °C for later analysis after flash freezing in liquid nitrogen. Picoeukaryotes and cyanobacteria were directly counted with a Flow Cytometer (Epics Altra II, Beckman Coulter) following the method of Marie et al. (1999) and Brussaard (2004b). SYBR Green I (Molecular Probe) was used as the staining dye for heterotrophic bacterioplankton and virioplankton counting on the same flow cytometer. The data acquisition and analysis were performed with EXPOTM [32] MultiCOMP software and FCM Express software.

### 2.3 Prokaryotic production and lytic viral production

Prokaryotic production was determined from ^{3}H-Leucine incorporation rates following the methods described by Kirchman (2001). Briefly, triplicate 10 mL samples and one control (fixed with 2 % final concentration of formaldehyde) for each sampling had 10 nM ^{3}H-Leucine (specific activity, 80 Ci mmol^{−1}, Amersham) added. After 4 h incubation in

the dark at in situ temperature setup with dry bath incubators (MK-20, Hangzhou Allsheng, China), the samples were fixed and then filtered onto 0.22-µm-pore-size cellulose nitrate filters (Millipore). Later, analysis was performed on a liquid scintillation counter (280TR, PerkinElmer, USA) and prokaryotic production rates were calculated based on the average rates of the triplicate leucine incorporation rates corrected for the control.

Lytic viral production was estimated using the reduction and reoccurrence assay following Weinbauer et al. (2010). About 600 mL of seawater was filtered using tangential flow filtration (TFF) with a 0.22 µm-pore-size PVDF cartridge (Labscale, Millipore) and reduced to 50 mL prokaryotic concentrate and about 500 mL filtrate. The filtrate was ultrafiltered using a 30 kDa polysulfone cartridge (Labscale, Millipore) and TFF again in order to obtain virus-free seawater. We then mixed the prokaryotic concentrate and virus-free seawater and incubated the mixture in triplicate (50 mL) in the dark at in situ temperature in dry bath incubators. Subsamples were collected at time 0 and every 3 h over 24 h of incubation, fixed with glutaraldehyde (final concentration 0.5 %), and stored at $-80\,^\circ$C for abundance analysis after flash freezing in liquid nitrogen.

The lytic viral production rate was assumed to be equal to the rate of viral accumulation in 24 h incubations with reduced natural viral abundance (Wilhelm et al., 2002). Rates of lytic viral production were calculated using the online program VIPCAL (http://www.univie.ac.at/nuhag-php/vipcal/; Luef et al., 2009), amending the deviation of the different piecewise-linear curves of concentration over time.

## 2.4 Virus-mediated prokaryotic mortality and carbon released by viral lysis

Virus-mediated prokaryotic mortality (VMM) was calculated by dividing the lytic viral production by a mean burst size of 24 for marine environments as suggested by Parada et al. (2006). The percentage of prokaryotic cells lysed was determined by dividing VMM by the prokaryotic abundance (standing stock of prokaryotes). To estimate the carbon released by viral lysis of the prokaryotic community, the VMM was multiplied by the previously determined average cell carbon content of 12.4 fg C cell$^{-1}$ for oceanic prokaryotic assemblages (Fukuda et al., 1998).

## 2.5 Statistical analysis

Analysis of variance (ANOVA) was performed to test possible differences among depth zones (epi-, meso- and bathypelagic) or depth layers (0, 200, 1000 and 2000 m). To examine any possible correlations between viral abundance and production and individual biological and environmental parameters, non-parametric Spearman rank correlation was performed. The relationship between the viral production/abundance and measured environmental variables was

investigated using distance-based multivariate multiple regression analysis DISTLM forward in Primer 6 (Primer-E, Plymouth, United Kingdom) with the PERMANOVA+ add-on package.

# 3 Results

## 3.1 Environmental parameters

The environmental variables at our sampling sites in the investigated areas of the western Pacific Ocean are shown in the Supplement Table S1. Sea surface temperature latitudinally increased from 27.76 °C at 18° N to 29.89 °C at 3° N, while salinity varied from 32.17 to 34.60. The surface Chl $a$ concentration, obtained from http://oceancolor.gsfc.nasa.gov/WIKI/OCProd.html, remained at a low level during the whole investigation (range, 0.0310–0.1834 mg m$^{-3}$). Water temperature decreased to, on average, $18.39 \pm 4.11$, $4.25 \pm 0.48$ and $2.19 \pm 0.33$ °C at the 200, 1000 and 2000 m depths. Salinity gradually increased with depth vertically to $34.63 \pm 0.22$ at 2000 m. The $NH_4^+$ concentration varied in a narrow range throughout the water column. Significant increase of $NO_3^- + NO_2^-$ concentration was observed between the surface and 200 m depth, while similar ranges were recorded at 1000 and 2000 m. The concentrations of $PO_4^{3-}$ and $SiO_3^{2-}$ significantly increased by one to two orders of magnitude from surface to deep sea. No spatial variation was observed for any nutrient pattern (Supplement Table S1).

## 3.2 Distribution of bacterioplankton and virioplankton

The horizontal and vertical distribution of bacterioplankton and virioplankton is shown in Fig. 2. In our study, prokaryote abundance for the surface water in the western Pacific Ocean ranged from $4.04 \times 10^8$ L$^{-1}$ at Station E-3 to $1.18 \times 10^9$ L$^{-1}$ at Station E-1, with an average of $5.89 \pm 2.25 \times 10^8$ L$^{-1}$. Prokaryotic abundance generally decreased with increasing depth at all the stations investigated except Station E-6, where higher prokaryotic abundance at the bottom of the euphotic zone than at the surface was observed. The abundance of bacterioplankton in the epipelagic layers (0–200 m) ranged from $2.17 \times 10^8$ L$^{-1}$ to $1.18 \times 10^9$ L$^{-1}$ (average $4.86 \pm 1.54 \times 10^8$ L$^{-1}$). In the mesopelagic (200–1000 m) and bathypelagic waters ($> 1000$ m), the number of bacterioplankton declined to $1.12 \pm 1.06 \times 10^8$ L$^{-1}$ and $0.72 \pm 0.71 \times 10^8$ L$^{-1}$. Other than the peak appearing at the surface of Station E-1, no clear spatial or latitudinal variation of prokaryotic abundance was observed in the western Pacific Ocean (Fig. 2).

The range of surface viral abundance in the western Pacific Ocean was $0.36$–$1.05 \times 10^{10}$ particles L$^{-1}$ with an average of $5.82 \pm 2.05 \times 10^9$ particles L$^{-1}$. The highest viral abundance in the surface water was detected at Station E-1 (129.60° E, 17.60° N), while the lowest was at Station E-2 (129.99° E, 14.92° N). Vertically, viral abundance maxima

**Table 1.** Spearman's rank correlation analysis of viral abundance against biotic and abiotic parameters. –, no data; $r_s$, correlation coefficient; $P$, probability that correlation is because of chance. Relationships considered significant in this study ($P < 0.05$) are given in bold.

| Parameter | 0 m $r_s$ | $P$ | 200 m $r_s$ | $P$ | 1000 m $r_s$ | $P$ | 2000 m $r_s$ | $P$ | Entire water column $r_s$ | $P$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Viral production | 0.139 | 0.701 | 0.467 | 0.205 | −0.150 | 0.700 | −0.183 | 0.637 | **0.72** | **0** |
| Prokaryotic production | −0.167 | 0.668 | 0.393 | 0.383 | 0.429 | 0.337 | 0.107 | 0.819 | **0.796** | **0** |
| Prokaryotic abundance | **0.648** | **0.043** | 0.139 | 0.701 | 0.200 | 0.580 | 0.533 | 0.139 | **0.86** | **0** |
| Picoeukaroytic abundance | 0.255 | 0.476 | 0.488 | 0.153 | – | – | – | – | – | – |
| *Synechococcus* | 0.467 | 0.174 | 0.344 | 0.331 | – | – | – | – | – | – |
| *Prochlorococcus* | −0.456 | 0.185 | **0.745** | **0.013** | – | – | – | – | – | – |
| Chl *a* | 0.233 | 0.546 | – | – | – | – | – | – | – | – |
| Salinity | −0.345 | 0.328 | 0.515 | 0.128 | −0.491 | 0.15 | −0.655 | 0.055 | **−0.383** | **0.016** |
| Temperature | −0.115 | 0.751 | 0.6 | 0.067 | 0.03 | 0.934 | 0.4 | 0.286 | **0.892** | **0** |
| $NH_4^+$ | 0.261 | 0.467 | 0.236 | 0.511 | −0.328 | 0.354 | −0.31 | 0.456 | 0.048 | 0.775 |
| $NO_3^- + NO_2^-$ | 0.627 | 0.071 | 0.236 | 0.511 | 0.188 | 0.603 | −0.405 | 0.32 | **−0.83** | **0** |
| $NO_2^-$ | −0.16 | 0.659 | 0.253 | 0.48 | 0.364 | 0.301 | −0.368 | 0.369 | −0.262 | 0.112 |
| $SiO_3^{2-}$ | −0.152 | 0.676 | −0.612 | 0.06 | −0.103 | 0.777 | 0.048 | 0.911 | **−0.886** | **0** |
| $PO_4^{3-}$ | −0.244 | 0.497 | **−0.701** | **0.024** | 0.134 | 0.713 | 0.238 | 0.57 | **−0.808** | **0** |
| Depth | – | – | – | – | – | – | – | – | **−0.913** | **0.000** |
| Longitude | −0.523 | 0.121 | −0.164 | 0.650 | −0.182 | 0.614 | −0.176 | 0.651 | −0.11 | 0.504 |
| Latitude | 0.273 | 0.446 | 0.503 | 0.138 | 0.273 | 0.446 | −0.15 | 0.7 | 0.117 | 0.478 |

appeared at 50–100 m in most of our sampling stations. Viral abundance significantly decreased with depth from epipelagic waters to meso- and bathypelagic waters (Fig. 2; ANOVA on rank, $P < 0.001$). In the mesopelagic zone, viral abundance was $1.32 \pm 0.87 \times 10^9$ particles $L^{-1}$ and, in the bathypelagic zone, $0.68 \pm 0.36 \times 10^9$ particles $L^{-1}$. Spatially, there was no significant trend in each pelagic zone (Fig. 2). Throughout the water column, viral abundance was significantly and positively correlated with viral production, prokaryotic abundance, prokaryotic production and temperature and negatively correlated with depth, salinity and inorganic nutrient concentrations such as $NO_3^- + NO_2^-$, $PO_4^{3-}$ and $SiO_3^{2-}$ (Table 1). In specific layers, significantly positive correlation was found between viral abundance and prokaryotic abundance in the surface water, and between viral abundance and *Prochlorococcus* abundance in the 200 m waters, while significantly negative correlation of viral abundance with $PO_4^{3-}$ was recorded in the 200 m waters. DIS-TLM analysis selected temperature, picoeukaroytic abundance, salinity, prokaryotic abundance, depth and longitude as the best set of environmental parameters explaining the variations (91.469 %) of viral abundance in whole water column (Table 2). In photic zones, biological parameters such as *Prochlorococcus*, prokaryotic abundance and picoeukaroytic abundance can explain 52.909 % of variation, followed by abiotic factors (temperature and salinity). Temperature was

the only environmental parameter explaining 24.825 % of the variation in aphotic waters (Table 2).

The VPR showed a distinct pattern compared to prokaryotic and viral abundance (Fig. 2). Throughout the water column, the highest VPR (24.15) was recorded at 75 m at Station E-3. The VPR at the surface ranged from 7.21 at Station E-2 and 16.22 at Station E-5, with an average of $10.08 \pm 2.41$. The average ratio increased to $13.31 \pm 4.25$ in mesopelagic and to $14.68 \pm 6.71$ in bathypelagic waters. However, the difference in the VPR from the epi to the bathypelagic layer was not statistically significant (ANOVA on rank, $P = 0.636$).

### 3.3 Prokaryotic production and lytic viral production

Overall, prokaryotic production and lytic viral production showed a wider range than did abundance throughout the water column. At the surface, prokaryotic production varied from 10.41 at Station E-1 and to $87.84 \, \mu g \, C \, L^{-1} \, h^{-1}$ at Station N-1, with an average of $26.87 \pm 23.73 \, \mu g \, C \, L^{-1} \, h^{-1}$. The prokaryotic production declined to a level of about 10 % of the surface rate at the boundary of the photic zone (200 m) with an average of $4.40 \pm 7.36 \, \mu g \, C \, L^{-1} \, h^{-1}$. At 1000 m, the prokaryotic production was $0.74 \pm 0.61 \, \mu g \, C \, L^{-1} \, h^{-1}$ and at 2000 m it was $0.97 \pm 0.91 \, \mu g \, C \, L^{-1} \, h^{-1}$. Prokaryotic production significantly decreased from the surface to 2000 m (ANOVA on rank, $P < 0.01$), and there was no clear spatial

**Table 2.** Relationship between viral abundance/production and environmental variables analysed throughout the water column (total) and in specific pelagic layers revealed by multivariate regression analysis with forward selection (DISTLM forward).

| Viral parameters | Selected variables | Pseudo-F | $P$ | $r^2$ | Cumulative |
|---|---|---|---|---|---|
| Abundance (total) | Temperature | 769.36 | 0.0001 | 0.87590 | 0.87590 |
| | Picoeukaroytic abundance | 13.313 | 0.0005 | 0.01362 | 0.88952 |
| | Salinity | 6.0453 | 0.0182 | 0.00591 | 0.89543 |
| | Prokaryotic abundance | 7.2946 | 0.0086 | 0.00673 | 0.90216 |
| | Depth | 8.2806 | 0.0049 | 0.00715 | 0.90932 |
| | Longitude | 6.5564 | 0.0103 | 0.00538 | 0.91469 |
| Abundance (photic) | *Prochlorococcus* | 35.036 | 0.0001 | 0.35738 | 0.35738 |
| | Prokaryotic abundance | 13.529 | 0.0006 | 0.11511 | 0.47249 |
| | Picoeukaroytic abundance | 7.3326 | 0.0094 | 0.05661 | 0.52909 |
| | Temperature | 5.0117 | 0.0313 | 0.03630 | 0.56540 |
| | Salinity | 5.7852 | 0.0181 | 0.03881 | 0.60421 |
| Abundance (aphotic) | Temperature | 14.53 | 0.0006 | 0.24825 | 0.24825 |
| Production (total) | Depth | 358.18 | 0.0001 | 0.54648 | 0.54648 |
| Production (photic) | Salinity | 59.692 | 0.0173 | 0.41664 | 0.41664 |
| | Latitude | 25.502 | 0.0387 | 0.17800 | 0.59464 |
| Production (aphotic) | Temperature | 4.0652 | 0.0607 | 0.26984 | 0.26984 |

The response variable was log-transformed and the resulting data converted into Euclidian distance similarities matrices. The Pseudo-F and the $P$ values were obtained by permutation ($n = 9999$).

**Table 3.** Spearman's rank correlation analysis of lytic viral production rates against biotic and abiotic parameters. –, no data; $r_{\mathrm{s}}$, correlation coefficient; $P$, probability that correlation is because of chance. Relationships considered significant in this study ($P < 0.05$) are given in bold.

| Parameter | 0 m | | 200 m | | 1000 m | | 2000 m | | Entire water column | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $r_{\mathrm{s}}$ | $P$ | $r_{\mathrm{s}}$ | $P$ | $r_{\mathrm{s}}$ | $P$ | $r_{\mathrm{s}}$ | $P$ | $r_{\mathrm{s}}$ | $P$ |
| Viral abundance | 0.139 | 0.701 | 0.467 | 0.205 | −0.150 | 0.700 | −0.183 | 0.637 | **0.72** | **0** |
| Prokaryotic production | −0.283 | 0.460 | 0.257 | 0.623 | 0.179 | 0.702 | −0.31 | 0.456 | **0.617** | **0** |
| Prokaryotic abundance | 0.370 | 0.293 | 0.067 | 0.865 | −0.267 | 0.488 | 0.333 | 0.381 | **0.751** | **0** |
| Picoeukaroytic abundance | 0.219 | 0.544 | 0.042 | 0.915 | – | – | – | – | – | – |
| *Synechococcus* | 0.37 | 0.293 | **0.695** | **0.038** | – | – | – | – | – | – |
| *Prochlorococcus* | 0.055 | 0.881 | 0.467 | 0.205 | – | – | – | – | – | – |
| Chl *a* | 0.500 | 0.170 | – | – | – | – | – | – | – | – |
| Salinity | −0.442 | 0.2 | 0.417 | 0.265 | 0.05 | 0.898 | 0.287 | 0.422 | −0.253 | 0.125 |
| Temperature | −0.224 | 0.533 | 0.517 | 0.154 | 0.35 | 0.356 | −0.03 | 0.934 | **0.783** | **0** |
| $NH_4^+$ | 0.248 | 0.489 | −0.417 | 0.265 | −0.142 | 0.715 | −0.1 | 0.798 | 0.106 | 0.531 |
| $NO_3^- + NO_2^-$ | 0.22 | 0.569 | −0.033 | 0.932 | 0.367 | 0.332 | 0.117 | 0.765 | **−0.652** | **0** |
| $NO_2^-$ | −0.049 | 0.893 | 0.341 | 0.37 | −0.516 | 0.155 | 0.051 | 0.896 | **−0.325** | **0.05** |
| $SiO_3^{2-}$ | −0.333 | 0.347 | −0.317 | 0.406 | −0.15 | 0.7 | 0.067 | 0.865 | **−0.772** | **0** |
| $PO_4^{3-}$ | **−0.72** | **0.019** | −0.336 | 0.376 | 0.042 | 0.915 | 0.067 | 0.865 | **−0.715** | **0** |
| Depth | – | – | – | – | – | – | – | – | **−0.795** | **0.000** |
| Longitude | −0.018 | 0.960 | 0.092 | 0.814 | 0.008 | 0.983 | 0.553 | 0.597 | 0.125 | 0.454 |
| Latitude | 0.152 | 0.766 | 0.083 | 0.831 | −0.100 | 0.798 | −0.285 | 0.425 | −0.065 | 0.697 |
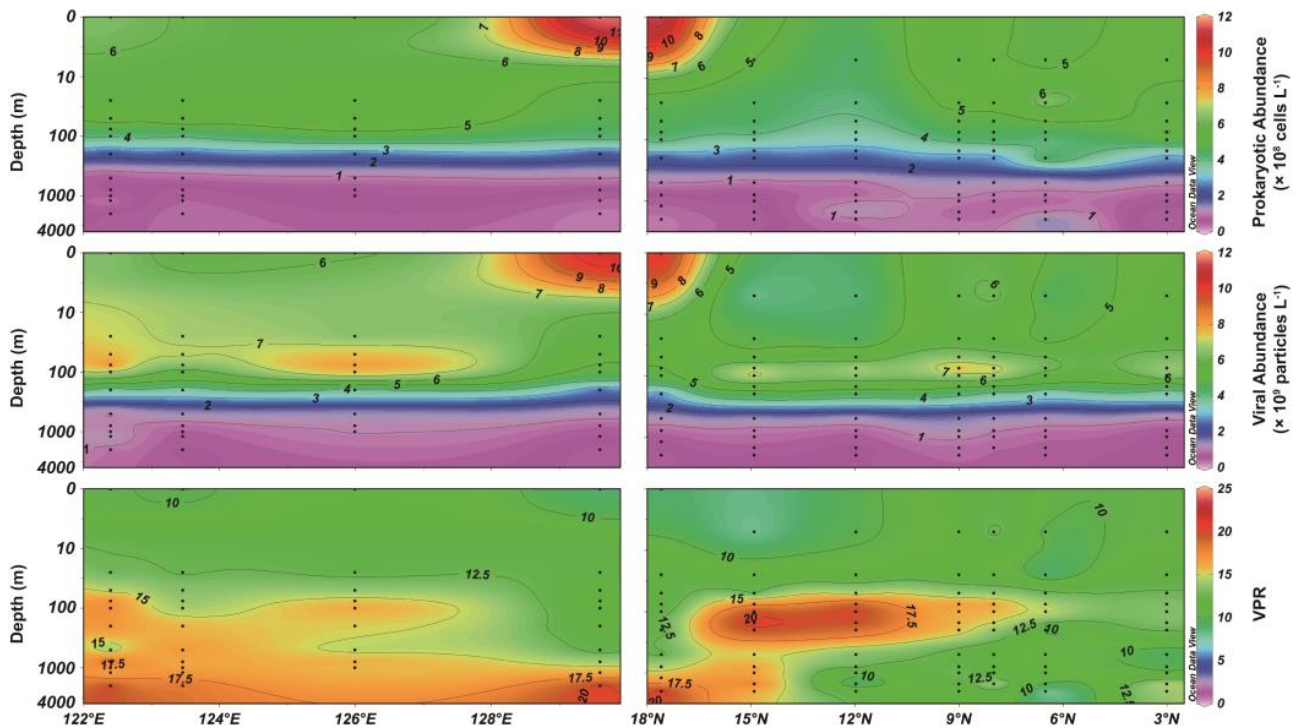
**Fig. 2.** Distribution of prokaryotic abundance, viral abundance and the VPR (virus : prokaryote ratio) in the (sub)tropical western Pacific Ocean.

variation of prokaryotic production at each depth in our investigation.

Except for one Station (E-3), the highest viral production at each station was usually observed in the surface layer, and ranged from 15.20 to 274.58 $\times 10^8 \, L^{-1}$ day$^{-1}$ (an average of $103.37 \pm 78.62 \times 10^8 \, L^{-1}$ day$^{-1}$). However, in Station E-3, the 200 m depth showed the highest viral production rate ($88.94 \times 10^8 \, L^{-1}$ day$^{-1}$). Low viral production rates were observed at 1000 m and 2000 m with averages of $12.67 \pm 13.47 \times 10^8 \, L^{-1}$ day$^{-1}$ and $5.74 \pm 4.41 \times 10^8 \, L^{-1}$ day$^{-1}$. Statistically, there was a significant difference among the four depths investigated in the western Pacific Ocean (ANOVA on rank, $P < 0.01$). Throughout the water column, viral production significantly and positively correlated with temperature, prokaryotic abundance and production and viral abundance, while significantly negative correlation was observed for VPR, depth and inorganic nutrient concentrations such as $NO_3^- + NO_2^-$, $NO_2^-$, $PO_4^{3-}$ and $SiO_3^{2-}$ (Table 3). In each layer, significantly negative correlation was found only for viral production with $PO_4^{3-}$ at the surface and significant negative correlation with *Synechococcus* abundance at 200 m (Table 3). The variability in lytic viral production was explained by depth in whole water column analysis (DISTLM test, $r^2 = 0.54$; Table 2). At two photic layers, salinity and latitude can explain 59.464 % of variability while there is no environmental factor that can be significantly related to vi-

ral production variation at aphotic layers based on DISTLM analysis (Table 2).

## 3.4 VMM and carbon released by viral lysis

Table 4 shows the VMM, the percentage of cells lysed by viruses and the carbon released by lysis calculated based on a mean burst size of 24 for marine viruses and an average estimate of 12.4 fg cell$^{-1}$ for oceanic prokaryotic assemblages (Parada et al., 2006; Fukuda et al., 1998). At the surface of the western Pacific Ocean, the VMM varied from 6.33 at Station E-3 to $114.41 \times 10^7$ cells L$^{-1}$ day$^{-1}$ at Stations N-2, with an average of $43.07 \pm 32.76 \times 10^7$ cells L$^{-1}$ day$^{-1}$. The average number of cells lysed by viruses was significantly reduced to $18.98 \pm 14.43 \times 10^7$ cells L$^{-1}$ day$^{-1}$ at the bottom of the euphotic zone and then decreased further to $5.28 \pm 5.61 \times 10^7$ cells L$^{-1}$ day$^{-1}$ at 1000 m and to $2.39 \pm 1.84 \times 10^7$ cells L$^{-1}$ day$^{-1}$ at 2000 m (ANOVA on rank, $P < 0.01$).

Surface water showed the highest average value of the percentage of cells lysed by viruses (average of 70.31 % with a range of 12.53 to 174.62 %) while there was on average a 64.95 % cell loss due to viral lysis in the 200 m waters. Relatively lower proportions of prokaryotic cells were lysed by viruses at 1000 m and 2000 m (on average 31.74 and 47.56 %, respectively). The percentage of cells lysed did not decrease significantly from the surface to 2000 m (ANOVA on rank, $P = 0.850$). In addition, three unusually high values

**Table 4.** Virus-mediated prokaryotic mortality (VMM), estimates of percentage prokaryotic cells by lysis and estimates of viral lysis effect on carbon release. VMM was calculated by dividing lytic VP by a mean burst size of 24 for marine environments according to Parada et al. (2006) and the percentage of cells lysed were determined by dividing lytic VMM by total prokaryotic counts in our study. Carbon released by viral lysis = VMM × cell carbon content, and prokaryotic carbon content were based on an average estimate of $12.4 \, \mathrm{fg \, cell^{-1}}$ for oceanic prokaryotic assemblages (Fukuda et al., 1998).

| Station | Lytic VMM ($\times 10^7 \, \mathrm{cells \, L^{-1} \, day^{-1}}$) | | | | Percentage of cells lysed (% day$^{-1}$) | | | | Carbon released ($\mathrm{\mu g \, L^{-1} \, day^{-1}}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 m | 200 m | 1000 m | 2000 m | 0 m | 200 m | 1000 m | 2000 m | 0 m | 200 m | 1000 m | 2000 m |
| N-1 | 35.12 | 8.55 | 2.07 | 0.24 | 55.13 | 55.09 | 39.72 | 8.68 | 4.35 | 1.06 | 0.26 | 0.03 |
| N-2 | 114.41 | 29.16 | 2.66 | 4.14 | 191.71 | 168.65 | 78.17 | 182.13 | 14.19 | 3.62 | 0.33 | 0.51 |
| N-3 | 9.26 | 3.49 | 1.97 | 0.64 | 17.76 | 19.06 | 26.95 | – | 1.15 | 0.43 | 0.24 | 0.08 |
| N-4/E-1 | 69.29 | 37.96 | – | 0.48 | 58.63 | 88.28 | – | 26.08 | 8.59 | 4.71 | – | 0.06 |
| E-2 | 66.39 | 30.37 | 5.35 | 5.60 | 134.43 | 164.89 | 109.91 | 153.69 | 8.23 | 3.77 | 0.66 | 0.69 |
| E-3 | 6.33 | 37.06 | 3.98 | 1.25 | 15.66 | 236.03 | 32.13 | 31.23 | 0.79 | 4.60 | 0.49 | 0.16 |
| E-4 | 19.24 | 2.93 | 1.91 | 3.62 | 29.29 | 15.13 | 22.89 | 38.09 | 2.39 | 0.36 | 0.24 | 0.45 |
| E-5 | 36.41 | – | 18.72 | 1.78 | 82.36 | – | 307.42 | 65.61 | 4.51 | – | 2.32 | 0.22 |
| E-6 | 30.13 | 10.61 | 9.30 | 3.96 | 68.80 | 15.68 | 181.35 | 16.15 | 3.74 | 1.32 | 1.15 | 0.49 |
| E-7 | 44.14 | 10.70 | 1.55 | 2.21 | 85.51 | 52.57 | 26.76 | 70.26 | 5.47 | 1.33 | 0.19 | 0.27 |

–, no data

of the percentage of cells lysed by viruses were recorded at 1000 m in Stations E-2, E-5 and E-6, due to high viral production rates.

The carbon released by lysis significantly decreased from the surface to 2000 m, with average values from $5.34 \pm 4.06$ to $0.30 \pm 0.23 \, \mathrm{\mu g \, C \, L^{-1} \, day^{-1}}$ (ANOVA on rank, $P < 0.01$). In the surface waters, viral lysis released $0.79$–$14.19 \, \mathrm{\mu g \, C \, L^{-1} \, day^{-1}}$, while the range for 2000 m decreased to 0.03 to $0.69 \, \mathrm{\mu g \, C \, L^{-1} \, day^{-1}}$. At Station E-3, the carbon released by lysis was higher at the boundary of the euphotic layer than at the surface.

## 4 Discussion

### 4.1 Viral abundance and production

The abundance of virioplankton in epipelagic western Pacific waters fell within the range of data previously reported in marine environments (Wommack and Colwell, 2000). Vertically, in most of the stations, the viral abundance maxima observed at 50–100 m roughly matched the depth of the chlorophyll maxima in the western Pacific Ocean (Higgins and Mackey, 2000; Johnson et al., 2010). This subsurface peak of viral abundance is reported frequently (Boehme et al., 1993; Cochlan et al., 1993; Hara et al, 1996; Steward et al., 1996; Yang et al., 2010), suggesting the result of a high net viral production rate due to high productivity and a low viral decay/loss rate due to weak sunlight. The significant correlations observed between viral abundance and *Prochlorococcus* abundance ($r_s = 0.745$, $P = 0.013$) at 200 m depth (Table 1), as well as the highest percentage of variation of viral abundance explained by *Prochlorococcus* abundance (Table 2), indicated a large contribution of cyanobacterial viruses (cyanophages) to viral abundance in

the euphotic zone. Yang and coworkers observed a large proportion (57 %) of the variability in total virioplankton abundance were contributed by photoautotrophic picoplankton including *Prochlorococcus* in the central Pacific and Southern oceans (Yang et al., 2010). The time-series investigation performed in oligotrophic northwestern Sargasso Sea revealed a tight linkage between virioplankton and *Prochlorococcus* throughout 10-year timescale (Parsons et al., 2012). This also explained the lack of correlation between viral abundance and heterotrophic prokaryotic abundance at this layer, which was observed in the surface samples. In addition, DISTLM analysis clearly showed a more significant contribution of biological parameters, from both heterotrophic and autotrophic hosts, than abiotic factors to viral abundance variability in euphotic zone (Table 2; Yang et al., 2010). The viral abundance in the meso- and bathypelagic waters of the western Pacific Ocean is generally higher than that in the subarctic northern Pacific Ocean and Adriatic Sea (Hara et al., 1996; Corinaldesi et al., 2003), but in a similar range with those in the subtropical North Atlantic, the Gulf of Mexico, the central Pacific and the Southern Ocean (Parada et al., 2007; Hara et al., 1996; Boehme et al., 1993; Yang et al., 2014). The decreasing pattern of viral abundance from epipelagic waters to meso- and bathypelagic waters is commonly observed in the ocean worldwide (Arístegui et al., 2009). The variability of virioplankton abundance was larger in epipelagic waters than in deeper waters, indicating a more dynamic viral population in the upper oceans. The relatively larger variability in mesopelagic waters suggested the influence of biotic and abiotic factors in the euphotic zone on viral populations in the twilight zone. It is hypothesised that the distribution and standing stock of viral particles in the deep sea is the result of a complex balance of several processes, including allochthonous input from the epipelagic zone, convective

mixing, lateral advection, production, decay and life strategies (Magagnini et al., 2007; Parada et al., 2007; Yang et al., 2014). Our DISTLM analysis showed that all biological parameters can not significantly explain the variation of viral abundance in aphotic zones (Table 2), which is similar to previous studies performed in typical open oceans (e.g. de Corte et al., 2010, 2012).

Compared to prokaryotic abundance, the less pronounced decrease of viral abundance resulted in a generally increasing VPR pattern with depth (Fig. 2). A similar increasing VPR trend is previously described in the Atlantic Ocean and the Mediterranean Sea (Parada et al., 2007; de Corte et al., 2010, 2012; Magagnini et al., 2007) and recently observed in the Pacific and Southern oceans (Yang et al., 2014). The range of VPR in the entire water column of the western Pacific Ocean is generally smaller than that in the Atlantic Ocean, the Southern Ocean and the central Pacific Ocean (Parada et al., 2007; de Corte et al., 2010, 2012; Yang et al., 2014), but is slightly higher than values in the Mediterranean Sea, Gulf of Mexico and Arctic Ocean (Magagnini et al., 2007; Boehme et al., 1993; Hara et al., 1996). Spatial comparison of deep-sea VPR revealed a possible latitudinal pattern in the western Pacific Ocean. Higher deep-sea VPR was recorded in the northern ($> 15°$ N) part of our sampling area (Fig. 2). Although this was not supported by statistical analysis due to inadequate latitudinal coverage, we noted that in a large-scale latitudinal survey at the North Atlantic Ocean, VPR in meso- and bathypelagic zones is higher in 20–40° N than in tropical waters (de Corte et al., 2012) and Yang et al. (2014) also reports the similar latitudinal trend of VPR in deep central Pacific Ocean.

Surface viral production rates measured in our study for the western Pacific Ocean were generally higher than previous estimates in the central equatorial Pacific and Sargasso Sea (Rowe et al., 2008, 2012). However, approximately one magnitude higher surface viral production is reported in a spring phytoplankton bloom in the gyre of the South Pacific Ocean (Matteson et al., 2012) and North Adriatic Sea (Bongiorni et al., 2005). In addition, Rowe et al. (2008) assess a relatively similar but slightly smaller range of surface viral production in the North Atlantic Ocean compared to our data. Compared to accumulating viral production data from surface water, there are only three studies directly estimating deep-sea viral production (Umani et al., 2010; de Corte et al., 2010, 2012). In the western Pacific Ocean, lytic viral production in the 1000 and 2000 m waters were on average 12 and 6 % of the value in the surface water, respectively. A similar proportion of surface viral production is observed in the deep waters of the Atlantic Ocean (de Corte et al., 2010, 2012). Summarising this limited but typical data set, viral production generally decreased by one or two magnitudes from the epipelagic to the bathypelagic layer in the open ocean. The decrease of viral production with depth is comparable to that of bacterial production (Parada et al., 2007; Arístegui et al.,

2009). This suggested that viruses, as with bacteria and flagellates, are an active component of the deep-sea ecosystem.

In previous studies, several biotic and abiotic parameters correlated with viral production in surface and epipelagic waters (Rowe et al., 2008; Matteson et al., 2012; Holmfeldt et al., 2010). However, little information concerning the linkage between viral activity and other factors is available for the deep sea. In our study, viral production showed significant and positive correlation with microbial parameters such as viral abundance, prokaryotic abundance and prokaryotic production throughout the water column (Table 3). Similar positive correlation was observed with temperature in whole water column comparisons. However, owing to the general environmental gradient with depth from the surface to the deep sea, the significant correlations which appeared in the entire water column in our study, including the negative ones with nutrient concentrations, cannot be explained by any ecological or biological connections among these parameters. This might be the reason why they are not significantly correlated with each other in specific layers, except for the two weak correlations with *Synechococcus* abundance and $PO_4^{3-}$. Indeed, viral production and abundance were found to be significantly negatively correlated with water depth (Tables 1 and 3). This was supported by DISTLM analysis in which depth was the only parameter explaining the variation of viral production throughout the water column (Table 2). The lack of correlation between deep-sea viral production and biological factors has been shown previously. In the (sub)tropical Atlantic Ocean, various viral parameters, including production, are significantly correlated with depth (de Corte et al., 2010), and depth alone can explain the majority of the variations in viral production in a latitudinal study in the North Atlantic (de Corte et al., 2012). These two large-scale investigations did not show any environmental parameters to be correlated with viral production in specific layers of the deep sea. Similarly, none of the environmental parameters measured in our study were related to or significantly explain the deep-sea viral production (Tables 2 and 3), and so, the controlling mechanism of viral production in the deep sea is still unclear. However, as Yang and co-workers point out, the lack of a tight coupling between biological parameters with viral abundance/production in the deep sea does not mean the lack of virus–host relationship (Yang et al., 2014). The deep-sea virioplankton might have a different ecological behaviour and life strategy compared to their counterparts in the surface and epipelagic ocean, such as the prevalence of lysogeny, broad host range or large burst size (Weinbauer et al., 2003; Parada et al., 2006). All this information is severely limited for deep-sea environments and requires further investigation.

## 4.2 Contribution of deep water viruses to host mortality and carbon cycling

Marine viruses are known to be one of the most important factors related to host mortality and nutrient cycling (such

as C, N, P and Fe) in the surface ocean (Wilhelm and Suttle, 1999; Evans and Brussaard, 2012; Gobler et al., 1997; Poorvin et al., 2004). Our understanding of the ecological role of viruses in the dark ocean is, however, very limited. In a paper reviewing microbial oceanography in the deep sea, Arístegui et al. (2009) indicate that flagellates, rather than viruses, are a major source of bacterial mortality in meso- and bathypelagic waters. One supporting piece of evidence is that the prokaryote:flagellate ratio decreases with depth, whereas the VPR does not, suggesting a tight coupling of the prokaryote population with their predators. The authors argue that the abundant viral particles observed in the deep sea are in a state of maintenance and are not infective. However, our study showed that the viral activity in the deep waters of the western Pacific Ocean is not negligible, although the production rates at 1000 and 2000 m are on average one or two orders of magnitude lower than rates in epipelagic waters (Fig. 3). More importantly, the percentages of bacterial cells lysed by viruses in meso- and bathypelagic waters (on average 91.70 and 65.77 %, respectively) are comparable to those observed at the surface and bottom of photic zones (on average 73.93 and 90.60 %,respectively). This led us to hypothesise that in addition to a large amount of allochthonous viral input from sedimenting particles (Hara et al., 1996; Parada et al., 2007), there is an active viral population in the deep sea. Although the host density is low in the deep sea, which reduces the contact rate between viruses and hosts, the large population size, low decay rate and long turnover time of viruses will increase the chance of a successful infection. In an investigation simultaneously measuring top-down control of nanoflagellates and viruses on bacterioplankton in the bathypelagic Mediterranean Sea and Atlantic Ocean using dilution techniques, Umani et al. (2010) observe that 13.4 % of the prokaryotic biomass loss is caused by viruses. In addition, it is proposed that viruses may enter a lysogenic life cycle in order to adapt to sparsely populated environments such as the deep sea (Weinbauer, 2004). Although we did not investigate lysogeny in our study, significant lysogenic infection is observed in deep waters of the North Atlantic Ocean and Mediterranean Sea (Weinbauer et al., 2003; de Corte et al., 2012). Therefore, considering viruses in lytic and lysogenic conditions, one may expect viruses to be an important factor for host mortality in the deep sea.

With an average conversion factor of 12.4 fg C cell$^{-1}$ for oceanic prokaryotic assemblages (Fukuda et al., 1998), our calculation of carbon released by viral lysis in the upper water column in the western Pacific Ocean is generally higher than estimates for the Southern Ocean (conversion factor 18.7 fg C cell$^{-1}$; Evans and Brussaard, 2012). Despite the considerably variable marine prokaryotic carbon content observed in the ocean (Fukuda et al., 1998), viral lysis may be a significant pathway in carbon cycling at the surface and 200 m layer of the western Pacific area. In the deep sea, because of low viral production and low bacterial abundance, viral lysis released carbon in meso- and bathypelagic wa-
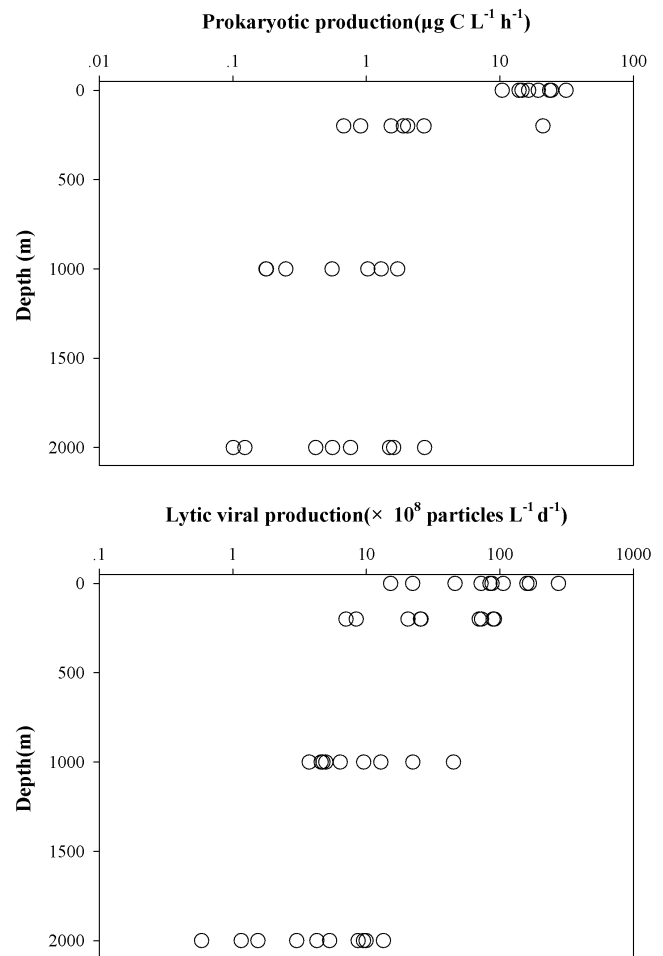


**Fig. 3.** Distribution of prokaryotic and lytic viral production rate throughout the water column of the (sub)tropical western Pacific Ocean at the different stations (see Fig. 1 for the locations).

ters at a one magnitude lower rate (Table 4). Previous studies showed that the majority of dissolved organic carbon (DOC) pool in the deep sea is recalcitrant with a residence time of thousands of years and it is not easily utilised by microbes (Jiao et al., 2010; Hansell et al., 2009). Comparably, although the amount of carbon released by viruses is small in the deep sea, the bio-availability of this carbon is labile. Materials from the bacterial cell wall and cell content, including carbohydrates, amino acids, DNA, glucosamine and diaminopimelic acid, are found in lysis products (Middelboe and Jorgensen, 2006; Weinbauer and Peduzzi, 1995; Brum, 2005). Previous studies suggest that the majority of these lysis products are rapidly (within days) degraded and belong to the labile DOC fraction (Gobler et al., 1997; Poorvin et al., 2004; Haaber and Middelboe, 2009). This highly bio-available carbon may relieve the limiting by organic carbon for heterotrophic prokaryotes and fuel prokaryotic activity in the deep sea. Similarly, other nutrient elements (e.g. N, P, Fe, etc.) released during virus-mediated cell lysis are also

organic and are more bio-available than those with high concentration but in inorganic form in deep-sea environments (Gobler et al., 1997; Poorvin et al., 2004; Mioni et al., 2005). Furthermore, the active viral processes in the deep sea will affect the efficiency of the biological pump and the microbial carbon pump, and subsequently, the carbon sequestration in the ocean, all of which have a global-scale impact (Suttle, 2007; Jiao et al., 2010). Taken together, our results demonstrated that the viral shunt may have significant impacts on the regeneration and cycling of carbon and other elements in the deep sea. Viruses in the deep ocean regime may be ecologically and biogeochemically important on both local and global scales.

**Supplementary material related to this article is available online at http://www.biogeosciences.net/11/ 2531/2014/bg-11-2531-2014-supplement.pdf.**

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