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NOTES AND CORRESPONDENCE

Seasonal variations in virioplankton and picoplankton in semi-enclosed and open coastal waters

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ABSTRACT

Viruses are known to be important agents of prokaryotic loss in diverse environments. However, only few studies to date have examined seasonal variations in virus-prokaryote interactions in marine environments. This study measured viral and prokaryotic abundance between January and November 2015 to assess seasonal variations in the relation between viruses and prokaryotes (heterotrophic bacteria, *Synechococcus* spp., and picoeukaryotes) in eutrophic semi-enclosed and less productivity open coastal waters. Viruses and prokaryotes were found to be significantly more abundant in productive semi-enclosed coastal waters. Using side scatter and green DNA dye complex fluorescence, we analyze flow cytometry (FCM) data to clearly distinguish between two groups of viruses, VLP1 and VLP2. VLP1, the most dominant, ranged from 84 - 89% and 67 - 78% in semi-enclosed and open coastal waters, respectively. Lower virus-to-bacteria ratios (VBR) were observed at semi-enclosed coastal waters (0.9 - 6.1), due to turbidity values of these two coastal waters being significantly different (4.5 - 6.2 NTU and 0.5 - 1.2 NTU, respectively) in summer, probably a result of higher suspended matter causing removal of viruses from the surface waters.

1. INTRODUCTION

Viruses are numerically abundant in aquatic environments, and their potential effects on community structure and dynamics have received much attention (Fuhrman 1999; Wommack and Colwell 2000; Weinbauer 2004; Bettarel et al. 2006). Although rates of virus-induced mortality in bacteria have been reported to range from 90 - 100% in freshwater systems (Fischer and Velimirov 2002; Colombet et al. 2006), most studies have found it to range between 10 and 50% in marine environments (Bettarel et al. 2005; Jacquet et al. 2005; Ory et al. 2010). Thus, viral lysis can be considered a major cause of bacterial mortality, in some instances, comparable to that caused by grazing (Jacquet et al. 2005).

Viruses control prokaryotic mortality and may help to maintain microbial diversity (Fischer and Velimirov 2002; Peduzzi and Schiemer 2004). In most aquatic environments in which both bacterial and viral abundance increase with trophic conditions, there is a significant relationship between the two (Hennes and Simon 1995; Wommack and Colwell 2000; Bettarel et al. 2004; Peduzzi and Schiemer 2004). However, one study of two lakes in the French Massif Central found the relationship between viruses and bacteria to be weaker in the more productive lake, where there was also found to be an increase in cyanophage hosts (Bettarel et al. 2004). The importance of viruses in aquatic ecosystems can be best understood through their interaction with their hosts (e.g., bacteria or cyanobacteria). However, variation in physical factors may significantly affect the distribution of viruses (Noble and Fuhrman 1997). Previous studies, for example, have shown viruses to be attached to particles in the water column (Suttle and Chen 1992; Maranger and Bird 1996; Noble and Fuhrman 1997; Hewson et al. 2001). According to Noble and Fuhrman (1997), suspended matter removes

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almost twenty percent of the viral population daily in the shallow waters of Santa Monica Bight, where relatively low concentrations of suspended matter have been found.

Several studies of seasonal variations in viral abundance have been performed for freshwater environments (Bettarel et al. 2004; Madan et al. 2005; Liu et al. 2006; Personnic et al. 2009) and they often find magnitudes of change to be greater than one order between summer and winter. However, there have been few comprehensive reports of seasonal variations in viral abundance in waters of different trophic status and even fewer investigating these variations in coastal waters. The aim of this study was to quantify spatial and seasonal variability in viral and picoplankton abundance (bacteria, *Synechococcus* spp., and picoeukaryotes) in two bodies of water with different trophic statuses, the semi-enclosed and open coastal waters of the subtropical western Pacific, from January to November 2015.

2. MATERIALS AND METHODS

2.1 Sampling

Surface water samples were collected weekly between January and November 2015 at an established station in semi-enclosed coastal waters (Station A) and a station in open coastal waters (Station B) in northeastern Taiwan (Fig. 1). Water temperature was measured immediately after the sampling bucket was cast. All samples were brought to the laboratory within 30 min.

The environment of Station B has been described previously based on data gathered from 1999 to 2001 (Tsai et al. 2005). Generally, in March, surface water temperatures average 16°C and they slowly rise to 29°C by July (Tsai et al. 2005). Annual salinity ranges from 33.1 - 34.3 psu, with lower values probably a result of runoff from rainfall. From November to May, nitrate concentrations are highest (up to 12 μmol L^{-1}), and from June to October they decrease (1 μmol L^{-1}). Station A has nitrate concentrations ranging from 5.2 - 143 μmol L^{-1} (average 28.9 μmol L^{-1}) (Chao et al. 2013). For the current study, turbidity at both stations was measured *in situ* at each sampling. For this, a Waterproof Portable Turbidity Meter (TN 100) was used.

2.2 Flow Cytometric Analysis

Picoplankton (bacteria, *Synechococcus* spp., and picoeukaryotes) and virus populations were identified and measured by flow cytometry (FCM) (BD FACSCalibur™). To perform FCM analysis, we first quickly thawed three viral and bacterial samples. We diluted them 1:10 with 0.2 μm filtered TE buffer (10 mM Tris, 1 mM EDTA), stained them with SYBR Green I solution (1:500 dilution; Molecular Probes) and incubated them at 80°C in the dark for 10 min (Brussaard 2004). We added fluorescent beads (1 μm) (Molecular Probes) to each sample to a final concentration of 10^5 beads mL^{-1} following Gasol and Del Giorgio (2000). We used phosphate-buffered saline (PBS) solution as a sheath fluid. We recorded forward-angle light scatter (FSC), side-angle light scatter (SSC), and green (SYBR-I) fluorescence values. *Synechococcus* spp. and picoeukaryotic cells were distinguished using pigment autofluorescence and FSC.

Cell side scatter (a proxy of cell size) and SYBR Green fluorescence (an indicator of nucleic acid) were used to distinguish viruses from heterotrophic bacteria (Marie et al. 1997; Brussaard 2004). Judging from their relative green fluorescence and SSC, we were also able to distinguish between two virus subpopulations: VLP1 and VLP2 (Marie et al. 1999). VLP1, which produced the lowest green fluorescence, and thought to represent mostly bacteriophages

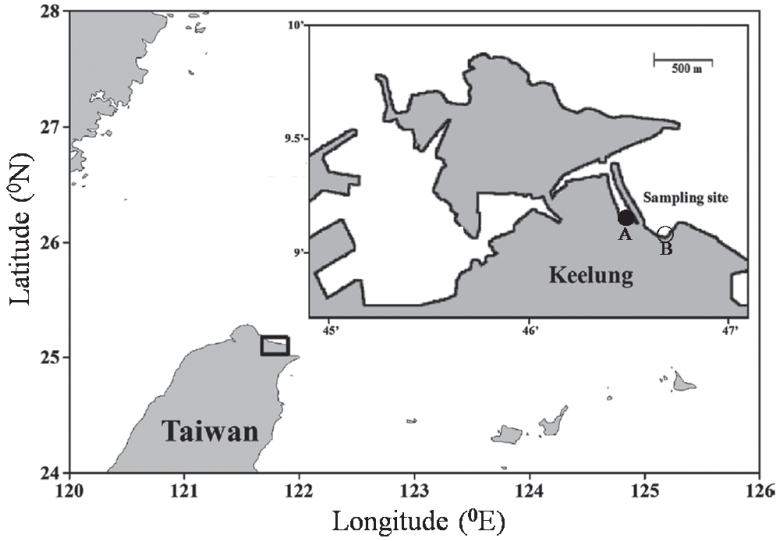

Fig. 1. Location of sampling sites (Stations A and B) in the coastal waters of western subtropical Pacific.

(Marie et al. 1999; Jacquet et al. 2002). VLP2, which produce a higher green fluorescence, is assumed to be a region typically indicating cyanophages (Sandaa and Larsen 2006; Personnic et al. 2009).

2.3 Statistical Analysis

Spearman rank correlations were used to analyze relationships between viral and picoplankton along with the physical parameters (temperature and turbidity) recorded for each station. The *t*-test was used to determine significant differences between stations during the study period. STATISTICA 7.0 software was used to perform all statistical operations. A probability value of < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Throughout the study, water temperature ranged from 15.5 - 30.5°C at Station A, the semi-enclosed waters area, and 16 - 31°C at Station B, in the open waters area. Temperature patterns were similar at both stations throughout the study period with no significant difference noted between the two stations (*t*-test, $p > 0.05$). Spatially, the abundances of all the microbial taxa, including bacteria, *Synechococcus* spp., and picoeukaryotes, were significantly higher at Station A than at Station B (*t*-test, $p < 0.05$) (Fig. 2). Temporally, at both stations, the highest bacterial abundances occurred between July and August, demonstrating clear seasonal variation (Figs. 2a, d). At Station A, *Synechococcus* spp. varied in abundance by about a factor of 1000, ranging from 2×10^2 to 1.5×10^5 cells mL^{-1} (Fig. 2b). At Station B, *Synechococcus* spp. abundance was lower, ranging from 2×10^2 to 6.9×10^4 cells mL^{-1} (Fig. 2e). However, seasonal patterns in picoeukaryotes were not significantly different between the two stations (*t*-test, $p > 0.05$) (Figs. 2c, f).

The total abundance of viruses ranged from 9.2×10^5 to 4.4×10^6 viruses mL^{-1} at Station A, a range significantly different from that found at Station B (4.5×10^5 to 5.1×10^6 viruses mL^{-1}; *t*-test, $p < 0.05$) (Figs. 3a, d). The viral abundances observed in this study were lower than those reported for other coastal sea areas (Weinbauer et al. 1993,

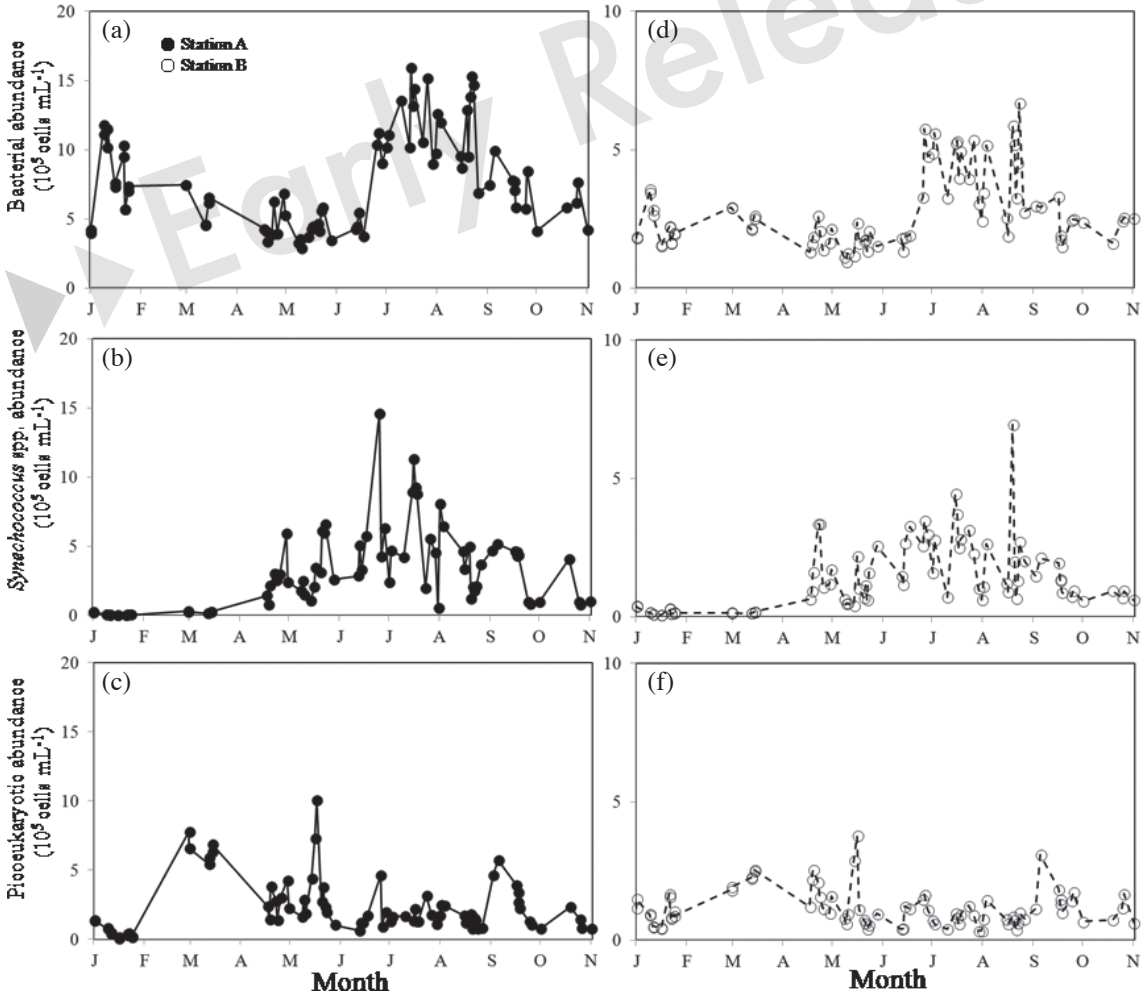

Fig. 2. Seasonal variations of bacterial (a) (d), *Synechococcus* spp. (b) (e), and picoeukaryotic abundance (c) (f) at Stations A and B, respectively.

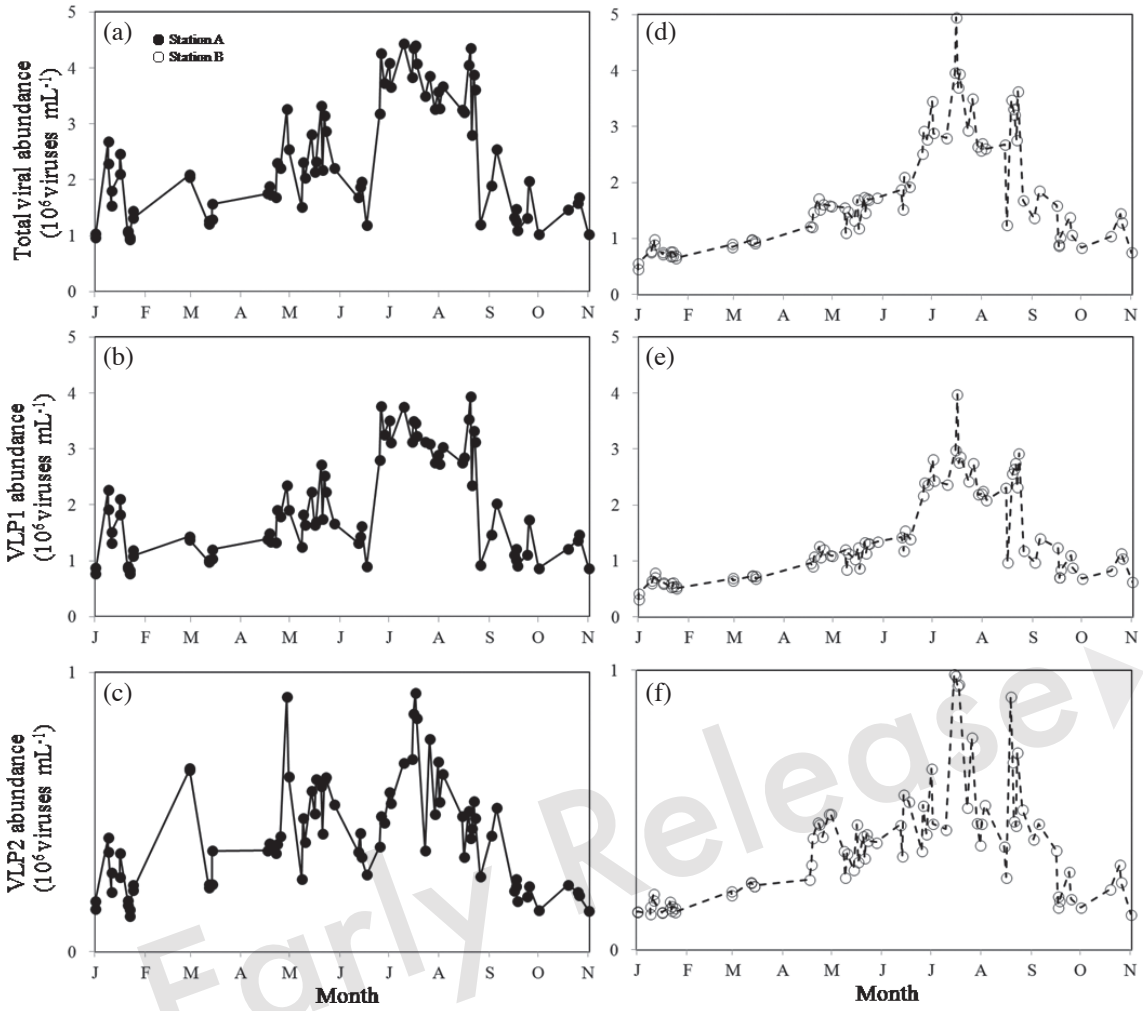

Fig. 3. Seasonal variations of total viral (a) (d), VLP1 (b) (e), and VLP2 abundance (c) (f) at Stations A and B, respectively.

1995; Alonso et al. 2001; Stopar et al. 2004; Bongiorni et al. 2005; Boras et al. 2009). For example, viral abundance in mid-Adriatic coastal waters ranges from 4.8×10^6 to 27.3×10^6 viruses mL^{-1} and from 3.9 to 11.6×10^6 viruses mL^{-1} in the southern Adriatic coastal waters (Ordulj et al. 2015). Viral abundance in the current study was similar to that found in other oligotrophic open ocean data sets (Rowe et al. 2012) for areas of the North Atlantic and Western Pacific oceans, which have similar Chl *a* concentrations and bacterial abundance.

Temperature had a significant effect on total viral abundance at both study sites (Station A: $p < 0.01$, Station B: $p < 0.01$) (Table 1). Furthermore, VLP1, which made up 84 - 97% of the total viruses at Station A and 67 - 78% at Station B, had similar seasonal patterns at both stations (Figs. 3b, e). VPL2 abundance was relatively constant with only small seasonal variations at both stations (Figs. 3c, f). Viral abundance was positively correlated with bacteria at both sites (Station A: $\rho = 0.61$, $p < 0.01$; Station B: $\rho = 0.75$, $p < 0.01$) (Table 1). Typically, VLP1 abundance was significantly associated with bacteria abundance at both sites (Station A: $\rho = 0.65$, $p < 0.01$; Station B: $\rho = 0.82$, $p < 0.01$) (Table 1). However, when comparing VLP1 abundance with other members of the microbial community, we found weaker correlations. For example, there was a weaker correlation between VLP1 and *Synechococcus* spp. abundance at both sites (Station A: $\rho = 0.52$, $p < 0.01$; Station B: $\rho = 0.58$, $p < 0.01$) (Table 1). VLP2, typically thought to represent picophytoplankton or cyanobacterial viruses, were significantly correlated with *Synechococcus* spp. abundance (Station A: $\rho = 0.55$, $p < 0.01$; Station B: $\rho = 0.78$, $p < 0.01$) (Table 1). However, no significant correlation was found between VLP2 and picoeukaryotes at either station (Table 1). VPL1, which dominates the viral community of other freshwater and marine environments (> 90%), are mostly comprised of bacteriophages (Li and Dickie 2001; Jacquet et al. 2002; Personnic et al. 2009; Paterson et al. 2012). Interestingly, we found a closer linear relationship between viruses and bacteria in the less productive waters where Station B was located than in those surrounding

2009a or 2009b?

Table 1. Spearman's correlation analysis (ρ) of abundance of total viruses, VLP1, VLP2, abiotic (temperature and turbidity), and biotic (bacterial, *Synechococcus* spp., and picoeukaryotic abundance) at Stations A and B during the study period. Non-significant values are denoted as n.s. ($p > 0.05$) and asterisks indicate significance as follows: ** $p < 0.001$, * $p < 0.05$).

	Viruses	VLP1	VLP2
Station A			
Temperature	0.61**	0.60**	0.25*
Turbidity	n.s	n.s	n.s
Bacteria	0.61**	0.65**	0.31*
Synechococcus	0.54**	0.52**	0.55**
Picoeukaryotes	n.s	n.s	n.s
Station B			
Temperature	0.69**	0.67**	0.38*
Turbidity	-0.35*	-0.45*	-0.21*
Bacteria	0.75**	0.82**	0.59**
Synechococcus	0.71**	0.58**	0.78**
Picoeukaryotes	n.s	n.s	n.s

Station A (Table 1). Bettarel et al. (2004) suggested that there is an increase in relative abundance of non-bacteriophage viruses such as cyanophages in more productive environments. This might explain the weaker linear relationship between viruses and bacteria at Station A. However, after deducting VLP2 (cyanophages) from total viral abundance, we also found a weaker correlation between VLP1 and bacteria at this station than that at Station B (Table 1). There may be environmental factors influencing the relationship between VLP1 and bacteria at Station A.

We used VLP1-to-bacteria ratio (VBR) as a proxy of virus-bacteria interactions in this study. VBR ranged from 0.9 - 6.1 and 1.7 - 10.6 at Stations A and B, respectively (Fig. 4). The highest VBRs were observed during May at both sites. Bettarel et al. (2004) suggested that VBR values increase along with increasing trophic status. Differences in these values may be related to burst size and infection rate of host cells (Wommack and Colwell 2000; Hewson et al. 2001). Bettarel et al. (2004) also suggested that high concentrations of phytoplankton and cyanobacteria may also increase VBR. However, Maranger and Bird (1995), conducting a study of samples taken from twenty-two lakes in Quebec, did not find trends in VBR to be related to the trophic status. With no agreement in the field, it is not possible to make any satisfactory generalization regarding the association between changes in VBR and trophic status of aquatic systems.

With regard to seasonal variations in VBR, one study reported the abundance of nanoflagellates to be high during the warmer part of the year at the coastal waters of Adriatic Sea, causing a lower VBR, compared to VBR in winter (Ordulj et al. 2015). One previous study of Stations A and B in the current study concluded that nanoflagellates exerted significant control of bacterial production during the warmer part of the year, whereas viruses controlled the bacterial production during the colder months (Tsai et al. 2013). If nanoflagellates consume virus-infected bacteria as readily as they do healthy ones, then viruses may theoretically contribute significantly to losses of bacteria. This would mean that decreases in viral production should result in lower VBR values. González and Suttle (1993) reported that since some nanoflagellates can consume viruses, the viruses may also serve as a source of nutrients, even though they would make up a very small proportion of the biomass.

To further study the effect of environmental factors on viral abundance, we also measured turbidity (Fig. 5, Table 1). During the study period, turbidity was generally lower at Station B (0.5 - 4.3 NTU) than Station A (4.5 - 9.4 NTU) and was negatively correlated with total viruses at Station B (spearman $\rho = -0.35$, $p < 0.05$) (Table 1). A preliminary study was conducted in June during the study period to understand the effects of suspended matter on variation in viral abundance. In that study, we used size-fractionation to decrease the amount of suspended matter in the samples. Viral abundance ranged from 4.5×10^6 to 4.3×10^6 cells mL^{-1} in unfiltered waters and from 4.3×10^6 to 8.6×10^6 cells mL^{-1} in 2 μm filtered waters at the end of the experiment (24 h) at Station A (Fig. 6a). However, there was no significant difference between the unfiltered waters and the 2 μm filtered waters at the end of that experiment (24 h) at Station B (Fig. 6b). The present study, however, found evidence of lower VBR at Station A, possibly resulting from higher concentrations of suspended matter and the removal of viruses from the surface waters through the sedimentation process. It has been reported that the higher the concentration of suspended matter, the greater the sedimentation rates of bacteria and viruses that sink along with these particles to which they are attached (Suttle and Chen 1992; Maranger and Bird 1996; Noble and Fuhrman 1997; Hewson et al. 2001). There is little documentation of the interactions between natural virus assemblages and suspended matter, particularly in marine systems where suspended matter is a ubiquitous and important factor.

In conclusion, in this study of variations in abundance of bacteria, picophytoplankton, and viruses in two coastal water sites, we found a strong correlation between viral and bacterial abundance, a finding that suggests most viruses are bacteriophages in these coastal waters. It is likely that the lower virus-to-bacterial ratios (VBR) observed at Station A resulted from the higher concentrations of suspended matter and their removal of viruses from those waters. Future studies may want to include viral production and mortality rates in their analyses when assessing the ecological role of phages and their potential in controlling bacterial dynamics.

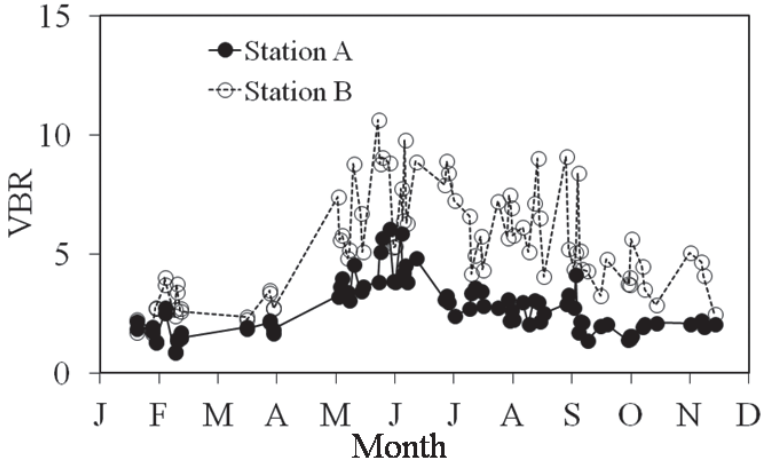

Fig. 4. Seasonal variations of VLP1-bacteria ratio (VBR) at Stations A and B, respectively.

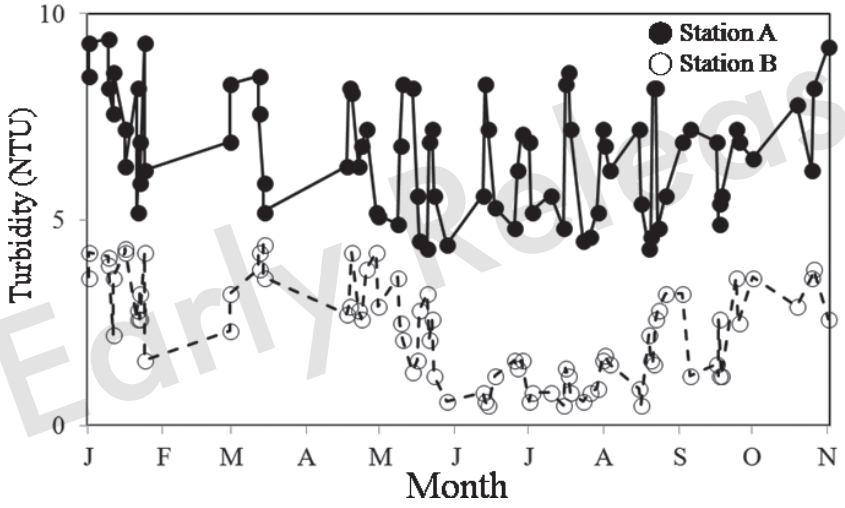

Fig. 5. Seasonal variations of turbidity at Stations A and B, respectively.

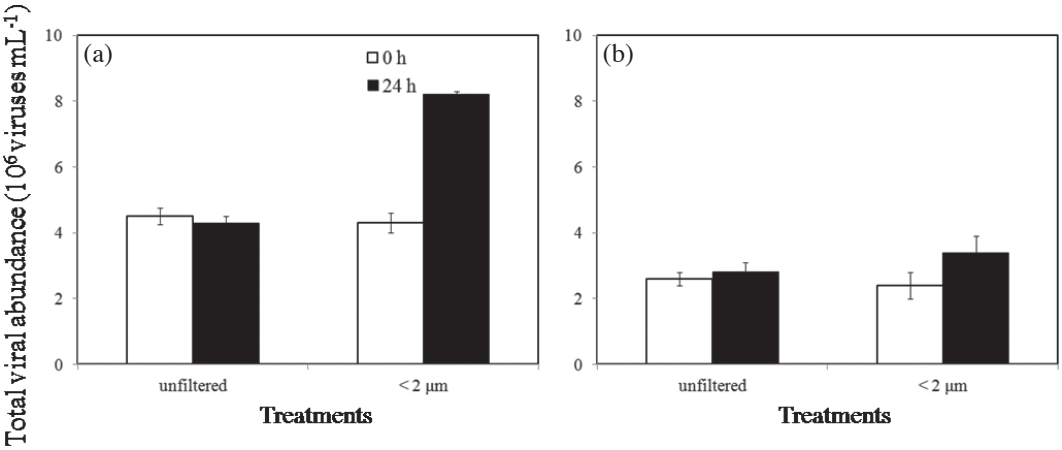

Fig. 6. Variations of total viral abundance in unfiltered and 2 μm filtered treatments, respectively. Error bars represent the SD estimated from triplicate incubations.

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