<Microbial Distribution and Community Composition of the Bohai Sea in the North of China>

by

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SUMMARY

Microorganism has been considered to be the most abundant community in the marine ecosystem, they mainly conduct their roles by acting as decomposers, consumers and producers. They are main drivers in the biogeochemical cycle, they can control the marine biomass and play a paramount role in microbial food web. So the role of marine microbial in the marine ecosystem has gained increasing attention in recent years. With the characteristics of high abundance and wide distribution, heterotrophic bacteria has been considered to be one of the most important factor in deciding the development of basic aquatic food web. It will be vital for marine resources protection to study the relationships between microbial distribution and environmental factors. Virioplankton mainly include bacteriophage and cyanophage which have been confirmed to be the most abundant marine plankton in the world. At present, the widely observed abundance of virioplankton range from 10^4 ml^{-1} (oligotrophic sea area) to 10^8 ml^{-1} (eutrophic sea area), which is nearly one order of magnitude higher than the abundance of heterotrophic bacteria. Virioplankton can modulate the conversion between particle organic matters and dissolved organic matters by the process of host cell lysis. So the virioplankton play a pivotl role in the process of biogeochemical cycles. Furthermore, virioplankton can also have an impact on the microbial diversity and community structure through the process of dynamic succession and genetic transformation. Bohai Sea is an important continental sea which located in the north of China with an average depth of 18m. It is an important fishing and spawning area which have taken great benefit to people nearby. So it will be of great importance to protect the ecology environment in this area. In this study, we used the flow cytometry to enumerate the number of virioplankton and bacterioplankton for evaluating the biomass of Bohai Sea. The results demonstrated that the macroscale distribution of virioplankton and heterotrophic bacteria had a close connection to salinity and concentrations of PO_4-P and NO_3-N. This thesis illustrated that the distribution of virioplankton and hetrotrophic bacteria in the Bohai Sea were impacted by the seasonal variation and nutrient availability. Also this thesis provides the first insight into the community structure of microbial in the Bohai Strait which is influenced by the open sea input such as the Yellow Sea Warm Current (YSWC). It was concluded that the YSWC flushes the microbial community in the Bohai Sea, and suggested that the YSWC entered the Bohai Sea with poor biomass and exited with rich biomass, converting the Bohai Sea nutrients to microbial biomass, which is flushed into the Yellow Sea and that enriches the Yellow Sea microbial loop.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed.....

Date.....23/08/2018.....

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It is done.

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ABBREVIATIONS

HNA	high nucleic acid
LNA	low nucleic acid
VLP	virus-like particle
VBR	virus to bacteria ratio
°C	degrees Celsius
µg	microgram
YSWC	the Yellow Sea Warm Current
TV	total virus abundance
TB	total bacteria abundance
CTD	conductivity temperature-depth equipment
POM	particle organic matters
DOM	dissolved organic matters
DMS	dimethyl sulphide
DMSP	dimethylsulfoniopropiomate
Chla	chlorophyII a
SSC	side angle light scatter
FSC	forward angle light scatter

ABSTRACTS ARISING FROM THE THESIS

Following is a list of the abstracts arising during the author's Doctor of Philosophy studies. A version of Chapter II was published in the journal of FEMS Microbiology Ecology as:

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A version of Chapter III is undergoing revision after previous submission for peer review at the journal of Estuarine, Coastal and Shelf Science as:

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CHAPTER I
INTRODUCTION

AN OVERIEW OF THE MARINE MICROBIOLOGY ECOLOGY

Marine microbiology is the scientific analysis and study of interactions between microorganisms and their living environment (Pedros-Alio *et al*., 2006). With high abundance and diversity, marine microbiology has been considered to be difficult to study. In recent years, with the development of progress in research methods and the deepening of the research, people began to realize the significance of marine microbiology in the process of biogeochemical cycles (Azam and Malfatti, 2007). Marine microbiology ecology not only has close relationships with industry, but also it has close connection with aquaculture and marine environmental protection. The study of marine microbiology ecology also helps to understand the microbial metabolic regulation and mechanism of microbial adaption to the environmental changes. The results will be of great significance for protecting the marine environmental resources and microbial diversities.

The main research content involve in marine microbiology ecology includes: (1) To study the microbial community composition and structure, distribution, temporal and spatial variation. (2) To study the types and functions of marine microorganisms in the extreme marine environment, such as hydrothermal, hypoxia and low temperature environment. (3) To study the correlation between marine microorganisms, marine plankton and animals. (4) To study the relationship between marine microbial and marine environmental changes. (5) To study the indicative function of marine microbial in the marine environment. (6) To study the marine microbial metabolism and active substances.

To study the marine microbial ecology, not only can we know the role of marine microorganisms conduct in the marine environment, but also it will be benefit for us to do comprehensive evaluation on the marine environmental variation. Through these, marine energy utilization and protection can be done well in the development of economic and society.

THE ECOLOGICAL FUNCTION OF MARINE BACTERIOPLANKTON

There are many kinds of bacteria species in marine environment. In order to adapt to different marine environment, these bacteria have their special physical characteristics. Heterotrophic bacteria play an important role in the process of microbial food web, their main role is to carry the recycling of energy and life elements in the biosphere. In the oligotrophic sea area it is much more prominent. Heterotrophic bacteria can return most of the primary productivity, and thus greatly improve the production efficiency of marine ecological system. What we are familiar with the food chain is about the process that phytoplankton produce particle organic matters by the process of

photosynthesis, it was then delivered to the next level by the zooplankton, and finally it was passed to the top through a serious of feeding process. But in fact, a considerable part of organic matters are not transferred into the top level of food chain, they are discharged into the environment and outside of food chain in the form of feces and secretion. Heterotrophic bacteria can break down this organic matter and take advantage of them, so it is called microbial food loop (Azam *et al*., 1993).

Because of the application of fluorescence microscope, a large number of bacterioplankton was detected. With the development of technology, people begin to realize the significance of heterotrophic bacteria. It was reported in the early study that the abundance of bacterioplankton was nearly 10^9 cells/ml in the eutrophic sea area (Hobbie, 1977). And it was also found that energy flow process of bacterioplankton in different location and season presented a different state. Among them, dissolved organic matter is the main food resource for heterotrophic bacteria.

THE ECOLOGICAL FUNCTION OF MARINE VIRIOPLANKTON

Virus is an important part in the marine microbial food web, nearly all the members in the marine microorganisms can be infected by the virus (Bench *et al*., 2007). The effect of virus lysis are the main reason for the bacteria mortality, which means that they may play an important role in the marine biogeochemical cycles. Virus can transform carbon and nutrients from particle organic matters (POM) to tiny and small dissolved organic matters (DOM). Fuhrman described the DOM regeneration process which was promoted by virus by the model establishment (Fuhrman, 1992). And then, some research related marine virus had proved that virus played an important role in the global carbon cycle (Wommack and Colwell, 2000; Wilhelm and Suttle, 1999; Fuhrman, 1999). Virus is the catalyst to speed up the nutrients transformation from particle state to dissolved state (Breitbart, 2012). Some previous studies had proved that organic matters can be recycled after cell lysis conducted by the virus, and then it can be utilized by microorganisms (Gobler *et al*., 1997; Middelboe and Lyck, 2002; Middelboe and Jørgensen, 2006).

Marine viruses also have an impact on the bacterial community composition and diversity (Weinbauer and Rassoulzadegan, 2004). Viruses can infect and crack special host to achieve the process of changing genetic diversity of microorganisms. So marine viruses can selectively infect the host and remove them from a particular niche, which increase the diversity of the whole microbial community.

Viruses also have an important influence on the global climate regulation, they can promote the release of Dimethyl Sulphide (DMS). DMS is one of the most active atmospheric gases, it is the most abundant ocean sulphide which accounts for 50% of the global natural sulphide emissions (Malin and Krist, 1997). It was demonstrated that marine viruses can infect *Micromonas pusilla*

which led to the release of Dimethylsulfoniopropionate (DMSP) and the increase of concentration of DMS in sea water (Suttle, 2005).

In recent years, plankton bloom occurs frequently in China Sea, it has bring serious threat to the aquaculture, ecology environment and human health. It had been studied that viral lysis can reduce the density of phytoplankton, we can prevent the occurrence of plankton bloom by restricting the increase of phytoplankton density (Brussaard, 2004; Gastrich *et al*., 2004). Nagasaki (Nagasaki *et al*., 1994) had found virus particles in *Heterosigma akashiwo*, they found virus particles increase with the decrease of *Heterosigma akashiwo* cell density by the Transmission electron microscopy, it was concluded that virus played a significant role in the process of red tide disappearance.

SUMMARY OF THE BOHAI SEA ENVIRONMENT

Bohai Sea is an semi-enclosed sea in the north of China which is located between N37°07'-41°and E117°35'-22°150'. Bohai Strait is considered as the dividing line between the south of Liaodong Bay and the north of Bohai Bay. Bohai Sea mainly includes Bohai Bay, Liaodong Bay and Laizhou Bay, the average depth of Bohai Sea is nearly 18m which makes it a shallowest coastal sea in the north of China. With the development of society and industry, a large number of pollutant is discharged into the Bohai Sea which is the main reason for the deterioration of water quality and phytoplankton bloom. Bohai Sea is one of the most serious polluted area in China and the polluted portion accounts for about 47% of the whole country (Dang *et al*., 2013). Some studies had been conducted in the sediment of Bohai Sea, it was concluded that microbial community can be significantly influenced by the impact of pollution (Wang et al., 2013). In view of the important role of the Bohai Sea, it will have key significance to study the dynamic changes of virioplankton and bacterioplankton in this area.

AIMS OF THIS THESIS

In this thesis I used the flow cytometry to enumerate the number of virioplankton and bacterioplankton in the surface water and bottom water of Bohai Sea and Bohai Strait to investigate marine microbial community, diversity and structure in a temporal and spatial variation.

Specifically I aim to:

Determine the macroscale distribution of virioplankton and heterotrophic bacteria in the Bohai Sea.

Determine the changes of water temperature and salinity in the Bohai Sea by using conductivity temperature depth system (CTD). Also by using this method I studied how the open sea input such as the Yellow Sea Warm Current (YSWC) impact the microbial community in the Bohai Sea.

Determine the relationships between virioplankton, bacterioplankton and environmental changes in the Bohai Sea.

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CHAPTER II MACROSCALE DISTRIBUTION OF VIRIOPLANKTON AND HETEROTROPHIC BACTERIA IN THE BOHAI SEA

ABSTRACT

In light of limited research in the relationship between the macroscale distribution and dynamic changes of microplankton in the shallow Bohai Sea, here, we used flow cytometry to analyze samples collected from the Bohai Sea channel in winter and summer. Results showed that both the average of viral abundance (VA) and bacterial abundance (BA) were lower in winter (3.61×10^7 cells/mL and 1.84×10^6 cells/mL, respectively) than in summer (7.47×10^7 cells/mL and 5.05×10^6 cells/mL, respectively). At all 16 stations, VA was one order of magnitude greater than BA, with a positive relationship between each other. In the horizontal distribution, variations in VA and BA followed a similar trend, and both were obviously higher near-shore than offshore. In the vertical distribution, variations in both VA and BA did not show a clear relationship with water depth. VA and BA in summer were 2.1 and 2.7 times those in winter, respectively. Spearman correlation analysis showed that both VA and BA were correlated with the concentration of PO_4-P in winter (positive) and NO_3-N in summer (negative). Additionally, BA showed a negative correlation with salinity. It is clear that the macroscale distribution of these two microbes in the Bohai Sea is related to seasonal variation and nutrient availability.

INTRODUCTION

Since the concept of microbial food loops was first developed (Azam, 1983), the role of marine microbes in ecosystems has been intensely studied. As a major part of the microbial loop, both viruses and heterotrophic bacteria are of great importance to marine ecosystems (Mitbavkar *et al*., 2012; Bouvy *et al*., 2011). These microbial components play an essential role in the production of biomass and the biogeochemical cycles of carbon and other nutrients (Weinbauer *et al*., 2004).

Heterotrophic bacteria constitute a major disintegrator in the ocean, due to their wide distribution and high abundance; thus, their survival and activities determine the development of the basic food chain (Fuhrman *et al*., 1980). Beyond that, heterotrophic bacteria are an important decomposer of marine pollutants (Kong *et al*., 2014). In particular, heterotrophic bacteria contribute significantly and effectively to the degradation of petroleum hydrocarbon pollutants in the ocean (Cappello *et al*., 2012; Brakstad *et al*., 2006; Jurelevicius *et al*., 2013). Therefore, clarifying the dynamic relationships between the abundance of heterotrophic bacteria and its influencing factors may be

beneficial to better understanding the development, utilization and protection of marine ecosystems (David *et al*., 2000).

Phytoplankton viruses mainly include phage (Contreras *et al*., 2002) and algae viruses (Boehme *et al*, 1993; Augesti *et al*, 1998) with approximately 4×10^{30} cells/mL which are the most abundant biological entities in aquatic environment (Suttle, 2005). Since high abundance ratios of phytoplankton viruses were first detected, they have been considered to be the most abundant and dynamic marine plankton detected so far (Bergh *et al*., 1989). Abundances of phytoplankton viruses range from 10^5 to 10^8 particles mL^{-1} of seawater, often tenfold more numerous than heterotrophic prokaryotes (Wommack *et al*, 2000). Their main role in the microbial community is regulating nutrient cycling by controlling the abundance and diversity of competitive advantages (Suttle, 2007). Thus, phytoplankton viruses can be considered a biological indicator in the marine ecological environment (Hara *et al*., 1991).

It has been shown that there is a close relationship between phytoplankton viruses and heterotrophic bacteria (Winter *et al*., 2012). Viruses can drive dynamic changes in bacteria by controlling bacterial abundance, diversity and production (Kopylov *et al*., 2011), making them a principal element of the marine ecosystem (Winter *et al*., 2012). Moreover, viruses and bacteria affect dynamic changes in the marine environment simultaneously, transforming inorganic carbon and nitrogen into a dissolved state (Gasol *et al*., 1997). Given the ubiquitous distribution of heterotrophic bacteria and viruses in marine environments, measurements of their dynamic changes have become a focus for marine ecology studies.

The common methods of studying marine bacteria and viruses, such as epifluorescence microscopy, have the disadvantages of slow speed and low accuracy (Duhamel *et al*., 2006; Larsen *et al*., 2001). The flow cytometry technique has emerged as a common and powerful tool for the study of viruses and heterotrophic bacteria in the marine ecological system (Bouvier *et al*., 2007; Gasol *et al*., 1999). By using flow cytometry, we can detect single cells at a rapid speed for processing samples with a large capacity (Wang *et al*., 2010; Yentsch *et al*., 2008; Vives-Rego *et al*., 2000). In the process of testing, particles of microscale (cells and cell fractions) are divided into different groups according to parameters such as size and color of emitting light and scattered light (Brussaard *et al*., 2010).

Flow cytometry separates different sub-populations of interest (cell sorting), mainly depending on the rapid and accurate characteristics of the cytometric population (Davey, 2010). Wang *et al*. (2010) evaluated the distribution of microbial populations and their relationship with environmental parameters in the coastal waters of Qingdao, China, and Ni *et al*. (2015) detected the abundance and community of picoplankton and virioplankton in the Pearl River Estuary and Daya Bay, South China. These studies proved that flow cytometry is an efficient tool for detecting the distribution of cells in cytometric populations in the ocean, especially for the determination of picoplankton.

The Bohai Sea is a semi-enclosed sea with a maximum depth of 86 m and an average depth of 25 m (Wang *et al*., 2014). Owing to its geographical location, the Bohai Strait is the only means of water exchange between the Bohai Bay and the Yellow Sea. This area is strongly affected by environmental factors and human activity (Zhang *et al*., 2013). Particularly in winter, the Yellow Sea Warm Current, which is controlled by local winds, and the Kuroshio Current, originating from the North Equatorial Current in the Western Pacific, have a significant impact on Bohai Sea water exchange (Pang *et al*., 2005). Currently, data are lacking regarding to the distribution of microplankton in Bohai Sea.

In this study, we examined the abundance and distribution of virioplankton and heterotrophic bacteria in the surface and bottom waters of Bohai Sea in winter and summer (Figure 1). The aims of this study were to (i) determine the dynamics of flow cytometrically defined populations of heterotrophic bacteria and virioplankton, and (ii) assess the potential links between the dynamics of these two microbes and environmental factors in the study area. This study provides evidence for the interaction of virioplankton and heterotrophic bacteria with their ecological environment in the studied area, and supports the fundamentally different ecological roles of these populations in the marine environment.

MATERIALS AND METHODS

Study site and sampling procedures

We selected the study site from coastal waters of the Bohai Sea (37.07°-37.41° N, 117.35°-121.10° E) located between Shandong province and Liaoning province in China near the Bohai Strait. Water samples were collected at 16 stations (K, E, L and R) in the Bohai Sea (37.593°-38.899° N, 120.495°-122.558° E) from 17 December to 26 December 2013 (winter) and from 27 August to 6 September 2014 (summer). These stations are frequently influenced by the transportation and aquaculture industries. At all stations, water samples were collected from the surface layer and 2 m above the bottom (Figure 1).

Triplicate water samples (2 ml) were collected using a CTD system (Sea-Bird Electronics Inc., USA) and transferred into 2-ml cryovials. Water samples were then fixed with glutaraldehyde (0.5% final concentration) for viral and bacterial counts. Samples were incubated at 4°C for 15 min, then transferred into liquid nitrogen to flash freeze. On return to the laboratory, the samples were stored at -80°C, ready for laboratory analysis. Sample processing was carried out within one month (Marie, 1999; Brussaard, 2004).

Vertical temperature and salinity were measured at each station using the CTD system (Sea-Bird). Carbon, nitrogen, phosphorus and chlorophyll content were determined as previously described (Guan *et al*., 2014).

Flow cytometric analysis

We enumerated virioplankton and heterotrophic bacteria using the flow cytometry technique (Paterson *et al*., 2013). Triplicate water samples were thawed and diluted at 1:10 with 0.2-μm filter membrane filtered TE buffer (10 mM Tris, 1 mM EDTA). The diluted samples were then stained with SYBR Green-I (Bioteke Corporation, Beijing, China) for 10 min in the dark at 80°C (Brussaard, 2004; Dann, 2014). Control samples were prepared with filtered Tris-EDTA buffer stained with 12.5 ml of SYBR Green-I. Yellow beads with 1 μm diameter (Molecular Probes) were added at a final concentration of about 10^5 beads mL^{-1} to each sample (Gasol and del Giorgio, 2000). The beads were fully vortexed before adding to samples. Phosphate-buffered saline was used as a sheath fluid and prepared in advance.

Flow cytometry was conducted using a BD Acurri C6 flow cytometer system (Becton-Dickinson, San Jose, CA, USA). We adjusted the flow cytometry parameters according to the concentration of yellow beads. After the optimum schedule for the machine was set up, we used forward-angle light scatter, side-angle light scatter and green fluorescence (SYBR Green-I) for collecting data. According to fluorescence and bead concentration, the flow cytometer settings were normalized to an appropriate value. Virioplankton and heterotrophic bacterial populations could be effectively distinguished due to the different distribution of the side scatter. Each sample was run at a medium flow rate for 2 min. All data were collected in the list-mode folder and processed using Microsoft Office software.

Statistical analysis

Correlations between viral abundance (VA), bacterial abundance (BA) and environmental factors were determined by Spearman's correlation test. Data were processed using a logarithmic transformation to meet the requirements of the normality assumptions in the least-squares regression analysis. Statistical analysis was performed using SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Variance analysis was performed by ANOVA testing. Values of $P < 0.05$ and $P < 0.01$ were considered statistically significant and highly significant, respectively. The distribution maps of abiotic variables and biotic groups were drawn using Surfer 11.0 (Golden Software Inc., Golden, CO, USA).

RESULTS

Virioplankton abundance

Horizontal distribution

The VA in winter ranged from 9.88×10^6 to 7.80×10^7 cells mL^{-1}, with a mean value of 3.60×10^7 cells mL^{-1}. In the summer, the VA ranged from 2.31×10^6 cells mL^{-1} to 3.92×10^8 cells mL^{-1}, with a mean value of 7.47×10^7 cells mL^{-1}. The VA in surface waters in summer was 2.4 times that in winter. Furthermore, the VA in bottom waters in summer was 1.5 times that in winter.

We took the Bohai Strait as a dividing line and found that in the surface waters in winter, the VA east of the Bohai Strait was about 1.7 times larger than the west. In summer, the situation was reversed: the VA east of the Bohai strait was about 1.6 times less than the west. In the bottom waters, the VA near the Shandong Peninsula was higher than that near the Liaodong Peninsula in both winter and summer. The VA near the Shandong Peninsula was about 1.04 and 1.26 times more than that near the Liaodong Peninsula in winter and summer, respectively (Figure 2).

Vertical distribution

We found that the VA in the bottom waters was markedly higher (~1.28 times) than that in the surface layer in winter. In contrast, the VA in the bottom was lower (~1.44 times) than that in the surface waters in summer. Furthermore, both in the surface (~2.75 times) and bottom (~1.49 times) layers, the VA in summer was much higher than in winter. There was a large discrepancy between the summer and winter groups of data (Figure 3).

Heterotrophic bacterial abundance

Horizontal distribution

The BA ranged from 3.51×10^5 to 5.15×10^6 cells mL^{-1} in winter, and from 5.09×10^5 to 3.44×10^7 cells mL^{-1} in summer. The average BA was markedly lower in winter (1.84×10^6 cells mL^{-1}) than in summer (5.05×10^6 cells mL^{-1}). However, station E2 showed a substantially higher value (4.77×10^6 cells mL^{-1}) at the surface in winter, and both stations E6 and R7 showed a high value (4.64×10^6 and 5.15×10^6 cells mL^{-1}) at the bottom in the same season. In summer, the results were different: the highest value in the surface water was at station R6, and the highest value in the bottom water was at station R3 (Figure 4).

Vertical distribution

The BA in summer was generally much higher (~2.83 times) than that in winter. In winter, the BA in the bottom waters was slightly higher (~1.17 times) than the surface waters. Conversely, the BA at the surface was higher (~1.14 times) than the bottom layer in summer (Figure 5). All these results

were consistent with the VA data (Figure 3).

Environmental parameters

Environmental conditions

Environmental factors of surface water and bottom water in winter and summer were respectively showed in Table 3 and 4. In winter, there was no significant difference in temperature between surface water and bottom water (Table 3). In summer, the temperature of surface water was higher than that in the bottom water (Table 4). Water mass with salinity and high levels of nutrients (nitrate, nitrite, ammonia, phosphate and silicate) and chlorophyll a (Chla) was detected in the waters of all of these stations.

Relationships between microbes and their environment

Spearman correlation analysis revealed that there was a positive correlation between VA and BA in both winter and summer (Tables 1 and 2). Both VA and BA were positively correlated to the concentration of PO_4-P in winter. In summer, there was a negative correlation between VA and NO_3-N concentration. Moreover, BA was negatively correlated with salinity and NO_3-N concentration at the significant and highly significant levels, respectively. And difference was very remarkable between winter and summer (Table 5).

DISCUSSION

Variation in the horizontal distribution of virioplankton and heterotrophic bacteria

In the Bohai Sea study area, the average abundance of virioplankton was 3.61×10^7 and 7.47×10^7 cells mL^{-1} in winter and summer, respectively; the average abundance of heterotrophic bacteria was 1.84×10^6 and 5.05×10^6 cells mL^{-1} in the two seasons. These numbers are significantly greater than survey results (the average number of VA was 1.37×10^7 cells mL^{-1}, the average number of BA was 1.64×10^6 cells mL^{-1}) from the North Yellow Sea (Bai *et al*., 2012). The total virioplankton abundance was one order of magnitude higher than the heterotrophic bacterial abundance in the current study. Research has indicated that viruses can control the biodiversity of bacterial populations (Sandaa *et al*., 2009), and they are usually considered to be a major factor in regulating the abundances of heterotrophic bacteria and phytoplankton in aquatic ecosystems (Weinbauer *et al*., 2004).

The abundances of virioplankton and heterotrophic bacteria in Bohai Sea were clearly influenced by the environmental factors including temperature, salinity and nutrients. In the Pearl River Estuary and Daya Bay in South China, river inputs play a key role in regulating the abundances of picoplankton and virioplankton (Ni *et al*., 2015). While in the coastal waters of Qingdao, in northeast China, the distribution of microbial populations is greatly affected by nutrients (Wang *et al*., 2010). In the Bohai Sea, water inputs from the open sea were driven by the Yellow Sea Warm Current which may play a pivotal role in regulating the abundances of virioplankton and heterotrophic bacteria. The Yellow Sea Warm Current (YSWC) is a compensational current formed in the center of the Yellow Sea under the forcing of winter monsoon (Yuan *et al*., 1984). It has important implications for the environment and ecosystem of the Bohai Sea and the climate in this region. YSWC can greatly influence the environmental factors of Bohai Sea, leading to increases in temperature and the concentration of salinity of sea water in the Bohai Sea (Mask *et al*., 1998; Xu *et al*., 2009). So the environmental changes induced by the YSWC may alter the abundances of viroplankton and heterotrophic bacteria.

Both the abundances of virioplankton and heterotrophic bacteria were markedly higher in summer than in winter at most stations. This may be due to the high degree of eutrophication of seawater which frequently occurs in the study area in summer, because water quality evolves as the temperature changes (Kong *et al*., 2014). In water bodies with a high degree of eutrophication, the concentration of heterotrophic bacteria and virus particles often increase accordingly (Liu *et al*., 2006; Jiao *et al*., 2006). Additionally, the distribution of heterotrophic bacteria and phytoplankton is influenced by the synergic effects of temperature, nutrient supply, ingestion pressure, and light (Shiah *et al*, 1994). In summer, strong light intensity, high water temperature and appropriate salinity can promote vigorous growth of heterotrophic bacteria and phytoplankton, leading to a high virus release. In winter, weak light and low temperature are the main reasons for the lower abundances of heterotrophic bacteria and phytoplankton (Auguet *et al*., 2005; Jiang *et al*., 1994).

Variation in the vertical distribution of virioplankton and heterotrophic bacteria

In the Bohai Sea study area, the average abundances of both virioplankton and heterotrophic bacteria was higher in the bottom waters than in the surface waters in winter. This result is unique compared with previous findings in other ocean areas (Riegman *et al*., 2003; Wang *et al*., 2010). According to the current analysis, this may be related to the large accumulation of organic matter (Lin *et al*., 2014) in the bottom waters during winter, which support the growth of abundant heterotrophic bacteria and virioplankton. Additionally, owing to the influence of the Yellow Sea Warm Current in winter, there is significant water exchange in this area, which greatly changes the ecological environment and brings warm and saline water towards Bohai Sea (Xu *et al*., 2009); thus, the abundances of heterotrophic bacteria and virioplankton are greatly changed.

In the summer, the average virioplankton abundance was lower in the bottom waters than in the surface waters. Consistent results were obtained for heterotrophic bacterial abundance. We consider that environmental factors, including opulent sunshine, high temperature, abundant nutrition and the rapid growth of algae (Table 3, Table 4, Table 5 and Table 6), play a major role in increasing the abundances of heterotrophic bacteria and virioplankton in summer (Jiao *et al*., 2006). Other studies have suggested that bacterial and viral abundances will change with water depth in both nonstratified (Wang *et al*., 2010) and stratified (Magagnini *et al*., 2007) conditions. However, in the current study, there was no relationship between the microbial abundances and water depth. We suspect that the relationship has been reduced in shallow waters of the Bohai Sea (<100 m).

Correlation between microbial abundances and environmental parameters

Across the whole survey area, we found that the horizontal distribution of heterotrophic bacteria was consistent with virioplankton. Spearman correlation analysis showed the abundance of virioplankton was positively correlated with the abundance of heterotrophic bacteria. This finding is in line with a previous investigation (Wommack *et al*., 2000), which indicates that virioplankton make up a large proportion of the plankton community in aquatic ecosystems.

In order to identify the major factors influencing the abundances of virioplankton and heterotrophic bacteria, we analyzed the relationship between microbial abundances and environmental parameters, including temperature, salinity, water depth, concentration of NO_2-N, NO_3-N, NH_4-N, PO_4-P, SiO_2, and chlorophyll a (Tables 1 and 2). The correlation between biotic and abiotic variables indicated that the abundances of heterotrophic bacteria and virioplankton are notably controlled by environmental factors in the study area.

The abundances of both heterotrophic bacteria and virioplankton showed a correlation with PO_4-P concentration in winter (positive) and NO_3-N concentration in summer (negative). This trend can be related to the presence of the Yellow Sea Warm Current in the Bohai Strait, which brings warm and saline water to Bohai Sea and is much stronger in winter than summer (Song *et al*., 2009; Xu *et al*., 2009). The negative correlation between viral/bacterial abundance and NO_3-N concentration may be caused by the indirect relationship between heterotrophic bacteria and phytoplankton and the resulting antagonistic effects (Moore *et al*., 1995). Moreover, the heterotrophic bacterial abundance showed a negative correlation with salinity. Together the above results signified that both salinity and nutrients are critical factors controlling microbial growth in the coastal waters of the Bohai Sea, and the role of the Yellow Sea Warm Current is particularly evident.

This large-scale study revealed that both virioplankton and heterotrophic bacterial abundances greatly depend on environmental variables involving the levels of nitrogen, phosphorus and salinity controlled by the Yellow Sea Warm Current. The impact of environmental variables on heterotrophic bacteria was stronger than the impact on virioplankton in the Bohai Sea. The

correlation between biotic and abiotic variables hints at the possibility of synergistic and antagonistic effects being present during the growth of microbes.

CONCLUSION

This study determined the spatial distribution of virioplankton and heterotrophic bacteria in the surface and bottom waters of the Bohai Sea. We found a tight linkage between the two groups of microbes and nutrients. The results suggest that the growth and distribution of these two microbial groups were influenced by the seasonal variation significantly. Specifically, the different levels of nitrogen, phosphorus and salinity affected the two populations most. To the best of our knowledge, we have provided the first evidence obtained by flow cytometry for the distribution of virioplankton and heterotrophic bacteria as influenced by the Yellow Sea Warm Current in the Bohai Sea. This study related the distribution trend of virioplankton and heterotrophic bacteria to key environmental variables in the Bohai Sea. The results will provide a reference for further studies on the eco-environment in the Bohai Sea.

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Table 1. Spearman correlation coefficients between viral abundance (VA), bacterial abundance (BA) and environmental parameters in the Bohai Sea in winter.

	VA	BA	Tem	Sal	Depth	NO_2-N	NO_3-N	NH_4-N	PO_4-P	SiO_2	Chla
Virus	1.000	0.946**	0.275	0.116	0.183	0.209	-0.246	-0.297	0.378*	-0.254	-0.186
Bacteria	0.946**	1.000	0.272	0.126	0.089	0.242	-0.263	-0.320	0.395*	-0.297	-0.232

Notes: Abbreviations: VA, Virioplankton Abundance; BA, Heterotrophic bacteria Abundance; Tem, Temperature; Sal, Salinity. *$P < 0.05$, **$P < 0.01$.

Table 2. Spearman correlation coefficients between viral abundance (VA), bacterial abundance (BA) and environmental parameters in the Bohai Sea in summer.

	VA	BA	Tem	Sal	Depth	NO_2-N	NO_3-N	NH_4-N	PO_4-P	SiO_2	Chla
Virus	1.000	0.642**	0.174	-0.287	-0.172	0.002	-0.449*	0.123	-0.136	0.048	0.312
Bacteria	0.642**	1.000	0.132	-0.405*	-0.137	0.203	-0.489**	0.023	-0.095	0.129	0.295

Notes: Abbreviations: VA, Virioplankton Abundance; BA, Heterotrophic bacteria Abundance; Tem, Temperature; Sal, Salinity.* $P < 0.05$, ** $P < 0.01$.

Table 3.

Statistical summaries of environmental parameters from surface water and bottom water in winter.

Layer		Tem (℃)	Sal (‰)	NO_3-N+NO_2-N (μg/L)	NO_2-N (μg/L)	NO_3-N (μg/L)	NH_4-N (μg/L)	PO_4-P (μg/L)	SiO_2 (mg/L)	Chla (μg/L)
	Max	9.94	31.38	211.1	14.1	203.8	166.9	10.4	0.536	1.0894
	Min	6.19	28.43	9.0	1.7	1.0	10.3	0.7	0.141	0.2155
S	Median	8.96	31.12	100.1	7.0	91.9	47.0	5.4	0.3005	0.4309
	Mean	9.67	30.86	102.3	7.3	95.0	55.7	5.1	0.322	0.5172
	SD	1.22	0.75	57.3	3.8	57.6	45.2	3.3	0.133	0.2463
	Max	9.81	31.44	232.4	28.8	10.7	135.0	13.2	0.691	1.0783
	Min	6.69	30.03	13.4	1.7	203.6	0.6	1.6	0.074	0.1860
B	Median	8.87	31.12	115.0	5.545	111.0	55.1	7.7	0.323	0.4309
	Mean	8.55	31.01	117.1	7.243	109.9	47.75	5.7	0.3384	0.4731
	SD	1.01	0.42	64.8	6.68	62.6	40.6	3.6	0.16	0.25

Notes: Abbreviations: Tem, Temperature; Sal, Salinity; S, Surface water; B, Bottom water.

Table 4.

Statistical summaries of environmental parameters from surface water and bottom water in summer.

Layer		Tem (℃)	Sal (‰)	NO_3-N+NO_2-N (μg/L)	NO_2-N (μg/L)	NO_3-N (μg/L)	NH_4-N (μg/L)	PO_4-P (μg/L)	SiO_2 (mg/L)	Chla (μg/L)
	Max	25.69	31.11	51.9	8.03	51.4	37.7	6.24	0.409	6.15
	Min	20.37	29.83	10.8	0.32	9.9	0.5	0.67	0.080	0.07
S	Median	24.29	30.53	27.4	0.8	27.1	11.1	2.3	0.176	1.10
	Mean	23.99	30.53	30.4	2.1	56.2	13.2	2.4	0.198	1.60
	SD	1.30	0.35	14.1	1.9	13.9	9.1	1.5	0.090	1.50
	Max	24.94	31.75	111.1	32.59	97.9	76.9	16.36	0.569	1.20
	Min	7.01	29.97	24.9	0.23	19.5	3.1	1.48	0.230	0.18
B	Median	16.97	30.82	51.4	5.32	41.9	25.65	8.56	0.315	0.48
	Mean	15.59	30.93	58.0	14.28	49.09	32.11	8.48	0.343	0.52
	SD	5.94	0.50	24.6	9.03	23.94	21.69	3.72	0.105	0.33

Notes: Abbreviations: Tem, Temperature; Sal, Salinity; S, Surface water; B, Bottom water.

Table 5. One-way analysis of variance (ANOVA) testing the relationships between winter and summer variations on the virioplankton abundance and heterotrophic bacteria abundance in Bohai Sea.

P Value	**VA(winter)**	**VA-B(winter)**	**VA-S(summer)**	**BA(winter)**	**BA-B(winter)**	**BA-S(summer)**
VA(summer)	0.030*					
VA-S(winter)		0.224	0.021*			
VA-B(summer)		0.024*	0.248			
BA(summer)				0.016*		
BA-S(winter)					0.487	0.074
BA-B(summer)					0.099	0.778

*$P < 0.05$, **$P < 0.01$

VA: Virioplankton Abundance

BA: Heterotrophic bacteria Abundance

VA-S: Virioplankton Abundance in Surface Water

VA-B: Virioplankton Abundance in Bottom Water

BA-S: Heterotrophic bacteria Abundance in Surface Water

BA-B: Heterotrophic bacteria Abundance in Bottom Water

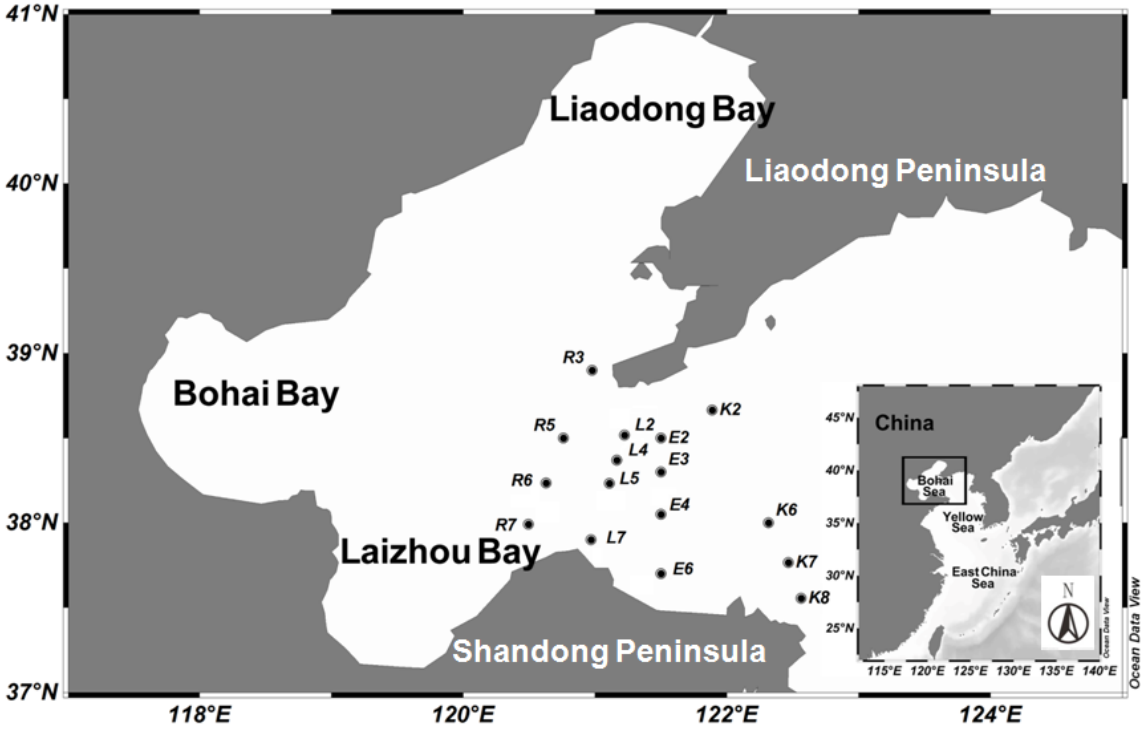


Figure 1 Sampling stations in the Bohai Sea study area.

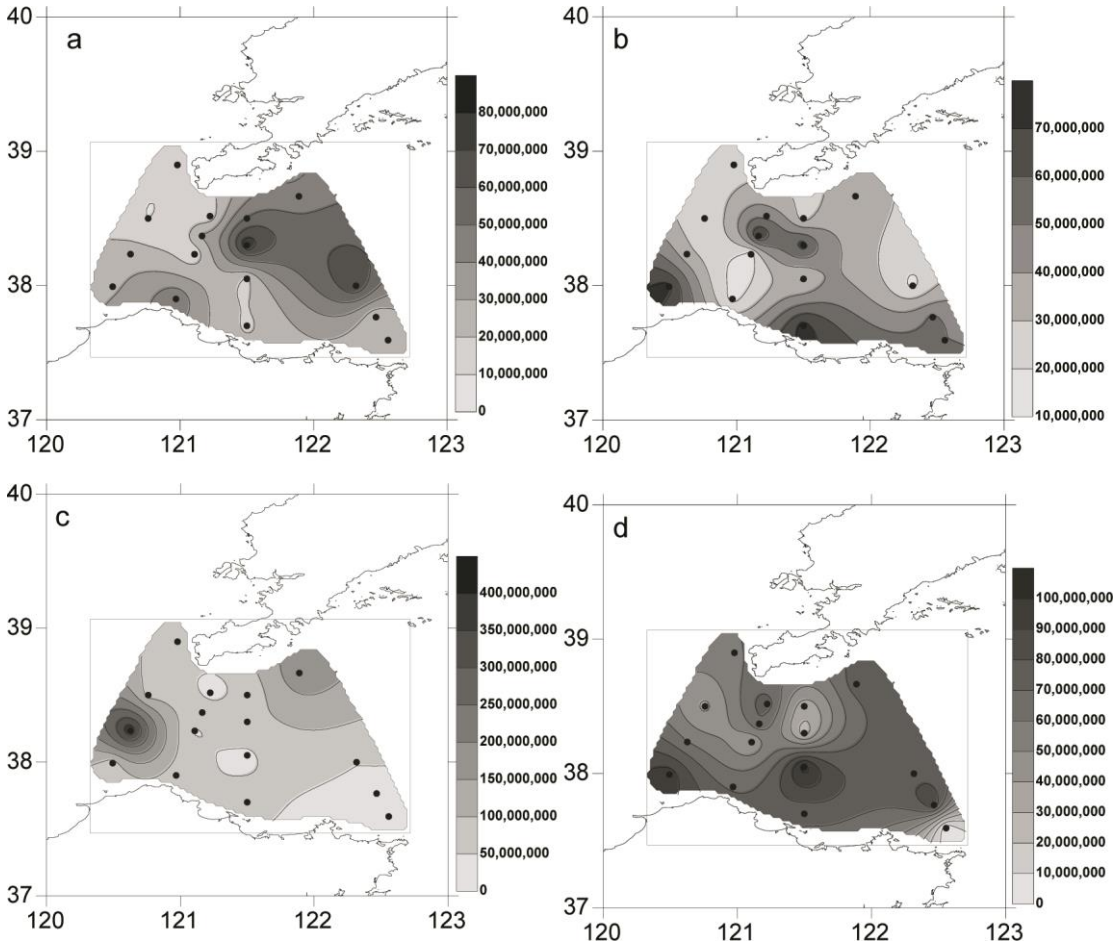
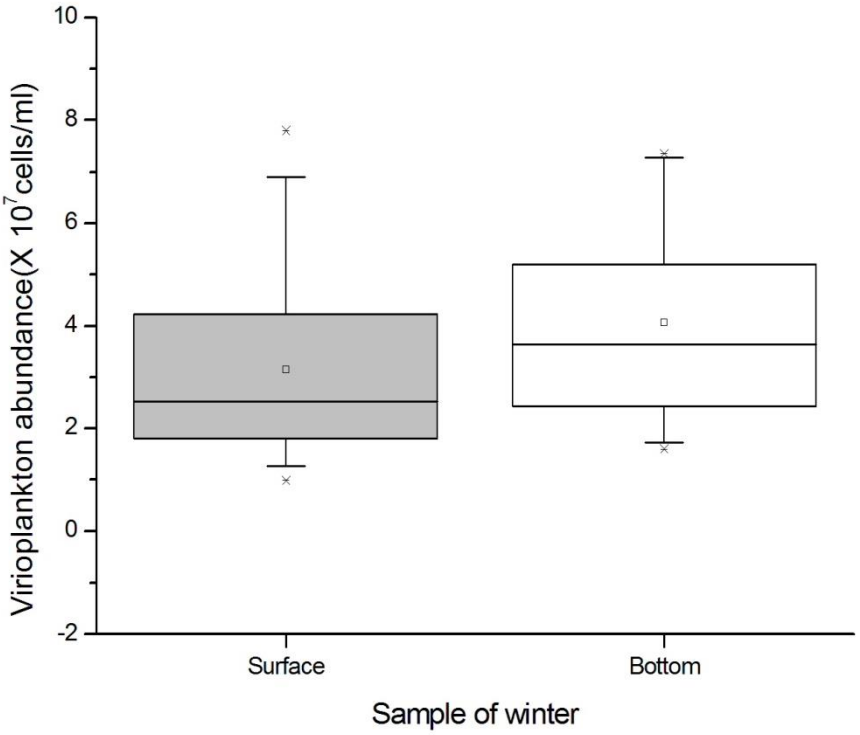
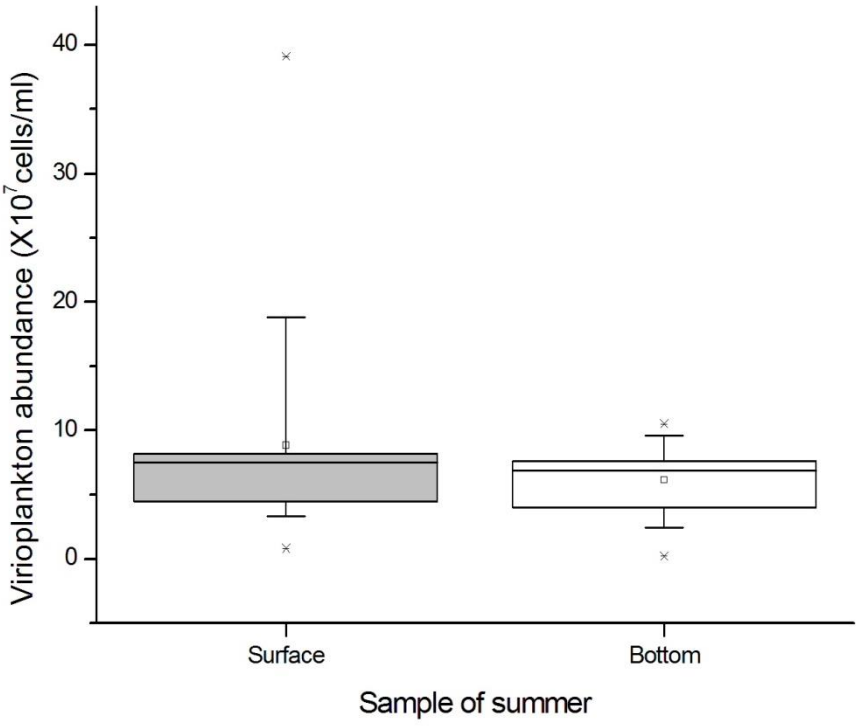


Figure 2 Horizontal distribution of virioplankton abundance in the Bohai Sea in winter 2013 and summer 2014 (Unit: cells/mL). (a) surface, winter; (b) bottom, winter; (c) surface, summer; (d) bottom, summer.



a



b

Figure 3 Vertical distribution of virioplankton abundance in the Bohai Sea in (a) winter 2013 and (b) summer 2014.

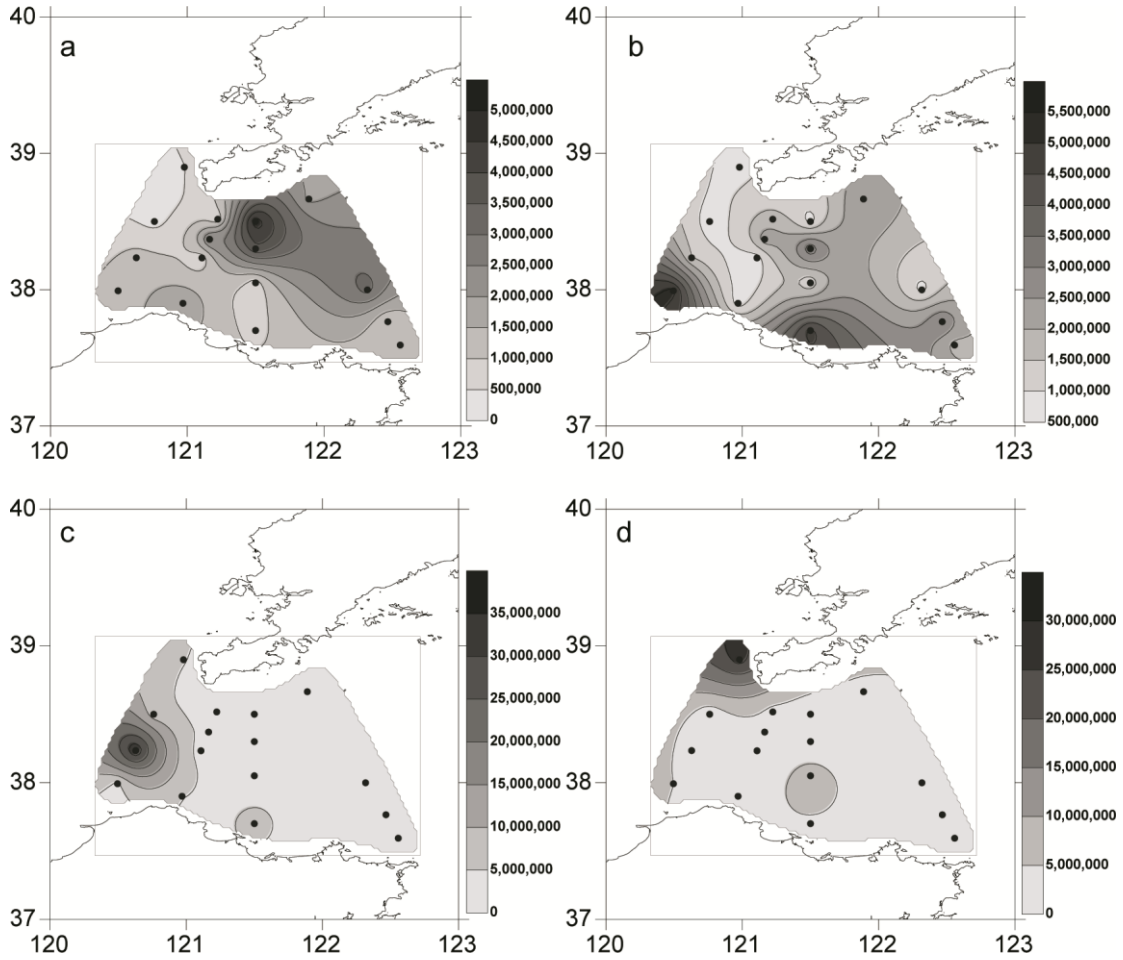
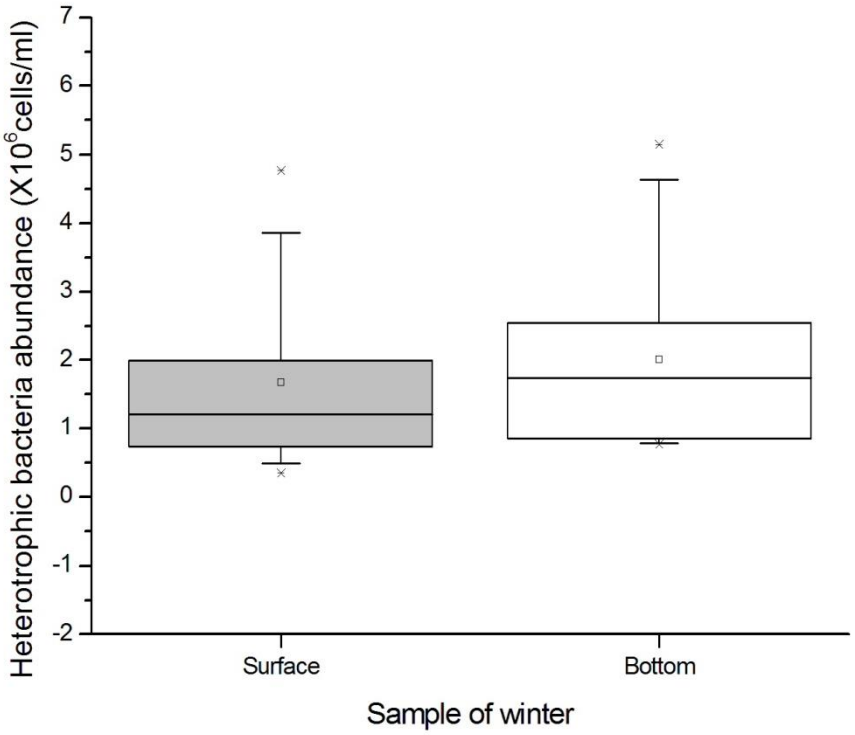
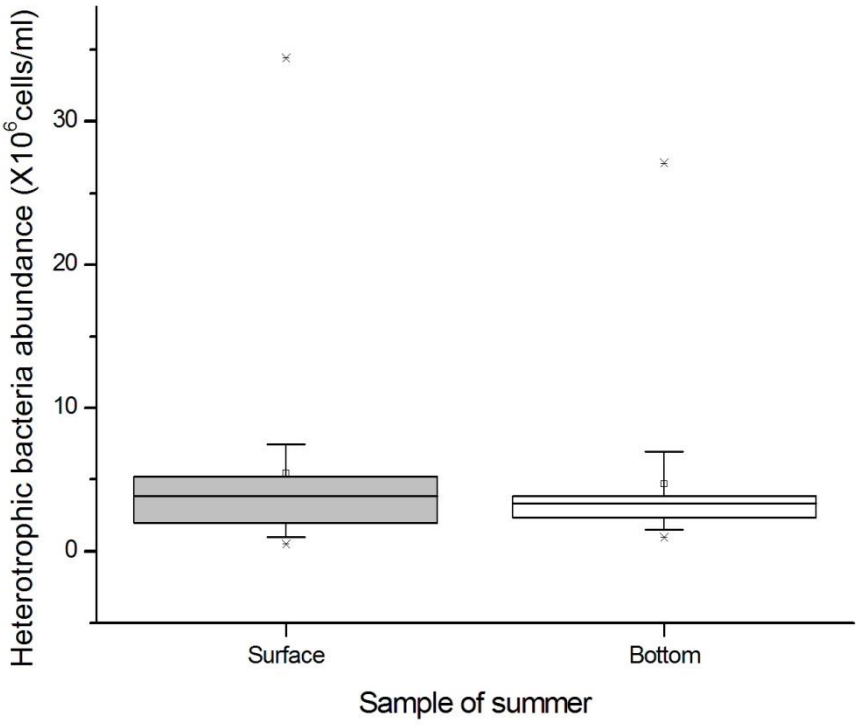


Figure 4 Horizontal distribution of heterotrophic bacterial abundance in the Bohai Sea in winter 2013 and summer 2014 (Unit: cells/mL). (a) surface, winter; (b) bottom, winter; (c) surface, summer; (d) bottom, summer.



a



b

Figure 5 Vertical distribution of heterotrophic bacterial abundance in the Bohai Sea in (a) winter 2013 and (b) summer 2014.

CHAPTER III THE YELLOW SEA WARM CURRENT FLUSHES THE BOHAI SEA MICROBIAL COMMUNITY IN WINTER

ABSTRACT

The effect of the Yellow Sea Warm Current (YSWC) on virio- and bacterioplankton communities in Bohai Sea is unknown. In this study, we measured the composition and dynamic changes of virio- and bacterioplankton at the entrance of the Bohai Sea to determine the influence of the YSWC on the Bohai Sea and vice versa. In the Bohai Strait there were east to west gradients of water chemistry and hydrology. Viruses and heterotrophic bacteria abundances decreased from the northeast to southwest. The average abundance of viruses and bacteria clustered geographically. The turbulent mixing between the deep northwest "warm" current, which is 9°C and the cold, nutrient-rich Bohai Sea water at 7 to 8°C appears to stimulate the abundance of viruses and heterotrophic bacteria. Viral and heterotrophic bacterial abundances at the junction of warm water and cold water were almost 10-fold greater than it in the low thermohaline areas, with abundances peaking where the temperature was high. The average viral and bacterial abundances in the Bohai Strait were 5.7×10^8 cells/mL and 7.1×10^7 cells/mL in the northeast area, and they were 2.1×10^8 cells/mL and 1.6×10^7 cells/ml in the southwest area, suggesting that the YSWC entered the Bohai Sea with poor biomass and exited with rich biomass, converting the Bohai Sea nutrients to microbial biomass, which is flushed into the Yellow Sea and that enriches the Yellow Sea microbial loop. Our results show the dramatic effect of a 1°C rise on microbial biomass, and indicate marine microbial communities will increase significantly with rising temperature and increasing eutrophication.

INTRODUCTION

The ubiquity of virioplankton and bacterioplankton has driven a continued and expanding interest in marine microbial dynamics (Proctor *et al*., 1990; Hobbie *et al*., 1977). Their abundance and metabolic flexibility (Sandaa, *et al*., 2009; Bouvy *et al*., 2011) mean heterotrophic bacteria play an essential role in many marine ecological processes (Azam, 1998), including the assimilation and decomposing of marine dissolved organic matter (Kong *et al*., 2014; Hung *et al*., 2016; Cappello *et al*., 2012; Burdige *et al*., 2016), and they function as trophic mediators, converting dissolved organic matter into microscopic particles. Simultaneously, free-floating viruses are also an important part in marine environments as indicators of environmental change (Hara *et al*., 1991). Strong obligate parasitic interactions between viruses and their host reveal that viruses integrate their own genome into the host genome by the process of lysogeny to maintain their survival

(Murray *et al*., 1992; Winter *et al*., 2012). Through the process of infecting microorganisms and viral lysis, viruses can release a large amount of dissolved organic matter into the environment to make it available for the other microorganisms (Winter *et al*., 2010; Suttle, 1994; Fuhrman, 1996). Also, viruses are important regulators in controlling bacterial abundances, diversity and production in aquatic ecosystems through “kill the winner” models, which maintains the growth and diversity of less competitive microorganisms (Kopylov *et al*., 2011; Thingstad and Lignell, 1997). Viruses not only directly affect the populations of bacterioplankton by cell lysis (Suttle, 2005), but also indirectly provide dissolved organic matter to the bacterioplankton communities, which plays an indispensable role in biogeochemical cycles (Wilhelm *et al*., 1999). The dynamic changes of virio- and bacterioplankton have drawn wide attention in microbial ecology. But, for the viral and bacterial communities in Bohai Sea we know little.

The Bohai Sea is an important spawning and fishing ground area which is located in the north part of China. It is a semi-enclosed sea, which is composed of Liaodong Bay, Bohai Bay and Laizhou Bay. As a sea surrounded by land, the Bohai Sea only connects with the Yellow Sea by the Bohai Strait (Li *et al*., 2015). The Gulf of Finland is similar. The dynamic saltwater wedge from Baltic Sea penetrates the Gulf of Finland to converge with the fresh water discharged from the east of the gulf. This convergence gives the area dynamic hydrographic and climatological properties (Lips *et al*., 2016). The Delaware Bay is a key focus area which is easily impacted by joint effect of inflow and outflow in the bay due to its coastal locations. Like the Gulf of Finland and the Delaware Bay, as a coastal sea, the Bohai Sea is influenced by the oceanic and anthropogenic inputs. Almost every year in winter, a stream of powerful, relatively warm and saline Yellow Sea Warm Current (YSWC) moves northward through the Yellow Sea Trough and flows into the Bohai Sea (Xu *et al*., 2009). Many previous studies had been focused on the nutrients, paths and zooplankton biomass migration that were associated with the YSWC (Lü *et al*., 2013). How the YSWC impacts virio- and bacterioplankton dynamics in the Bohai Sea is still not clear.

Phytoplankton and possibly jellyfish blooms in the Baltic Sea have increased with rising temperature (Paerl and Huisman, 2008; Sun, 2012). The YSWC transports warm, saline water from the north of the Yellow Sea into the Bohai Sea. To understand how these dynamic changes impact the Bohai Sea microbial community, we begin with biomass estimates from flow cytometric enumeration.

Flow cytometry is an established method for enumerating heterotrophic bacteria (Gasol *et al*., 1999). For flow cytometry, populations that appear on cytograms are often divided into high nucleic acid (HDNA) cells and low nucleic acid (LDNA) cells according to the intensity of the fluorescence signals (Falcioni *et al*., 2008; Gasol *et al*., 1999). The flow cytometry protocol that we used in the determination of bacterial populations has been used from freshwater to seawater and from eutrophic to oligotrophic environments (Gasol and del Giorgio, 2000). In the process of flow

cytometry working, particles are usually divided into different populations according to different parameters (e.g. size, color of emitting light and scattered light). It is similar to the bacteria, the viral populations are usually divided into different groups according to their differences in the green fluorescence and the side scatter. The populations of high fluorescence intensity are usually considered as cyanophage and the low fluorescent populations are usually thought to be bacteriophage (Li *et al*., 2001; Baudoux *et al*., 2007). Flow cytometric detection of virus and heterotrophic bacteria has been used in a wide range of the marine ecological environment (De Corte *et al*., 2016; Bouvier *et al*., 2007).

In this study, we used flow cytometry to enumerate populations of virio- and bacterioplankton in the Bohai Strait to evaluate how their spatial distributions and biomass are influenced by the YSWC, and to determine what factors alter viral and bacterial distributions in the Bohai Strait. Our results show that the Bohai Sea converts nutrients to biomass, which are subsequently flushed back into the Yellow Sea, contributing to its overall microbial loop biomass.

MATERIALS AND METHODS

Study site

The Bohai Sea is a semi-enclosed sea located between 37° 07-41° N and 117° 35-121° 10E. The southern, northern and western boundaries of the Bohai Sea are surrounded by land, it is linked with the Yellow Sea through the Bohai Strait. From northeast to southwest, the length of the Bohai Sea is about 550 kilometers, from east to west the width is about 350 kilometers. The area is approximately 77,000 square kilometers, with an average depth of 18 m. The deepest area is about 70m. It is the smallest and shallowest coastal sea in China.

Sampling procedures

Samples were collected on the RV “Xiang Yang Hong 8” from December 14th to December 25th along four parallel transects, located in the Bohai Strait (Fig. 1, Table 1, Table 2). Vertical temperature and salinity were measured in each station by using a conductivity temperature depth system (CTD). Samples for enumerating viruses and bacterial populations were taken from 2 to 5 m below the surface and from just above the seabed, and the sampling water depth ranged from 17 m to 71 m. After the completion of sample collection, water samples were immediately transferred into 2 ml cryovials and fixed with sterile glutaraldehyde (0.5% final concentration) and then incubated at 4°C for 15 min (Brussaard, 2004, Wang *et al*., 2016). All samples were flash preserved in liquid nitrogen, stored in a -80°C freezer upon returning to the laboratory, and analyzed within one month of sampling.

Flow cytometric analysis

The identification and enumeration of bacterial and viral populations were completed by using a Becton Dickinson Accuri C6^{TM} flow cytometer (FCM, BD biosciences, San Jose, CA, USA). Triplicate water samples were thawed in a water bath at 37°C for immediate analysis. Thawed samples were diluted according to the proportion of 1:10 with 0.2 μm filter membrane filtered TE buffer (10mM Tris, 1mM EDTA) and then stained with a SYBR-I Green solution (1:500 dilution; molecular probes) for 10 min in the dark at 80°C (Brussaard, 2004). Prior to sampling, yellow beads with a final concentration of 10^5 beads/ml were added to each sample as an internal size and concentration standard (Gasol and del Giorgio, 2000). Yellow beads with 1 μm particle size (Molecular probes) were fully mixed before being added to samples. Phosphate-buffered saline (PBS) was used as sheath fluid. After optimizing the machine, we used forward-angle light scatter (FSC), side-angle light scatter (SSC) and green fluorescence (SYBR Green-I) as means of monitoring data. All data were collected in list-mode folder and processed with FlowJo (TreeStar, San Carlos, CA, USA).

Based on side scatter distribution and fluorescence intensity, viral and bacterial populations were effectively distinguished. Bacterial populations were gated into high DNA and low DNA groups according to the green fluorescence intensity (Gasol *et al*., 1999), and viral populations were divided into two virus-like particle populations (Seymour *et al*., 2006; Larsen *et al*., 2008).

Statistical analysis

Correlations between viruses, bacteria and environmental factors were detected by Spearman's correlation test. Discrepancies between transects on water depths were determined by the Mann Whitney U test. While multiple comparisons among different transects and water depths were calculated by the Kruskal Wallis test (Zar, 2009).

RESULTS AND DISCUSSION

Environmental parameters

Temperature and salinity profiles of the water column were examined in surface and bottom waters (stations K, E, L, R) of all four transects (Fig. 3). From the east of Bohai Strait to the west, according to the data collected from transect K to transect R, the highest temperature was at least 3°C warmer than the lowest temperature. Moreover, from transect K to transect R (Fig. 1), temperature presented a significant decreasing trend along the path of the YSWC and the strength of YSWC is much stronger in winter than it is during any other times of the year (Song *et al*., 2009; Xu *et al*., 2009). The YSWC added warmth to the cold and nutrient rich Bohai Sea. The salinity

structure presented a similar trend with the temperature structure (Fig. 4A, 4B). And on the horizontal distribution, from the east of the Bohai Strait to the west, the salinity gradient decreased. This is due to the high salinity tongue of the YSWC moving largely to the west, which sets up this east - west gradient. As it was described in the Gulf of Finland, a large volume of in and out flow of water exchange would have a great influence on the nutrient and stratification characteristics in this area (Lehtoranta *et al*., 2017). The YSWC also make a remarkable influence on the hydrologic and environmental factors in the Bohai Sea.

Viral community

Total viral abundance

Total viral abundance ranged from 1.49×10^7 cells/ml to 131×10^7 cells/ml in the surface water, and from 2.69×10^5 cells/ml to 39.3×10^7 cells/ml in the bottom water (Table 1, Table 2). Significant differences in viral numbers were detected between different sampling depths and stations. From the eastern to the western sites, higher abundance of total viruses can be found in the stations of K6 (131×10^7 cells/ml), K7 (109×10^7 cells/ml), E3 (87.6×10^7 cells/ml), L2 (54.3×10^7 cells/ml) and R6 (87.3×10^7 cells/ml). These were almost 10-fold higher than the other stations in the surface water (Table 1). The same phenomenon also appeared in the bottom water of the Bohai Strait, a significant higher abundance of total viruses occurred in stations of K8 (33.8×10^7 cells/ml), E3 (17.4×10^7 cells/ml), L7 (39.3×10^7 cells/ml) and R6 (25.7×10^7 cells/ml).

The YSWC transported warm and saline water into the north of Yellow Sea and entered the Bohai Sea, forming a warm, saline tongue of water in winter (Fig. 2 and Ma *et al*., 2006). The result is mixing between the warm Yellow Sea water and the cold Bohai Sea water, which stimulates bacterial growth and in turn increases viral biomass.

The total viral abundance increased from one hundred to one thousand-fold in the surface water and bottom water at the stations with YSWC water (Fig. 5; Table 1, Table 2). Viruses are believed to have an essential role on controlling the bacterial community composition, and they account for large proportion of ocean biomass, which means they are a key component in ocean nutrient and energy cycles (Sorensen G., 2009; Suttle CA., 2007). As a consequence of their importance, viruses also regulate biogeochemical processes by causing and facilitating bacterial mortality, horizontal gene transfer and by influencing microbial metabolic pathways (Breitbart, 2012). In view of the vital function of viruses, they are increasingly considered to be main drivers of myriad ecosystem processes (Breitbart, 2012). Since viruses rely on bacterial growth, viral abundance and importance will be particularly acute in eutrophic aquatic ecosystems (Junger *et al*., 2017). Our results are consistent with the high viral numbers found in eutrophic systems and suggest that

Bohai Strait viral dynamics warrant further investigation to determine precisely how they regulate the Bohai Sea microbial ecosystem, and the Bohai Sea food web in general.

Individual viral population abundance

In Fig. 2, VLP1 and VLP2 populations were observed at all stations and depths of seawater. The abundances of VLP1 populations ranged from 0.95×10^7 to 62.31×10^7 cells/ml in surface water (Table 1) and from 0.02×10^7 to 18.25×10^7 cells/ml in bottom water (Table 2). VLP2 populations ranged from 0.55×10^7 cells/ml to 76.58×10^7 cells/ml in surface water and from 0.01×10^7 cells/ml to 21.00×10^7 cells/ml in bottom water. The general abundance of virioplankton investigated in this study was consistent with previous results from the Bohai Sea (Wang *et al*., 2016). The total average populations of VLP1 were significantly larger than VLP2 in surface water and bottom water ($P<0.05$) (Fig. 5A, B, C). The distribution patterns of viral abundances in the Bohai Strait were similar to the patterns studied in the coastal waters of the Bohai Sea (Wang *et al*., 2010). The abundance variation range for all stations was almost one thousand-fold. With average abundance of VLP1 and VLP2 are 2.99×10^8 and 3.18×10^8 in transect K, 1.39×10^8 and 1.21×10^8 in transect R, the abundances of VLP1 and VLP2 presented a significant difference ($P<0.05$) between the east and west of the Bohai Strait, and showed an obvious decreasing trend from the northeast to the southwest. Viral abundances are usually homogeneous in well mixed shallow sea (Pan *et al*., 2007; Guixa-Boixereu *et al*., 1999). Here, the distribution of viruses in the Bohai Sea was extremely heterogeneous. The results are consistent with mixing water masses and active viral production as discussed above. VLP1 and VLP2 abundances decreased from the northeast to the southwest of the Bohai Strait. This variation in the abundance of VLP1 and VLP2 leads to a great change in the community populations of viruses in the Bohai Strait which reflect the hydrological effect of water mass on the coastal viral community. It is consistent with the results that viral and microbial community can be controlled by the coastal shelf water interactions (Paterson *et al*., 2012).

Viral community composition

VLP1 accounted for about 55% and 57% of viruses in the surface and bottom waters (Fig. 5). The VLP1 and VLP2 abundances reflected the distribution patterns of total viral abundance. The average viral abundance of K transect (61.74×10^7 cells/ml) was almost three times higher than the other transects (E transect: 26.99×10^7 cells/ml, L transect: 18.86×10^7 cells/ml, R transect: 25.99×10^7 cells/ml) as a result of its location. We propose these differences are related to the variation occurred in temperature and nutrient of sea water which is caused by the water quality changes

that the YSWC brings with it (Kong *et al*., 2014). We note that viruses may be more sensitive to thermal stress than their host systems in marine water (Mojica *et al*., 2014). Particularly, increased levels of salinity and nutrients such as phosphorus and nitrogen can stimulate virus production by bacteria greatly (Kong *et al*., 2014; Lymer *et al*., 2006). In contrast, low nutrient concentrations conditions can lead to decreased burst size (Weinbauer *et al*., 2004). The YSWC changes the nutrient characteristic of Bohai Sea water, which had an important role on the water environment and stimulated the biomass and community in the central Yellow Sea (Liu *et al*., 2015).

Bacterial community

Total bacterial abundance

Total bacterial abundance ranged from 0.14×10^7 cells/ml to 17.09×10^7 cells/ml in the surface water, and ranged from 0.12×10^7 cells/ml to 4.53×10^7 cells/ml in the bottom water. There were significant differences in total bacterial abundance between different layers ($P<0.05$), it was concluded that the abundance of total bacteria in surface layer was almost four times higher than in the bottom layer. Incident light, nutrients, the comparatively high temperature and salinity in the surface water and the variation of depths can have a big impact on the distribution of bacterial abundance in different water areas (Tanaka and Rassoulzadegan, 2004; Jiao *et al*., 2006). A declining gradient in total bacterial abundance from transect K to transect R was observed along all stations in surface and bottom water (Fig. 6). Due to the influence of YSWC, from transects K to R, the water temperature in the east is about 3°C higher than the temperature in the west. Temperature is an important factor in bacterial dynamics (Bolnick *et al*., 2011). The YSWC originates from the Kuroshio water and Taiwan Warm Current, which have high relative temperature (Lin *et al*., 2011). This, combined with the nutrients, is the main reason for the result that bacterial abundance is much higher than the nearby Yellow Sea. Salinities are often much higher in the Bohai strait than inside of the Bohai Sea due to the offshore input from the open sea, and the high salinity tongue brought by YSWC leads to a decreasing salinity gradient from east to west in the Bohai Strait. Like the effect of temperature, salinity has also been considered to be an important determinant of controlling bacterial communities (Wu *et al*., 2006; Herlemann *et al*., 2011). Bacterial populations can be constrained by their own adaptability to the environment or by predators.

Individual bacterial population abundance

The diversity and abundance of bacteria are affected by environmental factors and viral particles

(Töpper *et al*., 2013). Three groups of bacteria populations (Fig. 2) were observed at all transects and depths. HDNA1 and LDNA populations were equal in abundance and HDNA2 populations made up lowest percentage of total bacteria. The average abundance of LDNA in all stations ranged from 0.05×10^7 cells/ml to 6.53×10^7 cells/ml in surface water and ranged from 0.03×10^7 cells/ml to 1.99×10^7 cells/ml in the bottom water (Table 1, Table 2). The average abundance of HDNA1 in all stations ranged from 0.05×10^7 cells/ml to 7.90×10^7 cells/ml in surface water and ranged from 0.05×10^7 cells/ml to 1.55×10^7 cells/ml in the bottom water (Table 1, Table 2). The average abundance of HDNA2 in all stations ranged from 0.03×10^7 cells/ml to 2.79×10^7 cells/ml in surface water and ranged from 0.04×10^7 cells/ml to 0.98×10^7 cells/ml in the bottom water (Table 1, Table 2). The patterns are similar to viral abundance patterns. The distribution of bacterial abundance followed the trend described in total virus abundance and decreased gradually from the YSWC affected east to west transects.

Bacterial community composition

The abundance of LDNA, HDNA1 and HDNA2 followed the trend observed in the total bacterial abundance. LDNA and HDNA1 dominated the community composition, contributing up to 89.1% in surface water and bottom water (Fig. 6 A, B). The percentage of HDNA2 was the smallest in all stations influenced by the YSWC. This is consistent with conclusions from Paterson *et al*., (2012) that fluctuations in the sea's physical conditions can cause sharp microbial community changes. Previous studies have suggested that environmental factors, such as temperature, are extremely important for the composition of bacterial populations and their abundance (Alonso-Sáez *et al*., 2007). The water temperature around the Bohai Strait decreases from east to west due to the effect of the YSWC which take a big change to the water environment of Bohai Sea. We observed that the bacterial abundance in transect K where the warm YSWC tongue penetrated first was much larger than the abundance in other west transects E, L and R. Therefore, the shift in the microbial community may indicate that these bacterial populations (LDNA, HDNA1 and HDNA2) might be adapted to the existent temperature conditions.

Virus to bacteria ratio

In this study, the value of virus to bacteria ratio (VBR) ranged from 1.39 to 24.35 in the surface water, and it ranged from 0.09 to 16.49 in the bottom water (Fig. 7). The average value of VBR was 10.18 in the surface layer and 8.40 in the bottom layer, respectively. VBR values are usually studied to demonstrate the relationships between viruses and heterotrophic bacteria. Viral-bacterial interactions are an indicator of water environmental change (Brum *et al*., 2016). In our study, the

wide range of VBR value showed a high tendency in the northwest and south east of Bohai strait, which present its response to the marine environmental change caused by the YSWC.

CONCLUSION

The YSWC has a major impact on the distribution of virioplankton and bacterioplankton across the Bohai Strait. We used flow cytometry to enumerate viral and bacterial abundance, and in doing so found that the YSWC flushes biomass into the Yellow Sea. The YSWC appears to increase microbial abundance by increasing temperature, with a concomitant increase in salinity. We suggest that in the future taxonomic studies be done on the virio- and bacterioplankton to demonstrate how the YSWC influences the community compositions of the Bohai Strait.

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Table 1. Abundance values of flow cytometrically defined viral and bacterial populations. S: surface sample, V1: VLP1, V2: VLP2, B1: LDNA, B2: HDNA1, B3: HDNA2, TV: Total virus abundance, TB: Total bacteria abundance, VBR: Virus to bacteria ratio.

Transect	**K**				**E**				**L**				**R**			
Type	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
	K2	**K6**	**K7**	**K8**	**E2**	**E3**	**E4**	**E6**	**L2**	**L4**	**L5**	**L7**	**R3**	**R5**	**R6**	**R7**
TV(×10^7cells/ml)																
Mean	3.23	131.10	109.70	2.93	4.76	87.59	2.66	12.95	54.26	1.49	8.92	10.77	2.36	3.53	87.34	10.75
SD	0.92	2.57	2.41	1.84	1.89	0.32	0.78	1.01	0.17	3.41	0.21	1.02	1.01	2.65	0.81	
V1(×10^7cells/ml)																
Mean	1.62	54.49	62.31	1.39	3.72	39.19	1.60	9.42	32.50	0.95	3.96	4.07	1.27	1.85	46.54	6.04
SD	0.03	1.73	2.65	0.05	0.12	0.95	0.09	0.10	2.36	0.06	0.10	0.10	0.26	0.18	1.73	0.17
V2(×10^7cells/ml)																
Mean	1.61	76.58	47.36	1.54	1.04	48.41	1.06	3.53	21.76	0.55	4.96	6.70	1.09	1.68	40.80	4.71
SD	0.13	3.00	2.10	0.12	0.20	0.90	0.22	0.10	1.70	0.09	0.19	0.17	0.11	0.20	2.00	0.35
TB(×10^7cells/ml)																
Mean	0.	17.	10.	0.1	1.	13.	1.	0.5	13.	0.	1.	1.0	0.1	0.2	6.9	0.8

	38	09	31	4	03	79	92	3	00	31	37	3	9	6	7	1
SD	0.06	0.69	1.90	0.04	0.40	1.23	0.13	0.06	1.51	0.07	0.12	0.18	0.05	0.06	0.19	0.05
B1(×10^6cells/ml)																
Mean	0.14	6.41	4.70	0.05	0.32	5.59	1.07	0.17	6.53	0.12	0.59	0.51	0.07	0.13	4.20	0.34
SD	0.49	3.87	2.52	0.46	0.76	2.63	0.82	0.58	2.65	0.98	1.08	1.22	0.52	1.38	2.61	0.95
B2(×10^6cells/ml)																
Mean	0.16	7.90	4.27	0.05	0.42	6.14	0.49	0.20	4.67	0.14	0.54	0.36	0.07	0.09	2.04	0.28
SD	0.88	3.96	1.80	0.37	0.58	2.17	0.59	1.03	2.41	0.44	2.33	1.60	0.60	0.83	1.00	0.80
B3(×10^6cells/ml)																
Mean	0.07	2.79	1.34	0.03	0.29	2.07	0.36	0.16	1.80	0.06	0.24	0.16	0.05	0.05	0.73	0.19
SD	0.60	1.73	2.00	0.31	1.78	1.73	1.76	0.88	2.60	0.43	0.87	0.89	0.35	0.53	2.00	1.73
VBR																
Mean	8.58	7.67	10.63	21.63	4.61	6.35	1.39	24.35	4.17	4.78	6.54	10.45	12.68	13.33	12.53	13.25
SD	2.65	3.61	1.73	2.65	1.00	2.11	0.56	1.82	0.97	1.73	1.13	2.00	1.73	2.36	2.68	1.73

Table 2. Abundance values of flow cytometrically defined viral and bacterial populations. B: bottom sample, V1: VLP1, V2: VLP2, B1: LDNA, B2: HDNA1, B3: HDNA2, TV: Total virus abundance, TB: Total bacteria abundance, VBR: Virus to bacteria ratio.

Transect	**K**				**E**				**L**				**R**			
Type	**B K2**	**B K6**	**B K7**	**B K8**	**B E2**	**B E3**	**B E4**	**B E6**	**B L2**	**B L4**	**B L5**	**B L7**	**B R3**	**B R5**	**B R6**	**B R7**
TV($\times 10^7$cells/ml)																
Mean	1.62	0.80	12.90	33.76	9.03	17.37	0.79	1.98	0.03	0.74	5.23	39.25	2.67	0.76	25.74	5.05
SD	0.33	0.08	1.65	2.69	0.38	2.65	0.14	0.65	0.03	0.21	1.01	2.65	1.48	0.22	1.73	2.26
V1($\times 10^7$cells/ml)																
Mean	1.03	0.44	5.07	13.62	6.27	8.87	0.59	1.01	0.02	0.49	3.47	18.25	1.28	0.62	9.89	2.51
SD	0.17	0.11	0.93	2.23	2.60	1.92	0.15	0.38	0.02	0.25	0.98	2.65	0.98	0.32	2.72	0.66
V2($\times 10^7$cells/ml)																
Mean	0.59	0.36	7.83	20.15	2.76	8.50	0.21	0.97	0.01	0.25	1.76	21.00	1.39	0.15	15.84	2.54
SD	0.10	0.08	2.08	1.73	0.77	1.73	0.06	0.17	0.02	0.11	0.47	2.65	0.18	0.03	2.74	0.44
TB($\times 10^7$cells/ml)																
Mean	0.1	0.	2.2	4.5	0.6	1.4	0.	0.1	0.	0.	0.	2.3	0.2	0.	3.3	0.3

	2	12	8	3	5	6	26	4	31	47	92	8	1	36	0	9
SD	0.07	0.11	1.08	0.79	0.10	0.62	0.06	0.07	0.11	0.08	0.84	0.82	0.10	0.07	1.25	0.03
B1(×10^6cells/ml)																
Mean	0.03	0.03	1.06	1.99	0.16	0.49	0.08	0.05	0.13	0.17	0.32	1.33	0.09	0.15	1.75	0.15
SD	0.10	0.04	1.73	2.65	0.52	0.98	0.07	0.10	0.53	0.53	0.98	1.73	0.10	0.53	2.65	0.30
B2(×10^6cells/ml)																
Mean	0.05	0.06	0.88	1.55	0.26	0.66	0.11	0.05	0.12	0.20	0.36	0.78	0.08	0.12	1.16	0.17
SD	0.02	0.03	0.70	2.79	0.36	0.56	0.17	0.03	0.22	0.20	0.82	0.82	0.05	0.20	2.65	0.31
B3(×10^6cells/ml)																
Mean	0.04	0.04	0.34	0.98	0.22	0.31	0.07	0.04	0.06	0.10	0.24	0.26	0.04	0.09	0.40	0.07
SD	0.09	0.04	0.26	1.11	0.26	0.26	0.04	0.02	0.09	0.17	0.26	0.35	0.03	0.05	0.30	0.02
VBR																
Mean	13.25	6.48	5.67	7.45	13.91	11.86	3.01	13.75	0.09	1.57	5.66	16.49	12.44	2.15	7.8	12.89
SD	2.65	1.73	2.00	1.40	2.00	1.73	0.89	2.63	0.60	0.44	1.73	2.43	1.74	1.48	1.87	1.03

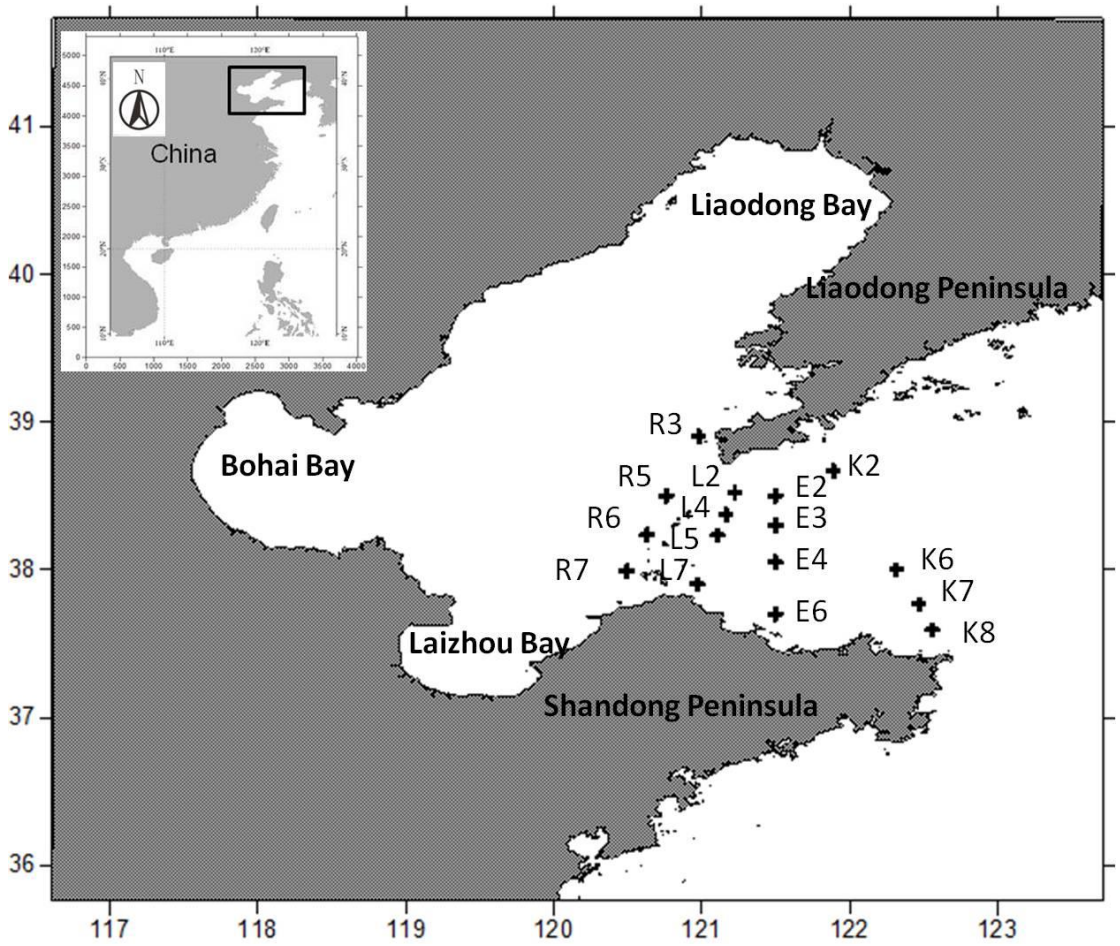


Figure 1. Sampling stations. The cross symbols represent the study area with sampling transects and stations located to the east, centre and west of Bohai strait.

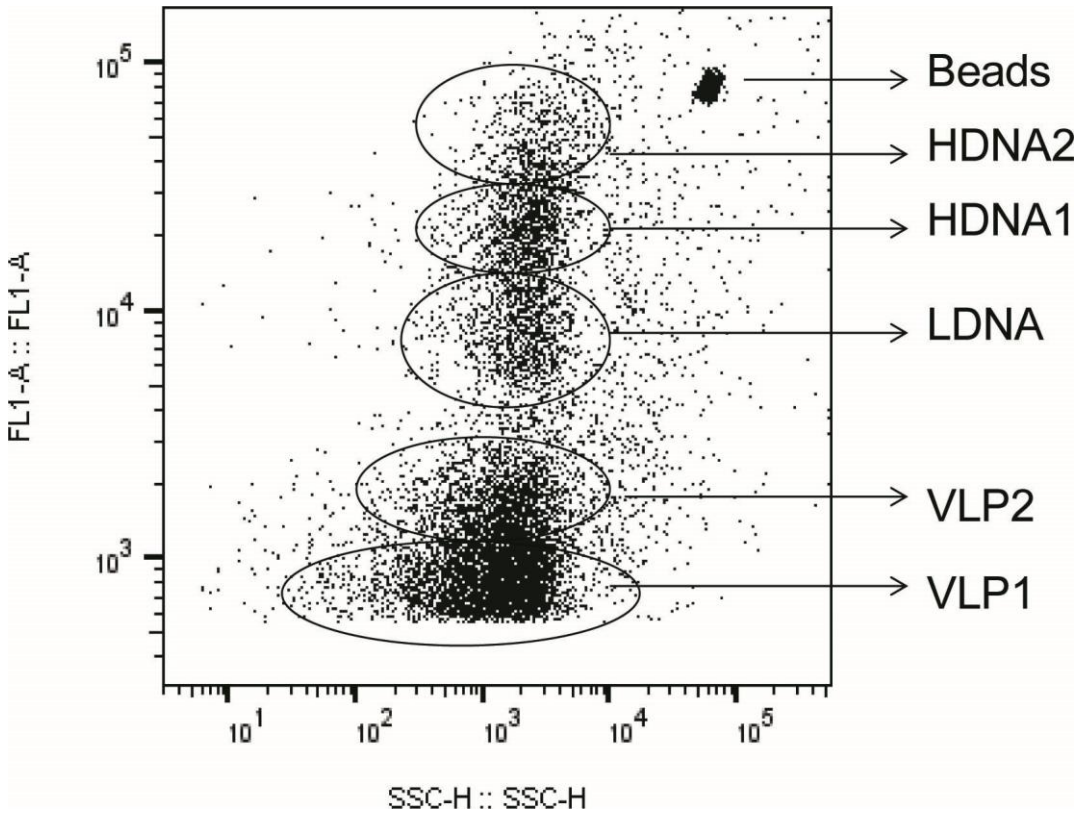


Figure 2. Archetypical cytograms were used to show viral and bacterial abundance within the Yellow sea warm current affected stations.

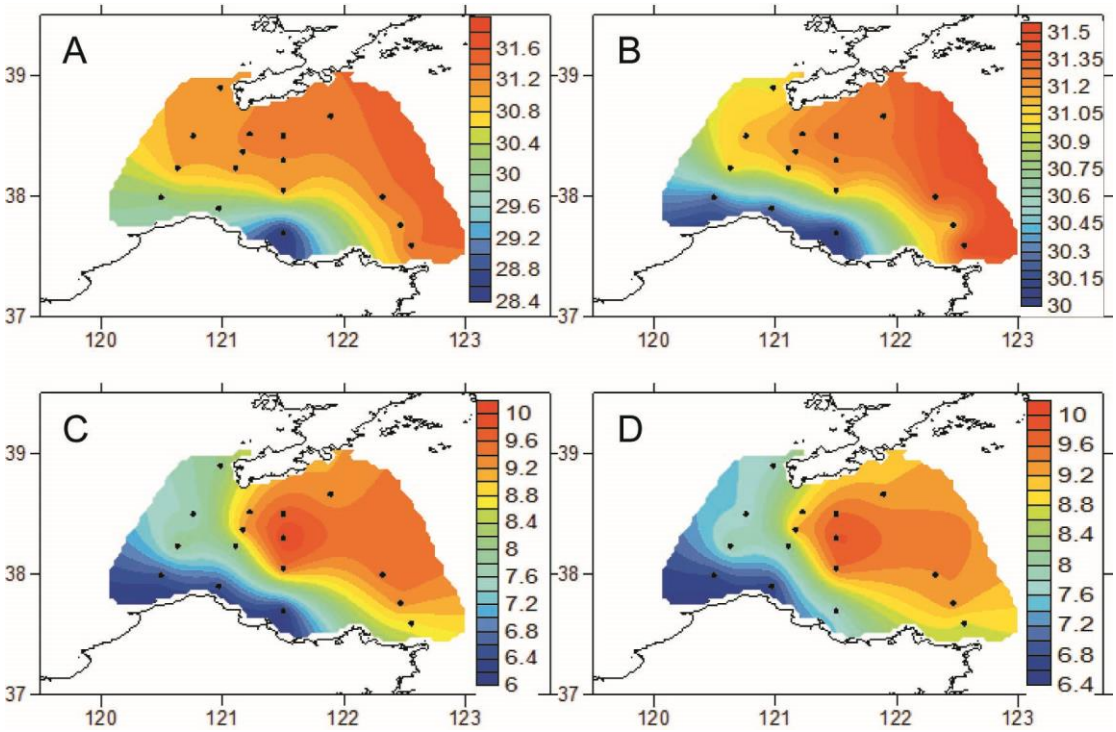


Figure 3. Sea surface salinity (A) and bottom salinity (B), surface temperature (C) and bottom temperature (D) of every stations in the Bohai strait.

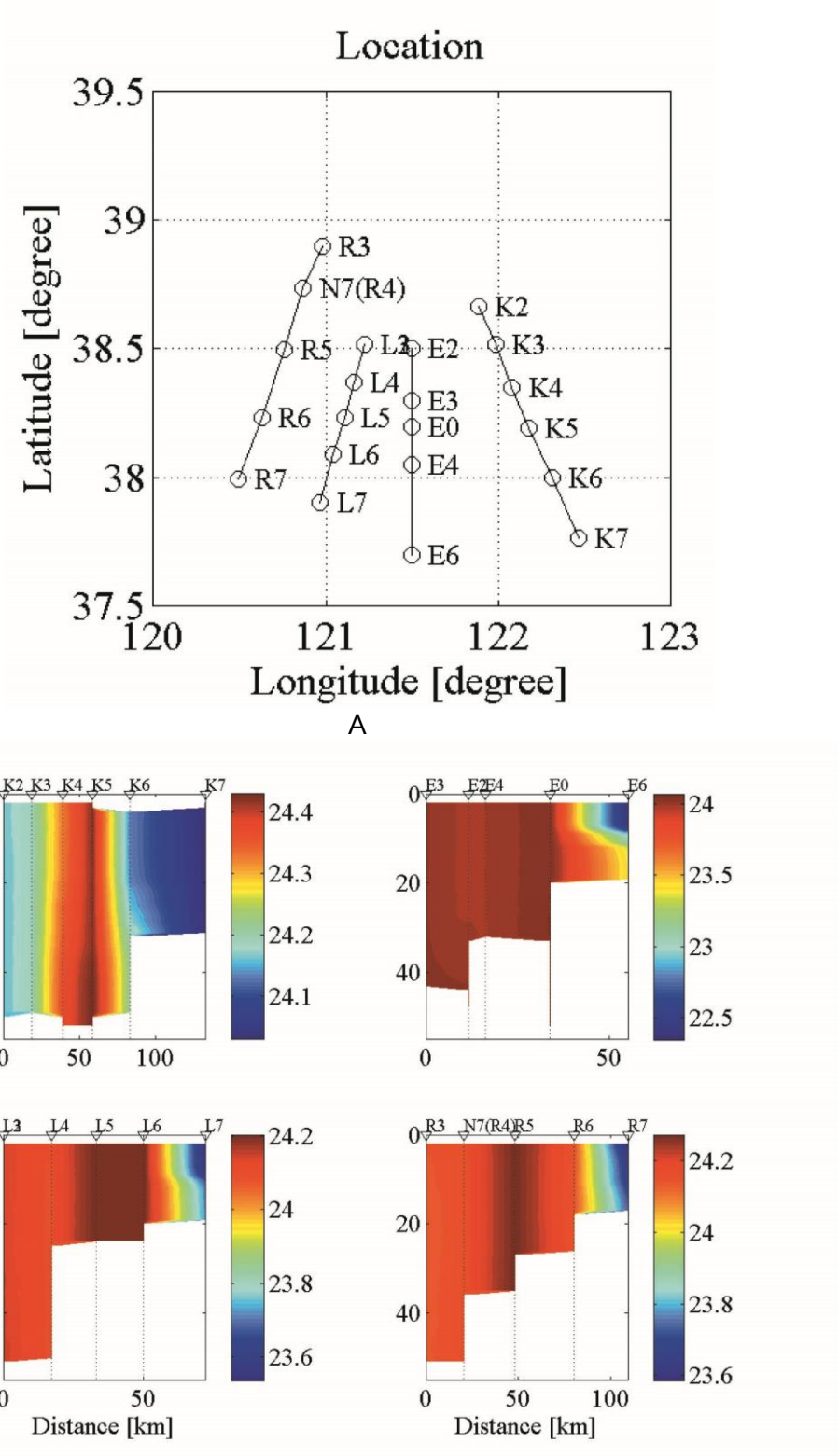
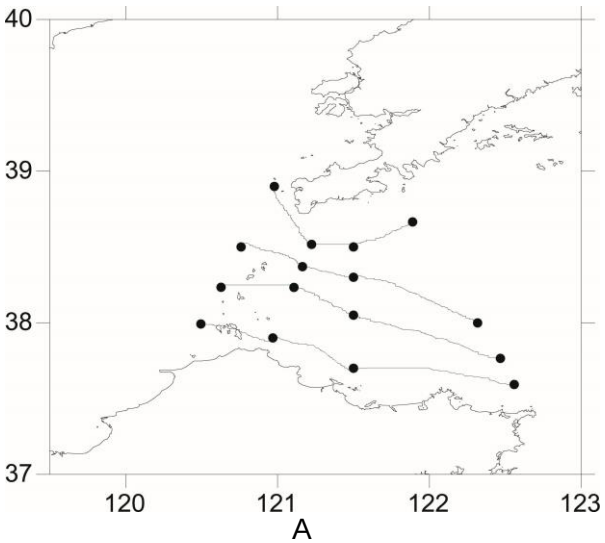
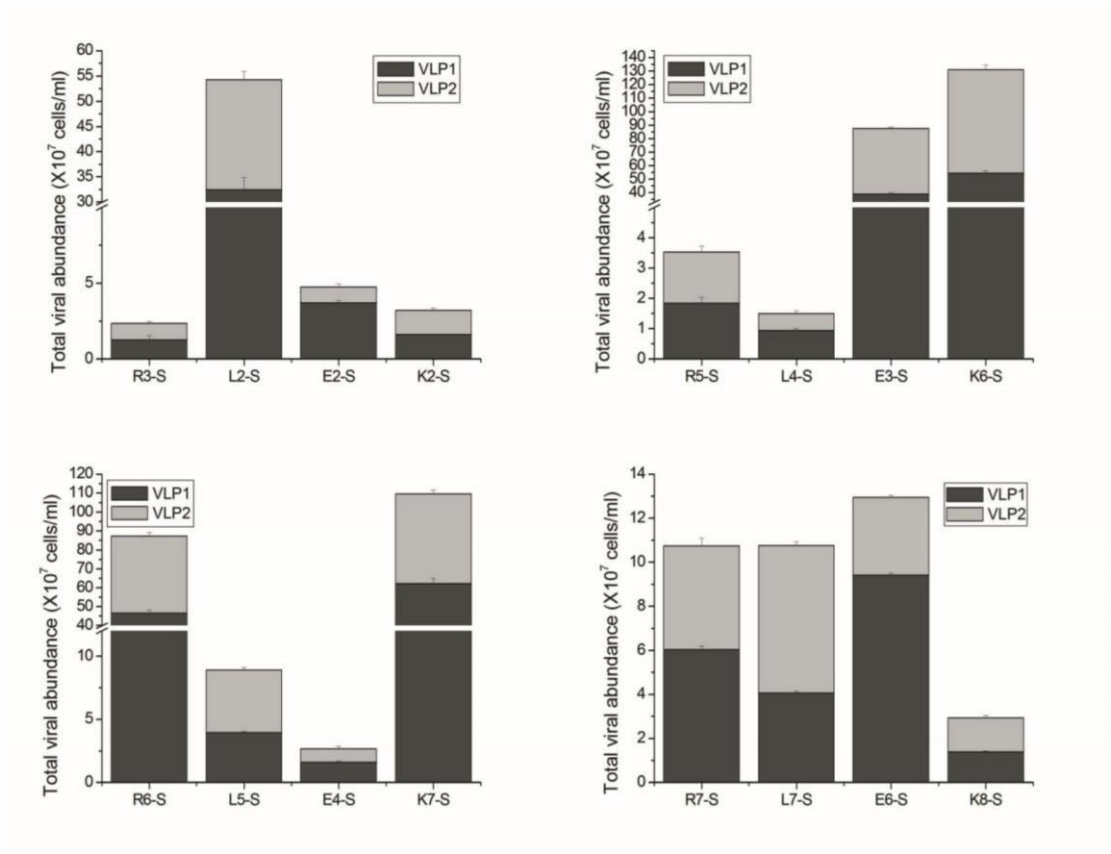


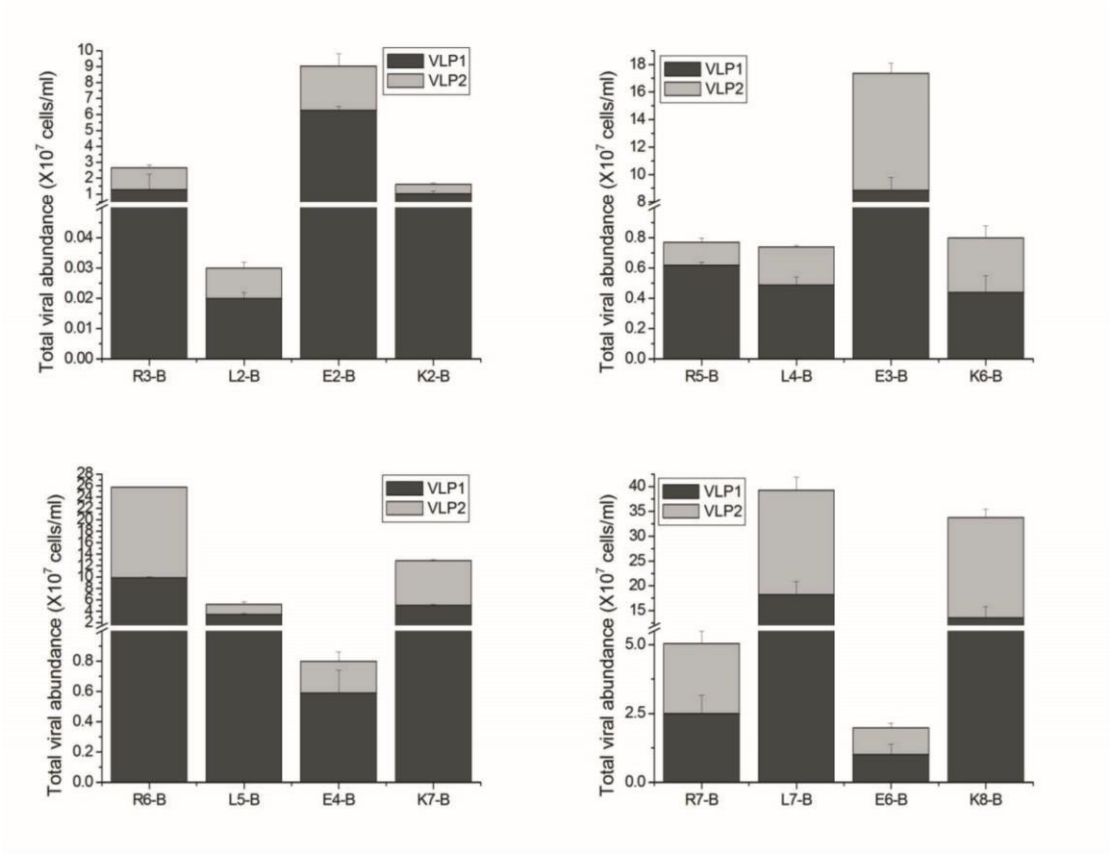
Figure 4. Horizontal distribution of each stations (A). Vertical profiles of four transects. Sigma-*t* of transects K, E, L, R in the Bohai Strait (B).


40
39
38
37
120
121
122
123

A

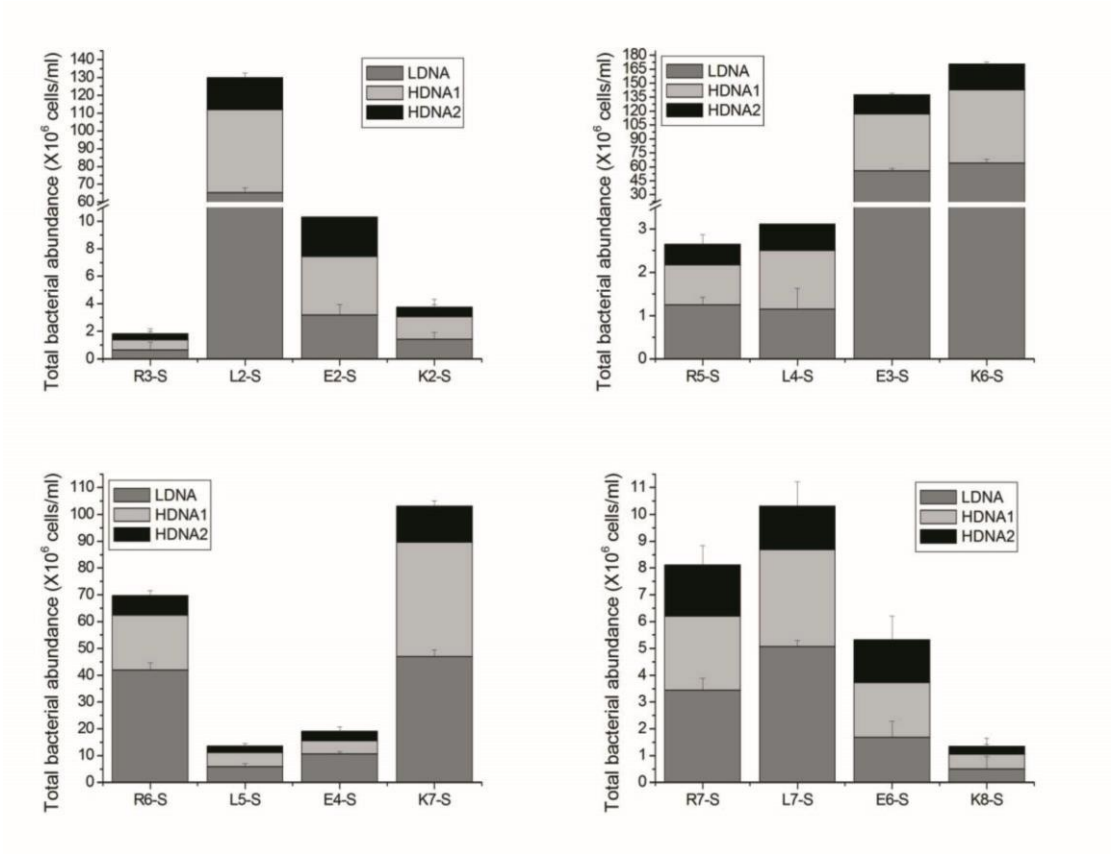

VLP1
VLP2
Total viral abundance (X10^7 cells/ml)
R3-S
L2-S
E2-S
K2-S
R5-S
L4-S
E3-S
K6-S
R6-S
L5-S
E4-S
K7-S
R7-S
L7-S
E6-S
K8-S

B

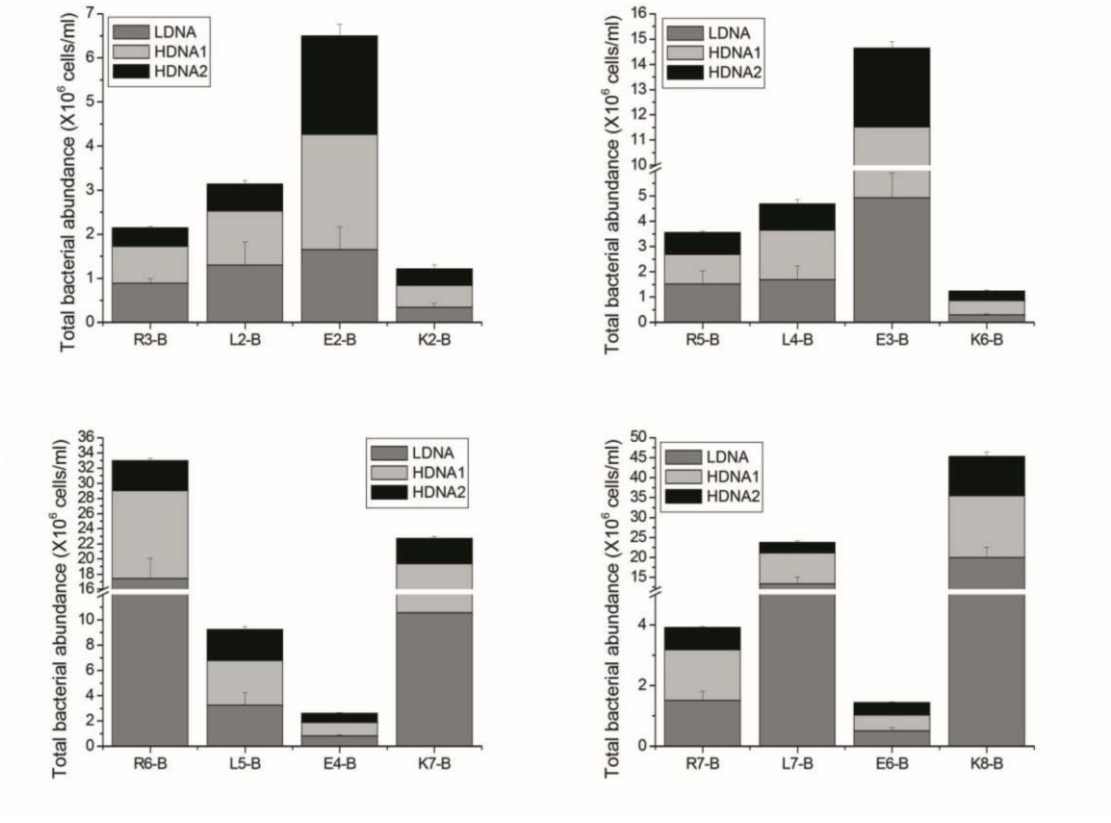


C

Figure 5. Total viral abundance and the groups of individual viral populations in surface water (K-S, E-S, L-S, R-S) and bottom water (K-B, E-B, L-B, R-B). A, Horizontal distribution of each stations presented as directions of the YSWC passed by. B represent total viral abundance in the surface water of station K, E, L, R, and C represent total viral abundance in the bottom water of station K, E, L, R.



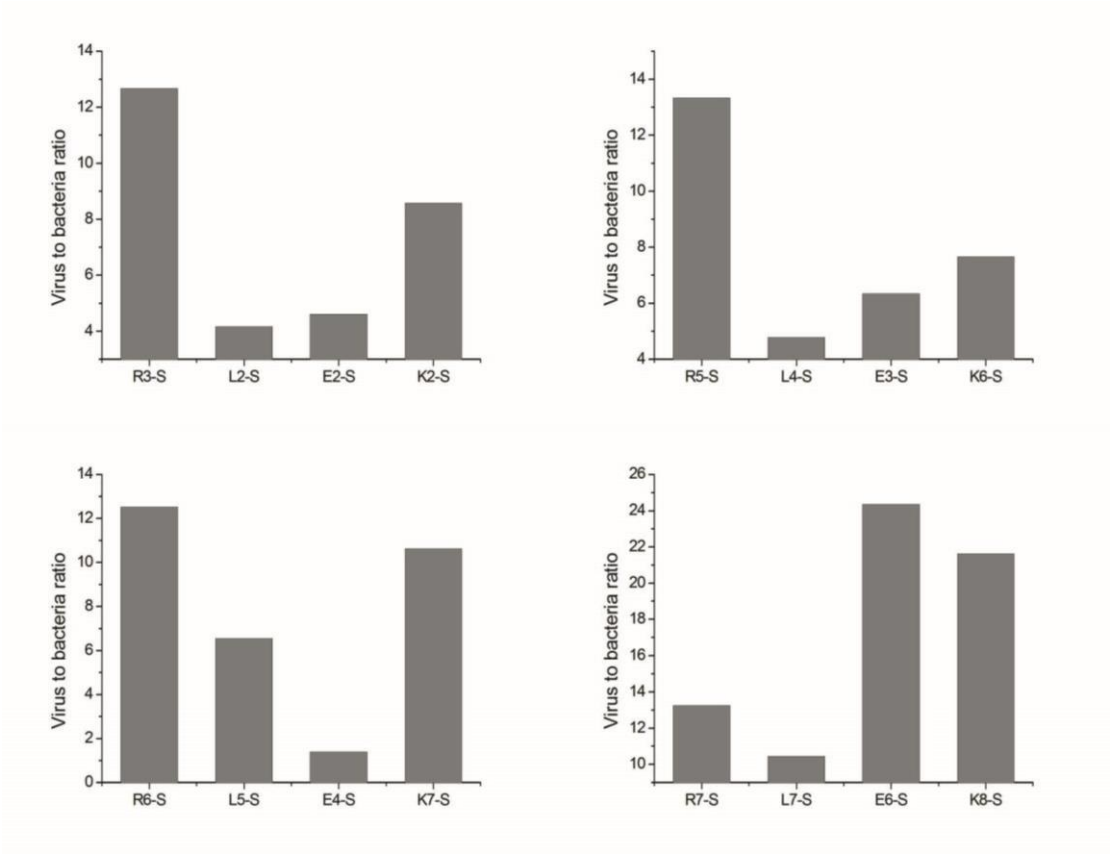
A



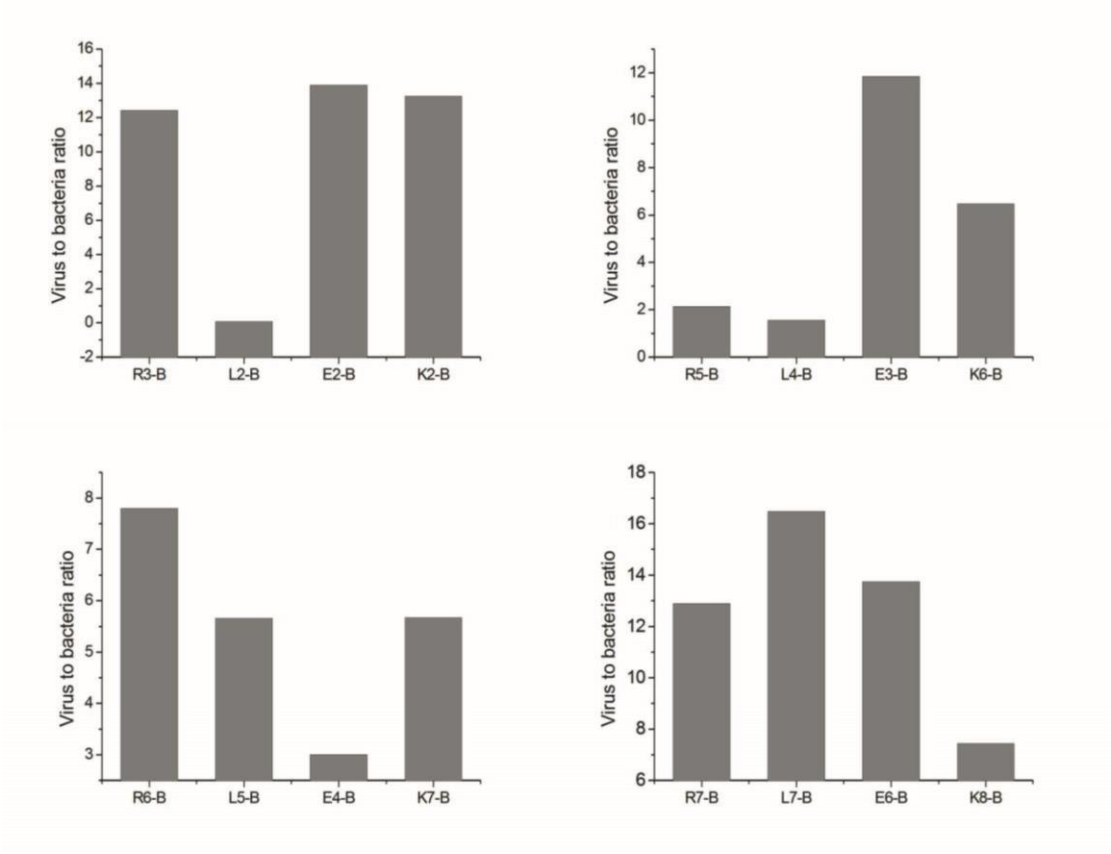
B

Figure 6. Total bacteria abundance and the groups of individual bacteria populations in surface water (K-S, E-S, L-S, R-S) and bottom water (K-B, E-B, L-B, R-B). A represent total bacteria

abundance in the surface water of station K, E, L, R, and B represent total bacteria abundance in the bottom water of station K, E, L, R.


Virus to bacteria ratio
R3-S
L2-S
E2-S
K2-S
R5-S
L4-S
E3-S
K6-S
R6-S
L5-S
E4-S
K7-S
R7-S
L7-S
E6-S
K8-S

A


Virus to bacteria ratio
R3-B
L2-B
E2-B
K2-B
R5-B
L4-B
E3-B
K6-B
R6-B
L5-B
E4-B
K7-B
R7-B
L7-B
E6-B
K8-B

B

Figure 7. Data about virus to bacteria ratio (VBR) were presented. A, The surface value of VBR in each stations of four transects. B, The bottom value of VBR in each stations of four transects.

CHAPTER IV COUPLING VIRIO- AND BACTERIOPLANKTON POPULATIONS TO RELATIONSHIPS WITH ENVIRONMENTAL CHANGES IN THE BOHAI SEA

ABSTRACT

Uncovering which environmental factors harbor significant fractions in governing microbial community are still key questions in coastal marine system. To test the interactions between environmental factors and distributions of virio- and bacterioplankton in trophic coastal area, we used flow cytometry to investigate the abundance of virio- and bacterioplankton covering 31 stations in the Bohai Sea of China. Our results suggested that the average value of total virus abundance (TV) (~2.29×10^8 particles/mL) in the surface water of winter were slightly lower than the average TV (~3.83×10^8 particles/mL) in summer. And the mean total bacteria abundance (TB) followed the trend observed in total virus abundance presented a much lower abundance (~2.54×10^7 particles/mL) in winter than (~5.43×10^7 particles/mL) in summer. Correlation analysis via redundancy analysis (RDA) and network analysis between virioplankton, bacterioplankton and environmental factors revealed a dependency between the abundances of viral and bacterial subpopulations and environmental factors. In winter, only temperature influenced the abundances of virio- and bacterioplankton significantly. While in summer, in addition to the effect of temperature, both salinity and nutrient (SiO_2) had remarkable impact on the distribution of virio- and bacterioplankton. This relative contribution showed a clear seasonal and trophic pattern throughout the whole water system. Overall, we presented a distinct network correlations of virio- and bacterioplankton in the euphotic layer of Bohai Sea, which revealed that warming and nutrient enrichment may play paramount role in the microbial distribution pattern.

INTRODUCTION

Microbial processes in marine environment has become increasingly obvious during these years. As the important decomposer of organic matters, bacterioplankton can transfer the dissolved organic matter into particle state, and hence act as a key role in cycling nutrients and remineralizing organic matters (Azam *et al*., 1983; Azam, 1998). Similarly, virioplankton are major players in controlling the microbial food web and in affecting the dissolved organic matter release via changing the virus-host interactions (Fuhrman, 1999; Suttle, 2005; Guidi *et al*., 2016). They controlled approximately 10-60% mortality of the bacterioplankton (Bettarel *et al*., 2004; Fischer and Velimirov, 2002; Jacquet *et al*., 2005), and constituted the most abundant biological particles in the sea. Virio- and bacterioplankton are found small but abundant components in the surface

water of ocean (Azam and Malfatti, 2007). It is reported that the typical bacterioplankton abundances are approximately 10^5-10^7 particles ml^{-1}, while the virioplankton abundances present the same patterns with bacterioplankton which are typically 10^7-10^9 particles ml^{-1} in the surface water (about 5-25times bacterial abundance) (Buitenhuis *et al*., 2012; Wommack *et al*., 2000). The abundance of bacterioplankton and virioplankton had been found large in many locations of marine environment (nearshore and offshore, tropical and polar, ice and sediment sea water), which awakened interest of scientists in this topic.

In view of the ubiquitous distributions and important roles in the marine ecology systems, virioplankton and bacterioplankton had been studied in a wide range of aquatic ecosystems in the past few years (Jiao *et al*., 2005; Liang *et al*., 2014; Pan *et al*., 2007). By using flow cytometry to analyze populations of picoplankton, we found that the populations of virioplankton is huge in aquatic systems which mainly includes bacteriophage and cyanophages two types of virioplankton. They usually infected the bacteria and algae by the process of cell lysis and cell destruction which led to the death of bacterioplankton and phytoplankton (Fuhrman, 1999). So viruses are in a specific position to influence the abundance and community of bacterioplankton and phytoplankton (Liu *et al*., 2006). While heterotrophic bacteria have often been grouped into two distinct categories which includes high nucleic acid (HDNA) and low nucleic acid (LDNA) (Longnecker *et al*., 2006; Bouvier *et al*., 2007). At present, there is still a big controversy over whether the LDNA is active or dead. Some studies supported that there are positive correlations between cell activities and nucleic acid contents. So it is generally accepted that HDNA cells are active while LDNA cells are dead (Gomes *et al*., 2015). But with the deepening of the research, people found that LDNA are not only active, but also it has important ecological significance (Gozdereliler *et al*., 2013). Bouvier (Bouvier *et al*., 2011) thought that LDNA cells had much higher capacity in avoiding virus infection than HDNA cells, it is also demonstrated that LDNA can cope with the adverse environment by using dormancy strategies, more specifically, in oligotrophic systems, the proportion of this kind of cells can reach 40% (Roesel *et al*., 2012). The composition of this kind of populations is thought to be important in controlling biological mechanisms and play essential roles in biogeochemical cycle. However, despite of the soaring awareness of the effects that virus and heterotrophic bacteria act in the microbial dynamics and biogeochemical cycles (Arrigo, 2005), there is still an exceeding lack of information on the relationships between viruses, bacterial populations about HDNA cells and LDNA cells and environmental factors.

As an important sea area in the north of China, Bohai Sea provides rich biological resources such as fishery, transportation and mineral products, all of them bring great convenience to people's daily lives. Although resources in Bohai Sea is very abundant, but the Bohai Sea is one of the largest ocean in the world which takes great ecological pressure. It is crucial for maintaining a sustainable fishery and marine biodiversity in consideration of ecological environment of Bohai Sea. However, the impacts of this environmental activities on the microbial food webs in Bohai Sea are

still not be fully evaluated. In this study, we characterized the distributions of different virus and heterotrophic bacteria groups in winter 2013 and summer 2014 of the Bohai Sea to enforce our understanding of the relationships between community structures and trophic coastal sea environment. From the abundance and relationships among virioplankton, bacterioplankton and environmental factors, we assess the elements that modulate viral and bacterial dynamics in relation to trophic conditions in the pelagic layer of Bohai Sea.

MATERIALS AND METHODS

Study area

Water samples were collected from surface water of Bohai Sea in December 2013 (Winter) and August 2014 (Summer) (Figure 1). Bohai Sea is a shallow semi-closed continental sea which is the main largest sea in the north of China. It includes three bays, namely Bohai Bay, Liaodong Bay and Laizhou Bay (Gao *et al*., 2014). The Bohai Sea connects with the Yellow Sea through Bohai Strait. The acreage of the Bohai Sea is about 7.7×10^4 km^2, and there are nearly 40 rivers flow from inland to the Bohai Sea. The average depth of Bohai Sea is about 18m with the deepest area is approximately 70m. In the Bohai Sea, water hydrology are mainly controlled by semidiurnal and diurnal tides, which takes up about 60% of the current variation and kinetic energy there (Chen *et al*., 2003). The influence of input from the open sea are mainly caused by the Yellow Sea Warm Current in winter and the Yellow Sea Cold Water Mass in summer which bring great impacts on the Bohai Sea physical and chemical environment (Ma *et al*., 2006; Xin *et al*., 2015).

Sampling

Water samples were collected from 31 stations of Bohai Sea by using a Niskin bottle during an open cruise of Xiang Yang Hong in December 2013 and August 2014. Surface water was filtered with nylon membrane and then transferred into 2 ml cryovials. Samples were immediately fixed with glutaraldehyde (the final concentration is 5%) for virus and bacteria counting which were first kept at 4°C for 15 min, and then transferred them to liquid nitrogen for instant freeze. The samples were stored at -80°C once coming back to the laboratory and analyzed samples within one month after collection (Brussaard *et al*., 2004; Wang *et al*., 2016). Temperature and salinity of the surface water were recorded by using a Conductivity Temperature-Depth equipment (SBE 25 Sea logger; Sea-Bird Electronic Inc., USA) (Figure 2). The dissolved inorganic nutrients such as (NO_3-N, NO_2-N, NH_4-N, PO_4-P and SiO_2) in surface water were measured using a nutrient auto-analyzer (AA3; Seal Analytical Ltd., UK) as previously introduced (Yu *et al*., 2016) (Figure 3).

Virio-and Bacterioplankton counting using flow cytometry

Virus and heterotrophic bacteria abundances were determined by using flow cytometry as method described previously (Seymour *et al*., 2005b; Paterson *et al*., 2013). Samples were defrosted and

diluted with TE buffer (filtered with 0.22μm membrane, 10 mM Tris, 1 mM EDTA) according to the scale of 1:10, and then stained with SYBR Green I (with final concentration of 1:500 Stock, molecular probes). The processed samples were incubated at 80°C for 10 minutes in the dark environment to optimize the counting method (Marie *et al*., 1997; Schapira *et al*., 2009). Fully vortexed beads were added at the final concentration of about 10^5 particles ml^{-1} as references to normalize the fluorescence and value.

We performed the experiments on a flow cytometry (BD Accuri C6, Becton-Dickinson, San Jose, CA, USA). We used phosphate buffered saline (PBS) solution as running sheath fluid, to evaluate the nucleic acid content in cells, each sample was recorded with forward-angle light scatter (FSC), side-angle light scatter (SSC) and green (SYBR-I) fluorescence. Bacterial populations were then divided into three groups (HDNA1 cells, HDNA2 cells and LDNA cells) according to the intensity of fluorescence (Li *et al*., 1995) (Figure 4). Viral populations were enumerated and classified into high fluorescence VLP1 and low fluorescence VLP2 (Larsen *et al*., 2008) (Figure 4). The total bacteria abundance was computed as HDNA1 + HDNA2 + LDNA, and the total viral abundance was calculated as VLP1 + VLP2, the populations of viral and bacterial abundance were shown in Figure 5 and Figure 6.

Statistical analysis

Surfer 8.0 (Golden software, Inc.) was used to create plots about the spatial distributions of viral and bacterial abundance. SPSS 13.0 (SPSS inc., Chicago, USA) software was used to analyze variance and independent t-test. When it is necessary data were processed using a logarithmic transformation to meet the requirements of the normality assumptions in the least-squares regression analysis. Redundancy analysis (RDA) and spearman correlation analysis were utilized to investigate the relationships between virioplankton, bacterioplankton and environmental factors in the surface water of Bohai Sea. This process was completed by using Canoco 4.5 software (Microcomputer Power, Ithaca, USA). Cytoscape 3.2.1 was used to analyze the network interactions between microbial community populations and environmental factors. In the pelagic environment of Bohai Sea, total viral abundance (TV), total bacterial abundance (TB), VLP1, VLP2, LDNA, HDNA1 and HDNA2 were created as factors analysis. Environmental variables which include temperature, salinity, concentrations of NO_3-N, NO_2-N, NH_4-N, PO_4-P and SiO_2 were used as variation assessment. All variations were logarithmically transformed and processed by Monte-Carlo test before RDA analysis to reduce the extreme values on ordination scores and to normal data distribution.

RESULTS

Distributions of environmental factors

A total of 62 samples from 31 stations were collected from surface water of Bohai Sea in winter 2013 and in summer 2014. The maximum, minimum, median, mean and standard deviation (SD) of the measured environmental variables from winter and summer were presented in Table 1. Surface water temperature in winter ranged from 3.2°C-9.95°C (Figure 2, Table 1), it presented an obviously decreasing trend from the east to the west of Bohai Sea. While in summer the highest temperature is 26.26°C, and the lowest temperature is 19.71°C, we found that the temperature is lowest in the north side of Bohai Strait through the total study area (Figure 2, Table 1). Comparing with temperature, the range of salinity in winter and in summer varied not greatly (Maximum and minimum salinity in winter is 32.95‰ and 29.11‰ respectively; Maximum and minimum salinity in summer is 31.31‰ and 29.32‰ respectively) (Figure 2, Table 1). The results showed that the high salinity area in winter and summer located near the north and east of the Bohai Strait.

The distributions of the dissolved organic matter (NO_3-N, NO_2-N, NH_4-N, PO_4-P and SiO_2) in winter and summer of Bohai Sea was shown in Figure 3. It exhibited that the concentrations of nutrients in stations located in the middle of Bohai Sea were much higher than the other areas. The high concentration distributions of NO_2-N, NH_4-N and PO_4-P was consistently observed in summer. But the concentrations of NO_3-N and SiO_2 appeared high concentration distributions in the west of Bohai Sea (Figure 3). In winter and summer, there are great discrepancy in the distribution of dissolved nutrient factors.

Distributions of virioplankton

In winter 2013, total viral abundance ranged from 1.87×10^7 particles ml^{-1} to 1.10×10^9 particles ml^{-1} at the surface water. And in summer 2014, total viral abundance of the surface water ranged from 1.06×10^6 particles ml^{-1} to 2.54×10^9 particles ml^{-1}. The average measured abundance of total virus in winter and summer is 2.29×10^8 particles ml^{-1} and 3.83×10^8 particles ml^{-1} respectively (Figure 5). In view of the average viral abundance, there was no significant difference between the two seasons include winter and summer. But for the distribution, high abundance area appeared in the east of Bohai Sea in winter and middle of the Bohai Strait in summer.

In regard to two viral populations (Figure 4, Figure 6, Figure 7A), VLP1 and VLP2 were observed to present at all stations of winter and summer in the Bohai Sea. The average abundances of VLP1 in winter were much lower than the abundances in summer. Along all stations of surface water in the Bohai Sea, the abundance of VLP1 and VLP2 in winter ranged from 1.27×10^7 particles ml^{-1} to 6.23×10^8 particles ml^{-1} and 5.10×10^6 particles ml^{-1} to 4.84×10^8 particles ml^{-1} respectively. While in summer the abundance of VLP1 and VLP2 ranged from 2.26×10^5 particles ml^{-1} to 1.87×10^9 particles ml^{-1} and 8.02×10^5 particles ml^{-1} to 6.67×10^8 particles ml^{-1}.

VLP1 dominated the composition of total viral community and represented up to 52% of total abundance in surface water of winter (Figure 7A, Figure 7C). The highest abundance of VLP1 in

winter was observed at stations of L2, K6 and R7. While in summer VLP1 dominated the composition of total viral community up to about 73%. The highest abundance of VLP1 appeared at the stations of E3, E4 and L5 (Figure 6).

Distributions of bacterioplankton

The total bacterial abundance ranged from 1.02×10^{6} particles ml^{-1} to 1.38×10^{8} particles ml^{-1} in winter and ranged from 6.74×10^{5} particles ml^{-1} to 4.24×10^{8} particles ml^{-1} in summer. The average abundance of bacteria in winter and summer were 2.54×10^{7} particles ml^{-1} and 5.43×10^{7} particles ml^{-1} respectively. The total bacterial abundance of summer was apparently much higher than the abundance of winter. Meanwhile, high abundance area could be found at the stations of K2 and L2 in winter. And in summer, there were significant increase in the abundance of stations such as E6 and N5 (Figure 5).

Bacterial community can be divided into three groups which included one low LDNA population and two high HDNA population (HDNA1 and HDNA2) by observing the whole stations (Figure 4, Figure 7B). The highest abundance of LDNA was found in the station of K6 in winter, and N5 in summer. In the surface water of Bohai Sea, the total abundance of bacteria in summer were much higher than it in winter. The abundance of HDNA2 accounted for the smallest portion both in winter and summer, but it presented an significant increase in summer which resulted in a high abundance of the total bacteria (Figure 7B). It is because of the increase of HDNA2 population which cause the total abundance of bacteria in summer get much higher than it in winter, which makes the average virus to bacteria ratio (VBR) much higher in winter (13.72) than in summer (5.72) (Figure 8).

To analyze the bacterial community composition, we concluded that the average abundance of HDNA1 in winter accounted for 48% in the abundance of total bacteria, and in summer this proportion is approximately 38% (Figure 7C). In the surface water of Bohai Sea, whether in winter or in summer the abundance of LDNA is the most abundant community composition (Figure 7B, Figure 7C).

Relationships among virioplankton, bacterioplankton and environmental factors

We used RDA analysis and network correlation analysis to assess the relationships between virioplankton populations, bacterioplankton populations and environmental factors in winter and summer of Bohai Sea. According to the results of RDA analysis, it was obvious that stations near the east part of Bohai Sea presented a close relationship with temperature in winter and transects near the Bohai Strait had a close connection with salinity in summer. There was a significant positive relationships between temperature and LDNA, HDNA1, HDNA2, total bacteria in winter (Figure 9A, Figure 10). Mean while in summer, there was a positive relationship between salinity,

and VLP1, VLP2, total virus and VBR, also there was a significant negative relationship between salinity, SiO_2 and LDNA, HDNA1, HDNA2 and total bacteria (Figure 9B, Figure 10).

DISCUSSION

Virio-and bacterioplankton play a key role in the process of nutrients uptake, export, transformation and biogeochemical cycle in the total water ecosystem (DeLong *et al*., 2005; Aristegui *et al*., 2009). While little research was conducted by using network analysis on the topic about relationships among virus, bacteria populations, host-virus interactions and environmental factors in the Bohai Sea. To figure out this problem, here we performed the survey related to the distribution of virio- and bacterioplankton populations, and relationships with environmental factors in the pelagic system of Bohai Sea.

Bohai Sea is a shallow semi-closed sea and generally constrained by the coastal river discharge, semidiurnal and diurnal tides and input from the open sea (Wang *et al*., 2008; Xu *et al*., 2009; Lie, 1984). This region has gradually become the hot spot that people study the dynamic relationships between environmental factors and picoplankton (Wang *et al*., 2016). As it is simultaneously affected by the coastal terraferma and the open sea. Our studies showed that nutrient environmental factors are indeed important factors that control the abundance of virio-and bacterioplankton, but in addition to this factors, seasonal patterns, interactions between virus and bacteria and different ecological niches determine its specific distributions.

Distributions of virio-and bacterioplankton populations and relationships with environmental factors

It had been shown that the dissolved organic matters can significantly stimulate the abundance of bacterioplankton (Nagata *et al*., 2000; Danovaro *et al*., 1998) and indirectly impact the populations of virus. As it was depicted in the previous studies, there were high abundances of virus populations and bacteria populations near the Liaodong peninsula where the dissolved organic nutrients such as the concentration of NO_3, NO_2, NH_3, PO_4-P and SiO_2 were very high due to the discharge of nearshore pollutants (Bai *et al*., 2012). It was also found that phosphorus was the main limiting factor that can control the growth of picoplankton in mediterranean sea. Bacterioplankton abundance and the cell activity were significantly increased when the concentration of PO_4-P in the water went up. So they believed that nutrients were indispensable for the growth of bacterioplankton (Lasternas *et al*., 2010). However, in our study virus and bacteria populations did not show particularly obvious relationships with nutrients in winter. All of communities include total virus, total bacteria, VLP1, LDNA, HDNA1 and HDNA2 showed a positive relationships with temperature. And VBR showed a significant negative correlation with temperature. We found that temperature is the main factor that impact the abundances of virio- and bacterioplankton in winter. While in summer, we found that abundance of virio-and

bacterioplankton were also rarely affected by the nutrients and only showed a negative correlation with concentrations of SiO_2. Among them, the abundance of total virus, total bacteria, VLP1, VLP2 and HDNA2 were negatively correlated to temperature. And there was only a significant positive correlation between VBR and salinity. As we known river inputs and coastal currents can change the environmental factors of marginal sea and indirectly influence the abundance of picoplankton (Katano *et al*., 2008). Also considering the hydrologic characteristics of Bohai Sea, the input from the open sea bring the warm and salinity water in winter and cause cold water in the east of Bohai Sea in summer, which will bring big changes in the hydrology environment of Bohai Sea (Li *et al*., 2006). So it might be one of the most possible factors that affect the distributions of virio-and bacterioplankton. With the rapid development of coastal industry, a large amount of pollutants were dumped into Bohai Sea every year, which lead to the eutrophication of Bohai Sea (Zhang *et al*., 2006; Li *et al*., 1996). All of these are the reasons for the high abundance of virus and bacteria in the Bohai Sea. We suspected that in addition to the synergetic effects of nutrient enrichment and warming, river input and coastal current might also be responsible for the specific distributions of virus and bacteria.

Correlation between virus and bacteria

Virus to bacteria ratio (VBR) usually has important significance in the theory about bacteriophage infection on the bacterial host community. Also high value of VBR was often related to the high productivity and nutrient-rich water areas (Wommack and Colwell, 2000; Weinbauer *et al*., 2004). In our study, the VBR value ranged from 1.39 to 42 in winter and ranged from 0.81 to 24.24 in summer. The average VBR value in winter and summer are 13.72 and 5.72 respectively. This concluded that viruses played an indispensible role in water ecosystem and there are extremely close connections between viruses and their host organisms. Previous studies suggested that if viruses stayed in a lysogenic condition for a long time before entering the lytic cycle which would cause a large wide range of VBR values (Jiang and Paul, 1994). Also it reported that viruses come from different stages of infection were released from the host bacteria cells at the same time can cause the VBR value increased greatly (Siokou-Frangou *et al*., 2010). Viruses can stimulate the growth of bacteria and there are close relationships between virus and bacteria which were convicted in many studies (Ruth-Anne *et al*., 2009; Bongiorni *et al*., 2005; Stroinov *et al*., 2011; Vrede *et al*., 2003). Now in our studies we also found that the abundance of viruses was positively related to the bacterial abundance. In the surface water of Bohai Sea, VLP1 (bacteriophages) accounted for a large portion in the community of virus which indicated that they are important components in controlling the abundance of virus.

CONCLUSION

Our studies suggested that the distributions of virio-and bacterioplankton in the surface water of trophic Bohai Sea were influenced by the nutrients in a certain extent. It was worth nothing that

VBR value had a significant relationships with environmental factors whatever it was in winter or in summer, supporting the viewpoint that the virus-bacteria interactions can make a quickly response to the environmental changes which may be a key factor in controlling the abundance of virio-and bacterioplankton indirectly. Additionally, our data showed a geographic distribution of the virio-and bacterioplankton which indicated that each populations of virus and bacteria communities have its independent niches, so to learn more about the roles they played in the ecological environment and biogeochemical cycles in the Bohai Sea, further researches about metagenomics are needed to be done.

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Table 1 Summary of environmental parameters from surface water of Bohai Sea in winter 2013 and summer 2014.

Season	**Tem (°C)**	**Sal (‰)**	**NO_2-N (μg/L)**	**NO_3-N (μg/L)**	**NH_4-N (μg/L)**	**PO_4-P (μg/L)**	**SiO_2 (μg/L)**
Winter							
Max	9.95	33.15	37.1	669.0	166.9	69.2	2.46
Min	3.20	29.11	1.7	1.0	10.3	0.3	0.14
Medium	6.27	31.18	6.9	159.8	60.7	3.1	0.45
Mean	6.69	31.19	7.6	224.8	68.7	8.2	0.67
SD	2.01	0.89	6.5	195.0	40.8	15.3	0.65
Summer							
Max	26.26	31.31	38.16	201.5	70.8	9.3	0.62
Min	19.71	28.93	0.27	1.7	0.5	0.67	0.06
Medium	24.92	30.45	1.20	19.83	10.8	2.5	0.21
Mean	24.16	30.29	5.81	40.33	15.87	3.20	0.26
SD	1.75	0.53	9.41	47.57	13.98	2.07	0.15

Table 2 Pearson correlation coefficients between total viral abundance (TV), total heterotrophic bacterial abundance (TB), bacteriophage, VLP1 (V1), cyanophages, VLP2 (V2), LDNA(B1), HDNA1(B2), HDNA2(B3) and environmental parameters in winter of Bohai Sea. *P < 0.05, **P < 0.01. Tem: Temperature, Sal: Salinity.

	TV	**TB**	**Tem**	**Sal**	**NO_2-N**	**NO_3-N**	**NH_4-N**	**PO_4-P**	**SiO_2**
TV	1.000	0.889**	0.332	0.394*	0.142	-0.262	-0.166	-0.077	-0.143
TB	0.889**	1.000	0.506**	0.314	0.213	-0.328	-0.191	-0.078	-0.196
V1	0.989**	0.866**	0.329	0.406*	0.109	-0.270	-0.155	-0.071	-0.150
V2	0.987**	0.893**	0.328	0.372*	0.174	-0.247	-0.173	-0.082	-0.131
B1	0.905**	0.983**	0.445*	0.348	0.234	-0.286	-0.133	-0.098	-0.160
B2	0.858**	0.986**	0.537**	0.272	0.198	-0.348	-0.240	-0.060	-0.213
B3	0.829**	0.985**	0.565**	0.284	0.161	-0.375*	-0.221	-0.052	-0.248
VBR	-0.215	-0.368*	-0.636**	-0.385*	-0.205	0.387*	0.001	-0.113	0.214

Table 3 Pearson correlation coefficients between total viral abundance (TV), total heterotrophic bacterial abundance (TB), bacteriophage, VLP1 (V1), cyanophages, VLP2 (V2), LDNA(B1), HDNA1(B2), HDNA2(B3) and environmental parameters in summer of Bohai Sea. *P < 0.05, **P < 0.01. Tem: Temperature, Sal: Salinity.

	TV	**TB**	**Tem**	**Sal**	**NO_2-N**	**NO_3-N**	**NH_4-N**	**PO_4-P**	**SiO_2**
TV	1.000	0.873**	-0.393*	0.245	-0.204	-0.223	-0.102	-0.170	-0.482**
TB	0.873**	1.000	-0.403*	0.219	-0.181	-0.167	-0.068	-0.235	-0.455*
V1	0.999**	0.854**	-0.388*	0.239	-0.198	-0.222	-0.104	-0.162	-0.476**
V2	0.992**	0.916**	-0.404*	0.258	-0.218	-0.225	-0.096	-0.191	-0.492**
B1	0.859**	0.983**	-0.332	0.185	-0.154	-0.124	-0.030	-0.216	-0.440*
B2	0.843**	0.992**	-0.357*	0.202	-0.183	-0.150	-0.061	-0.235	-0.442*
B3	0.832**	0.921**	-0.517**	0.263	-0.194	-0.231	-0.121	-0.235	-0.442*
VBR	0.404*	0.201	-0.145	0.511**	-0.124	-0.221	-0.257	-0.284	-0.503**

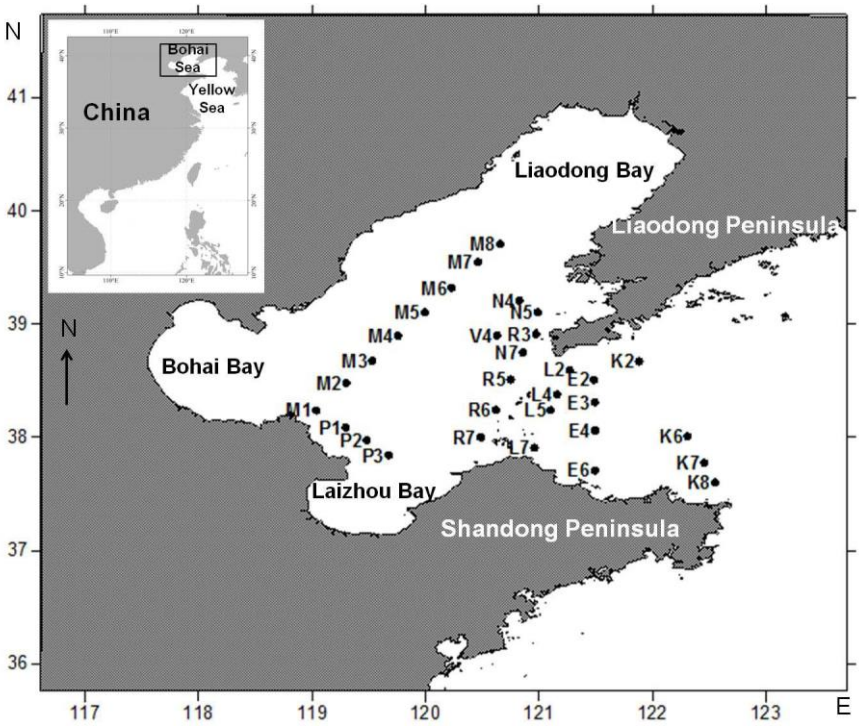


Figure 1 Sampling stations of winter 2013 and summer 2014 in the Bohai Sea study areas.

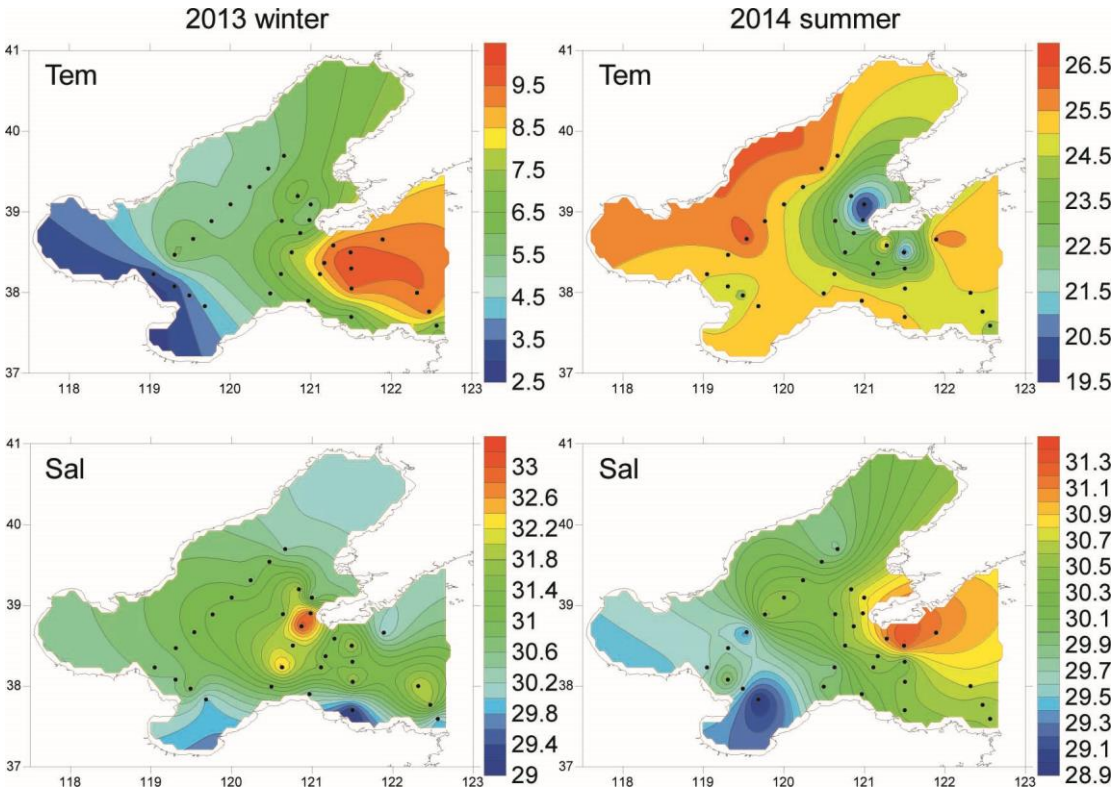


Figure 2 Distributions of temperature (Tem) and salinity (Sal) in the surface water of Bohai Sea (winter 2013 and summer 2014).

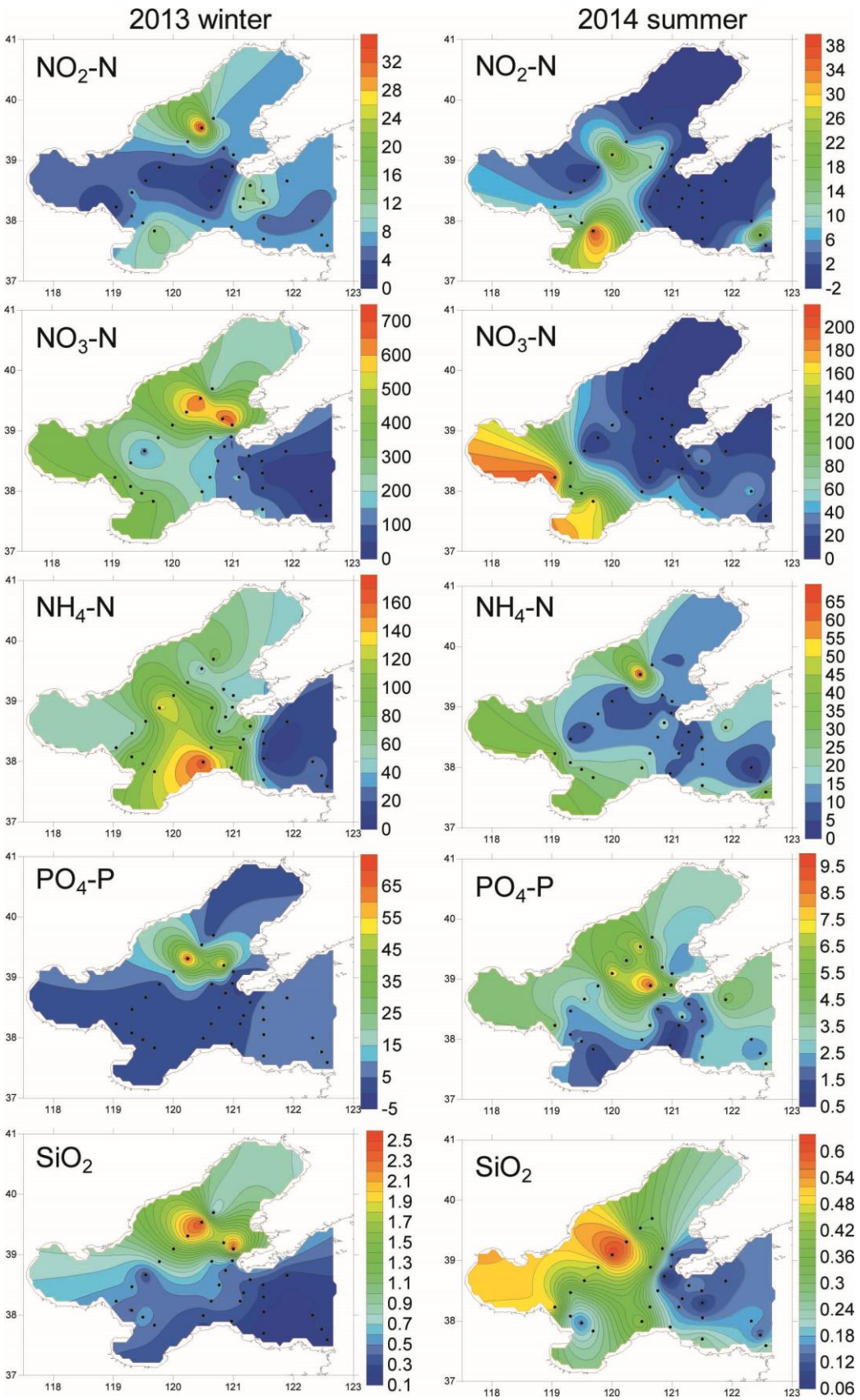


Figure 3 Distributions of nutrient factors along the transects in the Bohai Sea.

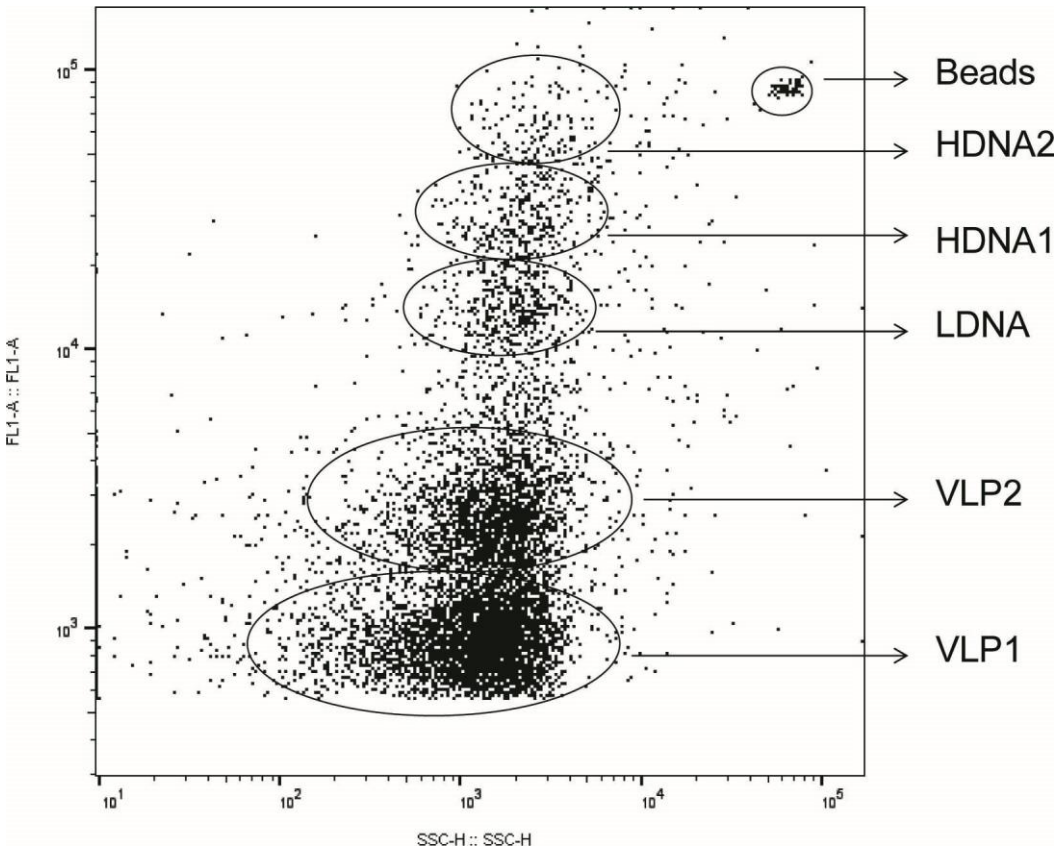


Figure 4 Archetypical cytogram was used to describe the community of viral and bacterial abundance.

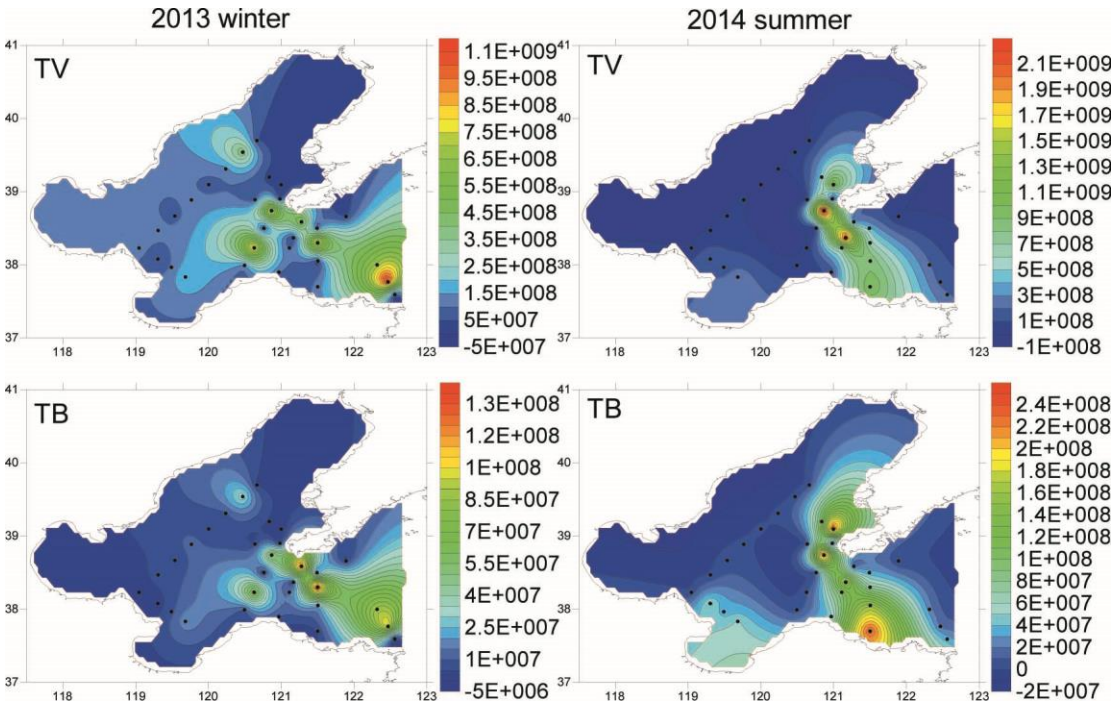


Figure 5 Total virus and total bacteria abundance in the surface water of Bohai Sea (winter 2013 and summer 2014).

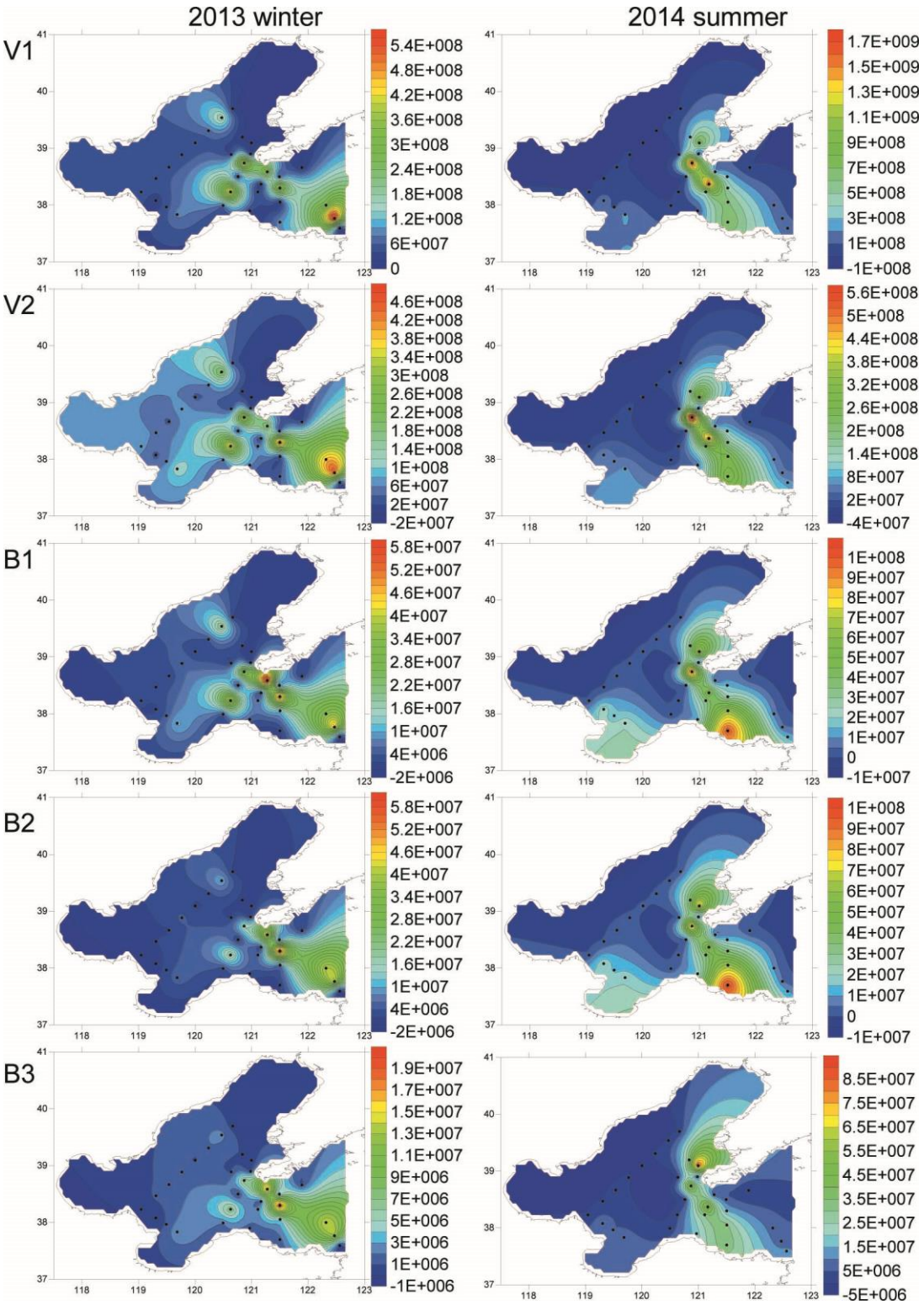
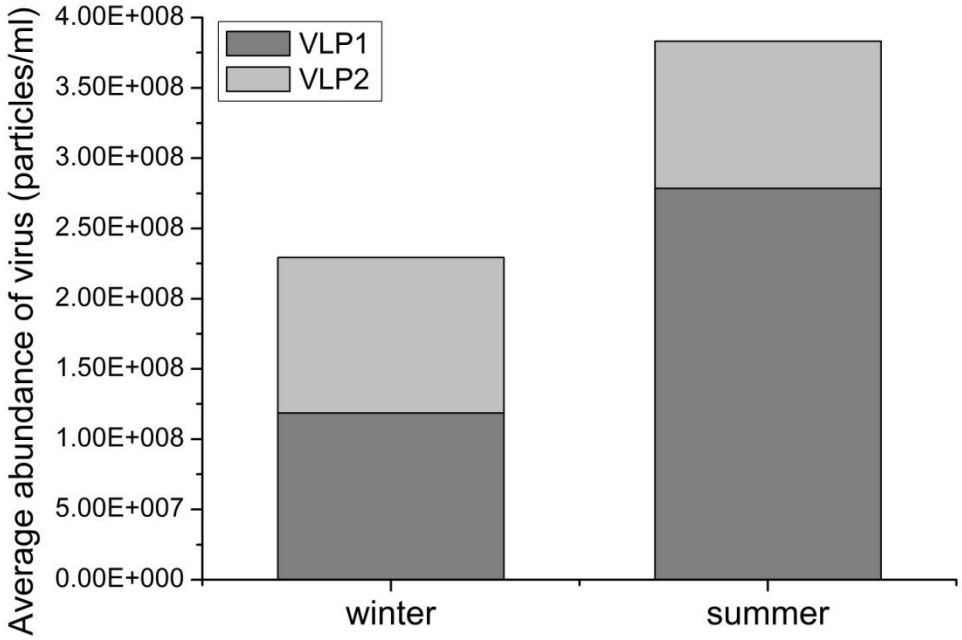
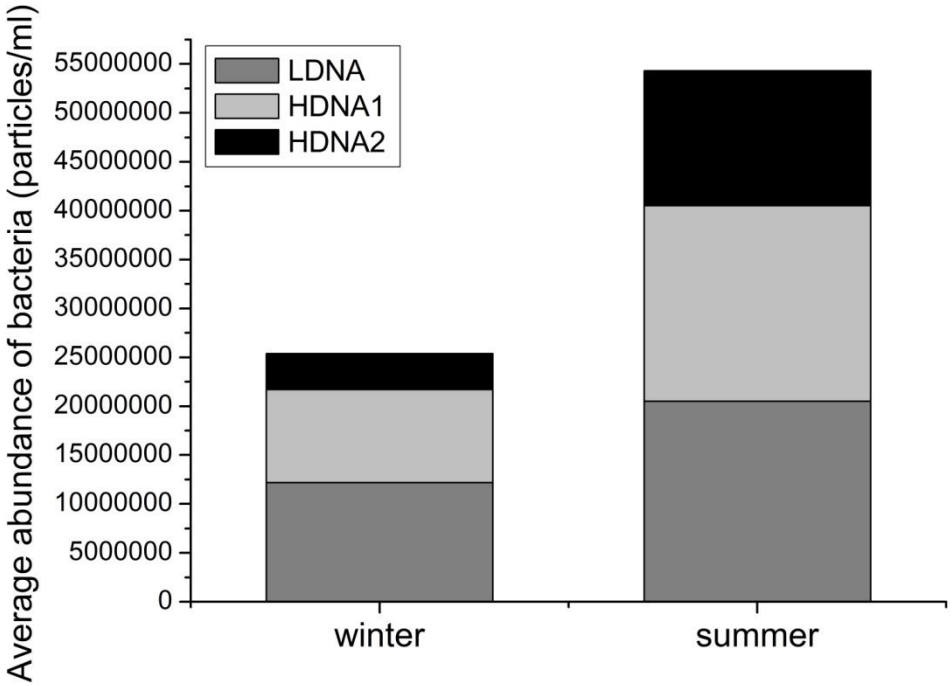


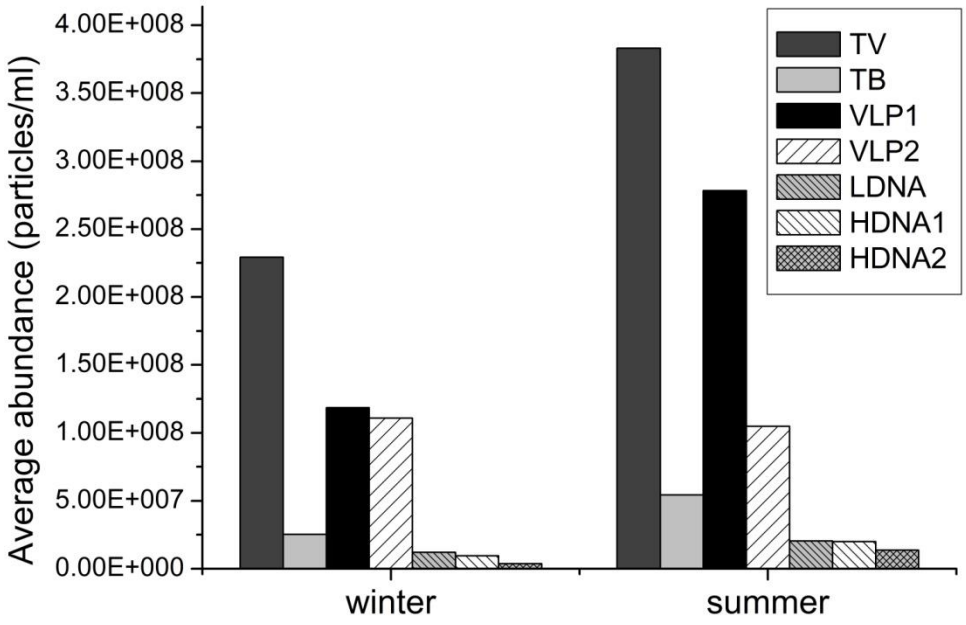
Figure 6 Community abundances of virus and bacteria in the surface water of Bohai Sea. (V1: bacteriophage, VLP1; V2: cyanophages, VLP2; B1: LDNA; B2: HDNA1; B3: HDNA2)


Average abundance of virus (particles/ml)
4.00E+008
3.50E+008
3.00E+008
2.50E+008
2.00E+008
1.50E+008
1.00E+008
5.00E+007
0.00E+000
VLP1
VLP2
winter
summer

A


Average abundance of bacteria (particles/ml)
55000000
50000000
45000000
40000000
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
LDNA
HDNA1
HDNA2
winter
summer

B



C

Figure 7 Average abundance of VLP1 and VLP2 in winter and summer (Figure 7A). Average abundance of LDNA, HDNA1 and HDNA2 in winter and summer (Figure 7B). Histogram was used to describe the average abundance of VLP1, VLP2, LDNA, HDNA1, HDNA2, TV and TB in winter and summer (Figure 7C). (TV: Total virus abundance, TB: Total bacteria abundance)

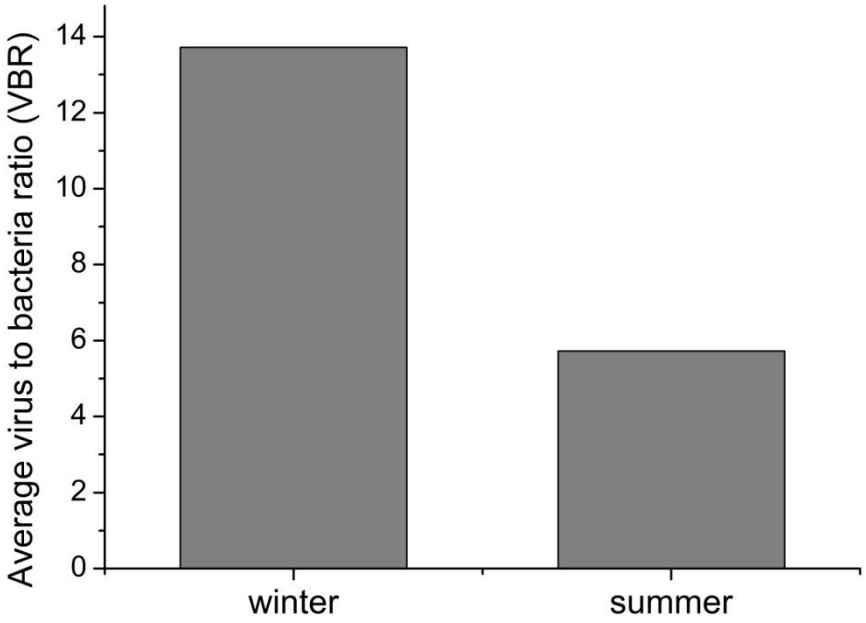
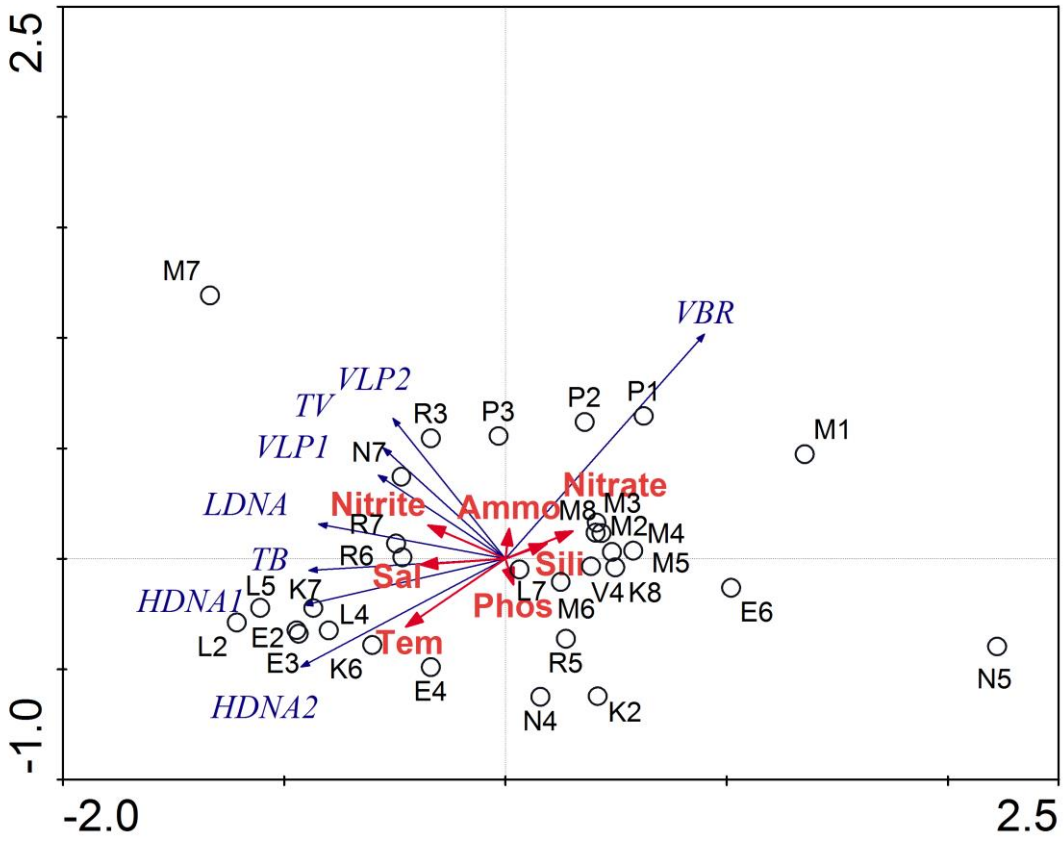
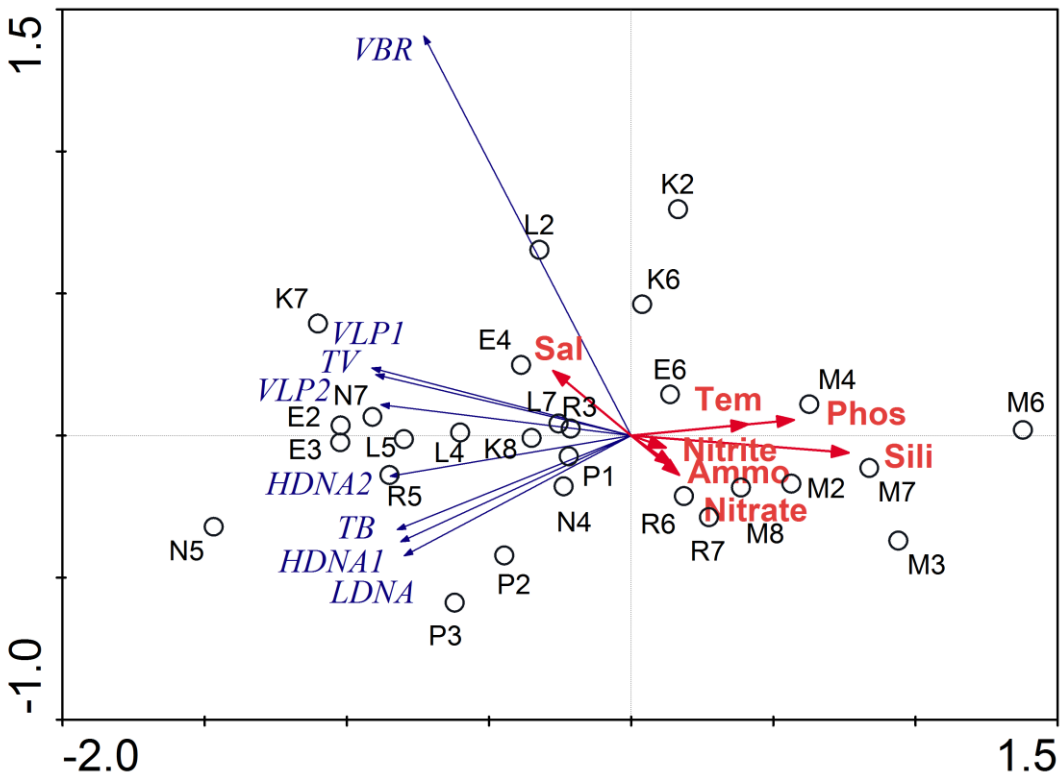


Figure 8 The average VBR in winter and summer. (VBR: virus to bacteria ratio)



A



B

Figure 9 RDA analyze of the viral, bacterial groups and environmental factors in the surface water of Bohai Sea. (Figure 9A: winter in 2013, Figure 9B: summer in 2014) (VBR: virus to bacteria ratio, Tem: Temperature, Sal: Salinity, Nitrite: NO_2-N, Nitrate: NO_3-N, Ammo: NH_4-N, Phos: PO_4-P, Sili: SiO_2), "○" represent stations in the Bohai Sea.

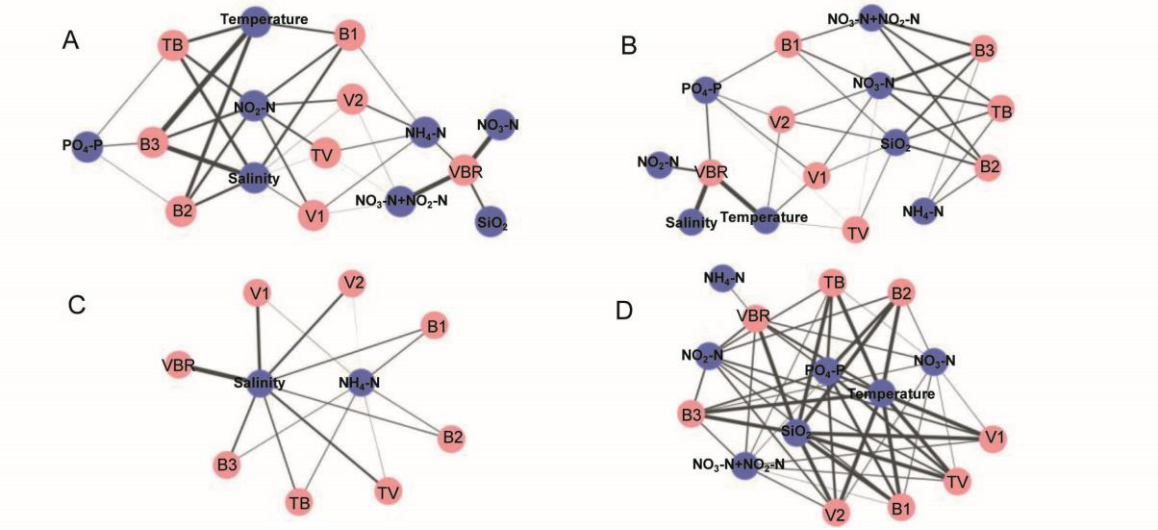


Figure 10 Network analysis for the correlation between total viral abundance (TV), total bacteria abundance (TB), VLP1(V1), VLP2(V2), LDNA (B1), HDNA1(B2), HDNA2(B3) and environmental factors which include temperature, salinity, NO_3-N+NO_2-N, NO_2-N, NO_3-N, NH_4-N, PO_4-P and SiO_2. A and B represent the positive and negative relationships in winter, respectively. C and D represent the positive and negative relationships in summer, respectively. The blue nodes represent the environmental factors. Edge with width of 4 represent that there are significant correlations between different populations and environmental variables ($p<0.01$). Edge with width of 2 represent that there are significant correlations between different populations and environmental variables ($p<0.05$). Edge with width of 0.1 represent that there are no significant relationships between different populations and environmental variables.

CHAPTER V
DISCUSSION

Since 1980s, flow cytometry technology was introduced to the area of marine picophytoplankton by Yentsch and Chisholm (Yentsch and Horan, 1989; Chisholm *et al*., 1988), flow cytometry had been considered as an developed method for enumerating the heterotrophic bacteria and virioplankton. This has promoted the development of the marine picoplankton research. We can divide the virio- and bacterioplankton into different groups by the different fluorescence in the flow cytometry (Falcioni *et al*., 2008). Because of the characteristics of wide distribution, high abundance, strong metabolic activity and rapid reproduction, virio-and bacterioplankton have been considered as playing crucial roles in marine ecology system (Fuhrman *et al*., 1989; Zhang *et al*., 2007). So more and more attention has been focused on the study of marine virio-and bacterioplankton.

In our study, Bohai Sea is an semi-enclosed sea which locates in the north of China. It is an important spawning and fishing area which takes a lot of economic and ecological benefit to people around it. It will be of great significance to study the distribution and dynamic changes of virio-and bacterioplankton in the Bohai Sea for protecting the sustainable and healthy environment of Bohai Sea.

In this thesis (Chapter II), we surveyed the macroscale distribution of virioplankton and bacterioplankton in the Bohai Sea, we found that the distribution of these two microbes were highly related to the seasonal variation and nutrient availability. As we known coastal sea is easy to be influenced by the riverine input and open sea input because of its special location which are often charactered as high nutrients and eutrophic area (El-Swais *et al*., 2015). The horizontal distribution of virioplankton and bacterioplankton were observed in winter and summer of Bohai Sea. The abundance of virioplankton and bacterioplankton were seriously impacted by the temperature, salinity and nutrient concentration which was consistent with the results concluded from the research in Daya Bay. River input play a key role in controlling the nutrient condition of coastal sea which in turn have an important influence on the abundance of picoplankton and virioplankton (Ni *et al.,* 2015). The similar study was also conducted in the coastal area of Qingdao, it was concluded that the distribution of microbial populations had close relationship with the nutrient conditions (Wang *et al.,* 2010). For the investigation of the vertical distribution of virioplankton and bacterioplankton, it was found that the abundance in the bottom layer was much higher than the surface layer. According to the current analysis, this may be mainly related to the large accumulation of organic matters in the bottom water of winter, which accelerate the growth rate of virioplankton and bacterioplankton (Lin *et al*., 2014). Also, there is an warm and salinity current

from the Yellow Sea in winter which will take great disturbance to the Bohai Sea water (Xu *et al*., 2009). Then the abundance of virioplankton and bacterioplankton will be influenced greatly.

In the Chapter III, we investigated the flush effect of the Yellow Sea Warm Current (YSWC) on the microbial community of Bohai Sea in winter. According to the environmental analysis, it was concluded that there was almost 1°C warmer in the east side of Bohai Strait than in the west side because of the impact of the YSWC. As it was described in previous studies, temperature rise is the main reason for the dynamic change of plankton bloom (Sun, 2012). The YSWC transport warm and saline water to the Bohai Sea which will have a great impact on the microbial community of Bohai Sea. From the eastern to the western sites of the Bohai Strait, the abundance of virioplankton presented a significant decreasing trend. The YSWC transported warm and saline water into the Bohai Sea and formed a warm and saline tongue in winter (Ma *et al*., 2006). There will be a mixture between the warm Yellow Sea water and the cold Bohai Sea water, which will stimulate the growth of virioplankton, which in turn increases viral biomass. Bacteria also followed the trend of virioplankton in the Bohai Sea. We concluded that the YSWC has a major impact on the distribution of virioplankton and bacterioplankton across the Bohai Strait. It appears that the YSWC increase microbial abundance by increasing temperature, with a concomitant increase in salinity.

Coupling the relationships between virio-and bacterioplankton abundance and environmental changes, we conducted the survey of the distribution of virioplankton and bacterioplankton in the whole Bohai Sea (Chapter IV). According to our results, we found that the virio-and bacterioplankton abundance showed a positive relationship with temperature ($p<0.05$). The concentration of SiO_2 and salinity also play a key role in controlling the abundance of virio-and bacterioplankton. It was consistent with previous studies that the dissolved organic matters can significantly stimulate the abundance of bacterioplankton (Nagata *et al*., 2000; Danovaro *et al*., 1998). So they believed that nutrients were indispensible for the growth of virio-and bacterioplankton (Lasternas *et al*., 2010). Also considering the hydrologic characteristics of Bohai Sea, the input from the open sea will bring big changes in the hydrology environment of Bohai Sea (Li *et al*., 2006), which might be one of the most possible factors that affect the distributions of virio-and bacterioplankton. With the increase of human activity near the coastal area, a large amount of pollutants were dumped into Bohai Sea annually, which contributes a lot for the eutrophication of Bohai Sea (Zhang *et al*., 2006; Li *et al*., 1996). All of these lead to the increase of abundance of virus and bacteria in the Bohai Sea. We suspected that in addition to the synergetic effects of nutrient enrichment and warming, river input and coastal current might also be responsible for the specific distributions of virus and bacteria.

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Zhang Z, Zhu M, Wang Z and Wang J (2006) Monitoring and managing pollution load in Bohai Sea, PR China. Ocean & Coastal Management 49:706-716

Appendices

The following publication is manuscript the author contributed to during her candidature in which she wrote the article, developed techniques and concepts that contributed to her thesis.

Wang CX, Wang YB, Paterson JS, Mitchell JG, Hu XK, Zhang H and Sheng YQ (2016) Macroscale distribution of virioplankton and heterotrophic bacteria in the Bohai Sea. FEMS Microbiology Ecology 92:fw017