FEMS Microbiology Ecology Advance Access published January 31, 2016

Microscale distribution of virioplankton and heterotrophic bacteria in the Bohai Sea

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Abstract: In light of limited research in the relationship between the microscale 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 microscale distribution of these two microbes in the Bohai Sea is related to seasonal variation and nutrient availability.

Keywords: Virioplankton; Heterotrophic bacteria; Flow cytometry; Microscale distribution; Bohai Sea

1. 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.

2. Materials and methods

2.1 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].

2.2 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.

2.3 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).

3. Results

3.1 Virioplankton abundance

3.1.1 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).

3.1.2 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).

3.2 Heterotrophic bacterial abundance

3.2.1 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).

3.2.2 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).

3.3 Environmental parameters

3.3.1 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.

3.3.2 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).

4. Discussion

4.1 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].

4.2 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).

4.3 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.

5. 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.

Acknowledgements

This work was supported by the Strategic Priority Research Programme of Chinese Academic of Sciences [No. XDA1102040303], the National Basic Research Program of China (973 Program) granted No. 2015CB453300.

Conflict of interest. None declared.

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Tables

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

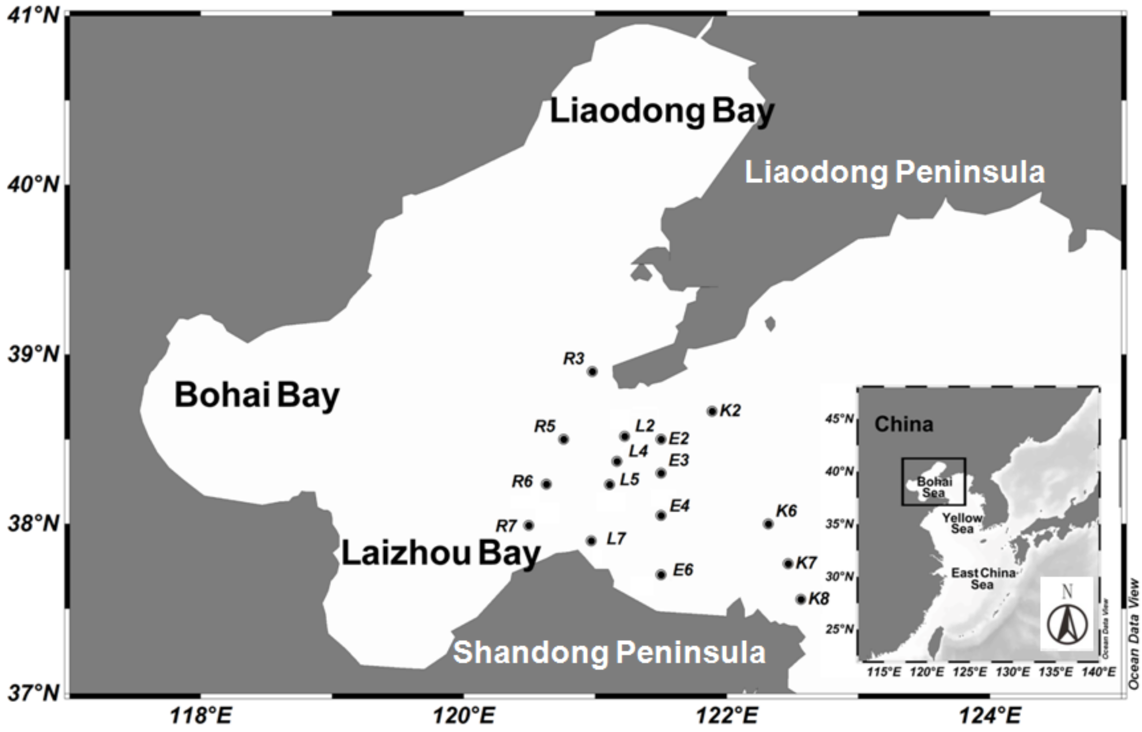


Figure 1 Sampling stations in the Bohai Sea study area.

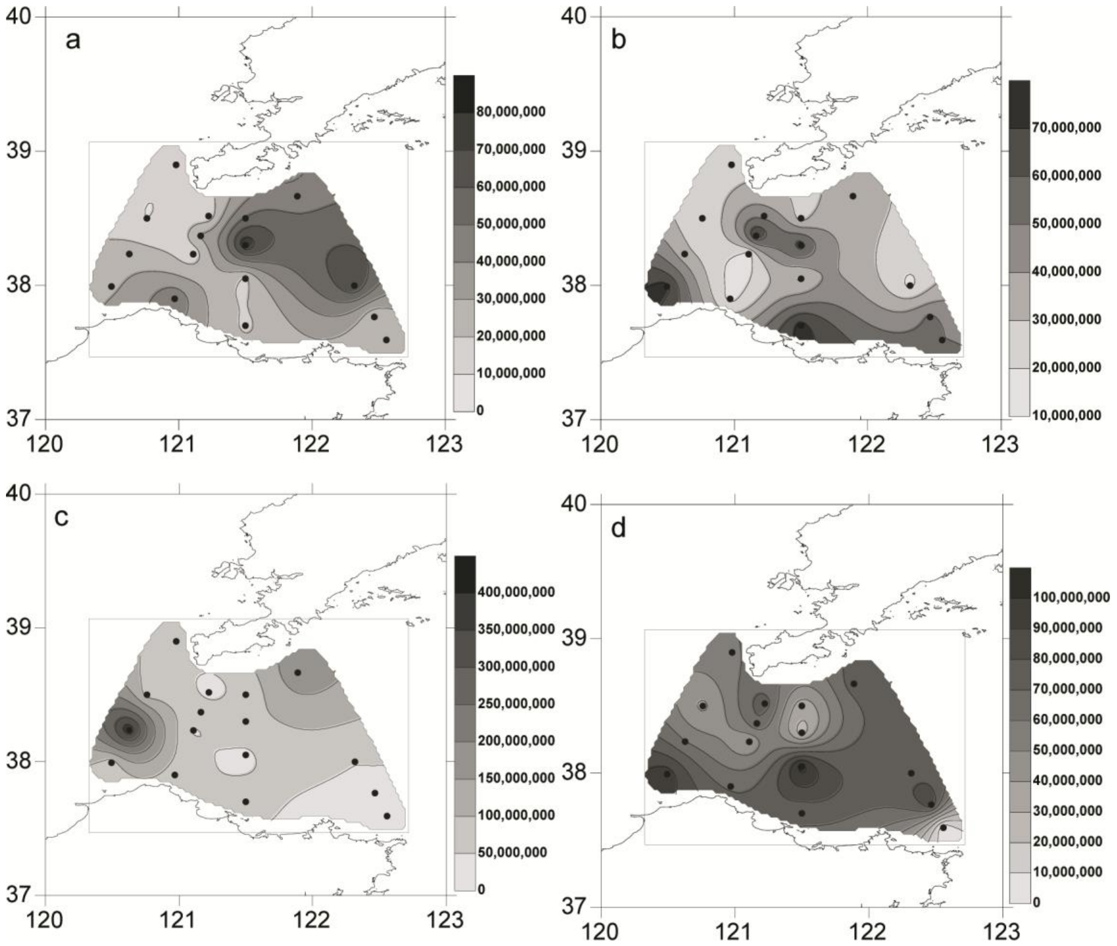
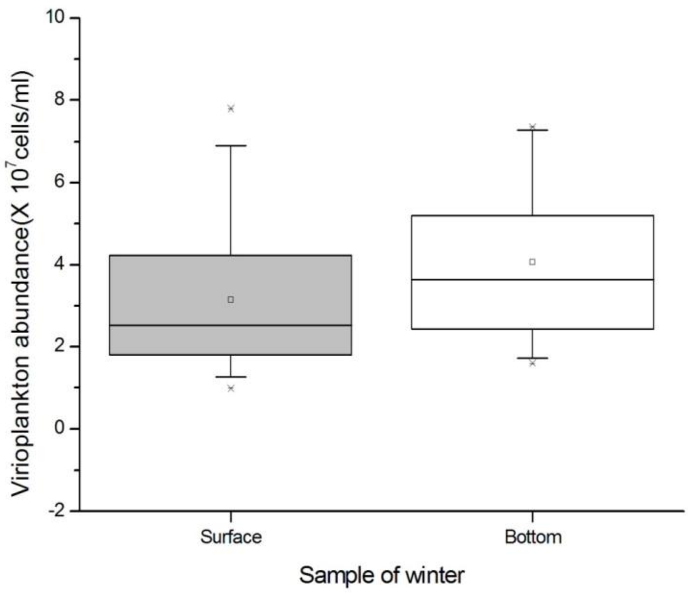


Figure 2 Horizontal distribution of virioplankton abundance in the Bohai Sea in winter 2013 and summer 2014 (Unit: cells/mL). (a) surface, winter; (b) bottom, winter; (c) surface, summer; (d) bottom, summer.



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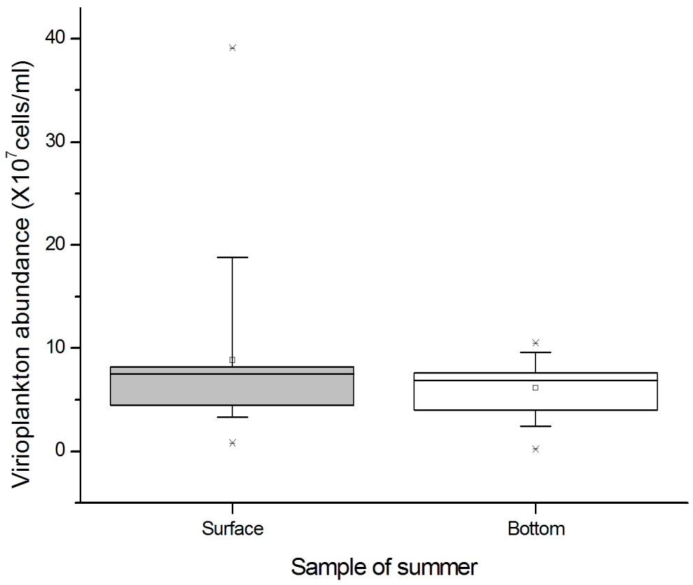


b

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

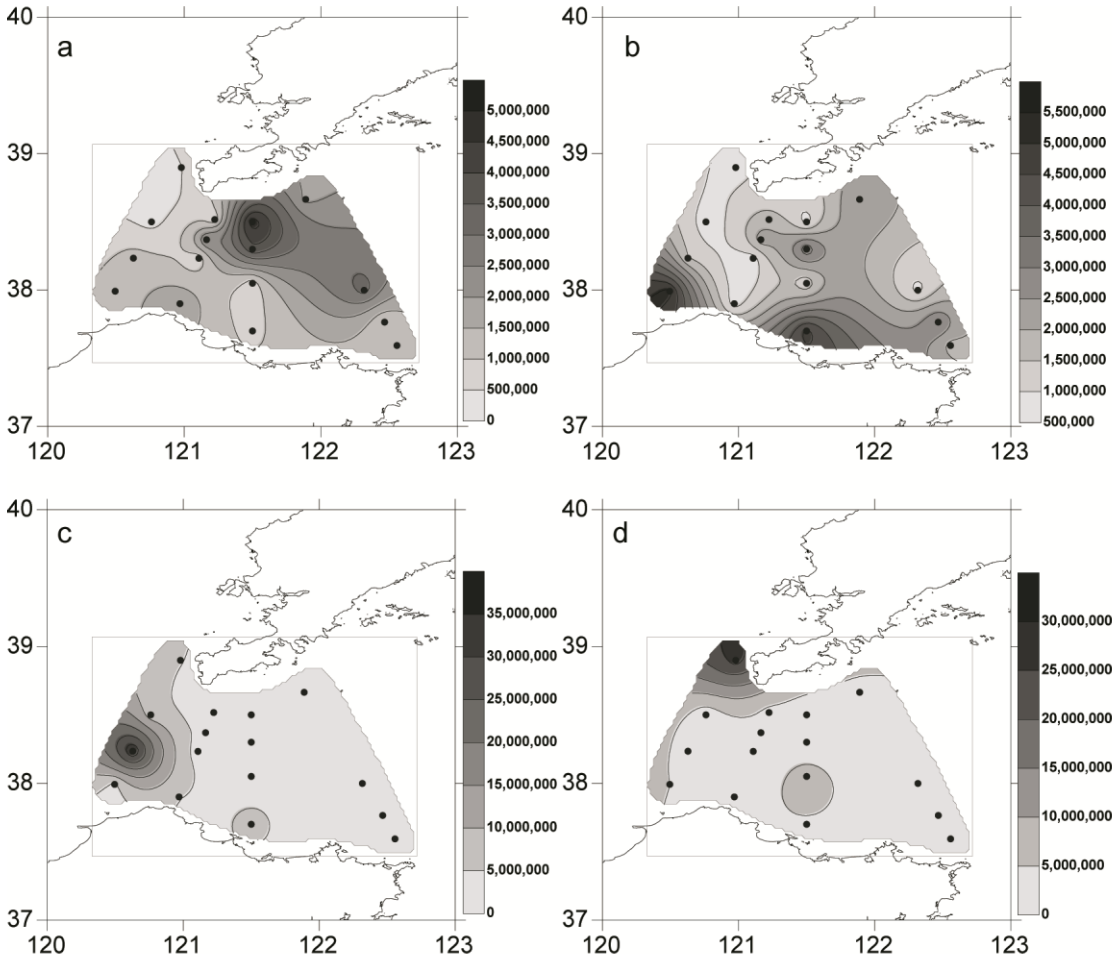
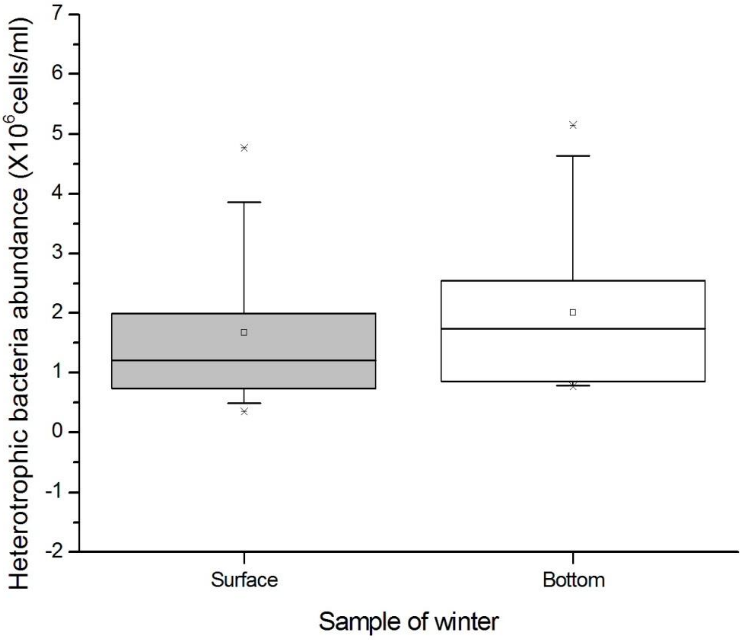


Figure 4 Horizontal distribution of heterotrophic bacterial abundance in the Bohai Sea in winter 2013 and summer 2014 (Unit: cells/mL). (a) surface, winter; (b) bottom, winter; (c) surface, summer; (d) bottom, summer.



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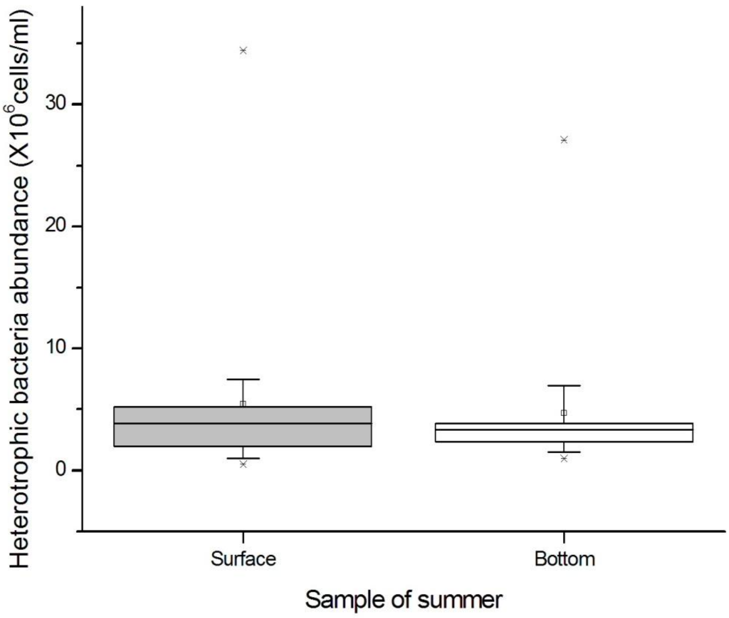

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Figure 5 Vertical distribution of heterotrophic bacterial abundance in the Bohai Sea in (a) winter 2013 and (b) summer 2014.