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T4-like myovirus community shaped by dispersal and deterministic processes in the South China Sea

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Summary
As the most abundant and genetically diverse biological entities, viruses significantly influence ecological, biogeographical and evolutionary processes in the ocean. However, the biogeography of marine viruses and the drivers shaping viral community are unclear. Here, the biogeographic patterns of T4-like viruses and the relative impacts of deterministic (environmental selection) and dispersal (spatial distance) processes were investigated in the northern South China Sea. The dominant viral operational taxonomic units were affiliated with previously defined Marine, Estuary, Lake and Paddy Groups. A clear viral biogeographic pattern was observed along the environmental gradient from the estuary to open sea. Marine Groups I and IV had a wide geographical distribution, whereas Marine Groups II, III and V were abundant in lower-salinity continental or eutrophic environments. A significant distance-decay pattern was noted for the T4-like viral community, especially for those infecting cyanobacteria. Both deterministic and dispersal processes influenced viral community assembly, although environmental selection (e.g. temperature, salinity, bacterial abundance and community, etc.) had a greater impact than spatial distance. Network analysis confirmed the strong association between viral and bacterial community composition, and suggested a diverse ecological relationship (e.g. lysis, co-infection or mutualistic) between and within viruses and their potential bacterial hosts.

Introduction
Viruses are the most abundant and diverse entities in marine ecosystems and considerably impact ecological, biogeographical and evolutionary processes in the ocean. Viruses could be responsible for reducing primary production in the oceans by up to 78%, and for an important percentage (~20%) of the mortality of marine heterotrophic bacteria (Suttle et al., 1990; Suttle, 1994). Therefore, it is becoming increasingly clear that we need to incorporate viruses and virus-mediated processes into our perception of marine ecology and oceanography (Suttle, 2007). Understanding the occurrence and mechanisms of the biogeographic patterns of marine viruses will help in predicting their influence on host populations and microbial community composition, and thus on large-scale oceanic processes (Stegen et al., 2012; Marston et al., 2013; Chow and Suttle, 2015; Gregory et al., 2019).

Recently, researchers have observed the existence of biogeographic patterns, often defined as the presence of spatial patterns of biodiversity resulting from deterministic (selection) and dispersal (spatial distance) processes, among a wide range of microorganisms (Hanson et al., 2012; Chow and Suttle, 2015). Selection varies the relative abundance of microbial species based on their ability to survive and reproduce. Potential variables influencing selection include the physical, chemical and biotic factors of a given environment. Dispersal refers to the movement, and subsequent successful establishment, of a microbial species to a new location (Hanson et al., 2012). Williamson et al. (2008) implicated that
marine viral communities display biogeographic patterns that are widely dispersed and influenced by local environmental selective pressures. Several other surveys also found a significant correlation between viral community structure and environmental parameters (Desnues et al., 2008; Marston et al., 2013). For instance, Angly et al. (2006) showed that salinity, temperature and oxygen concentration shape viral communities in the Gulf of Mexico, the Sargasso Sea and the Arctic Ocean. Frederickson et al. (2003) found that the physical structure of the water column influenced the structure of cyanophage communities in inlets in Canada. Studies on the influence of microbes on viral diversity and abundance both in coastal waters and in the open ocean have shown that viral community structure may be strongly influenced by their hosts’ communities (Fuhrman, 1999; Short and Suttle, 2002; Winter et al., 2005; Sandaa et al., 2007; Suttle, 2007; Parada et al., 2008; Short et al., 2011; Breitbart, 2012; Chow and Fuhrman, 2012; De Corte et al., 2016). While several studies have suggested that viral community diversity is mainly influenced by dispersal processes (Snyder et al., 2007; Winter et al., 2013), yet the influence of abiotic environmental variables, potential host community and spatial parameters are seldom investigated simultaneously in viral biogeographic studies.

The South China Sea (SCS), which is connected to the Pacific Ocean by the Luzon Strait, is one of the largest semi-enclosed marginal seas in the world. It has a wide continental shelf in the northwest area, which receives high volumes of runoff (3 × 10^11 m^3 v^−1) from the Pearl River, as well as a 4700-m-deep basin (Shaw and Chao, 1994; Zhang et al., 1999; Chen et al., 2001). The northern South China Sea (nSCS) is separated into three distinct regions based on bathymetric features: estuarine coastal plume (mostly eutrophic coastal region), continental shelf (transitional region) and open ocean (oligotrophic region) (He et al., 2009). Environmental variables such as salinity and nutrient availability often form a gradient in estuarine environments, producing a distance effect on spatial changes in microbial composition. The SCS is thus an ideal environment for investigating how viral community composition (VCC) and its control mechanisms alter with regard to distance.

T4-like phages are a subset of tailed phages that make up a significant fraction of the viral communities (Ackermann and Krisch, 1997; Breitbart et al., 2002; Brum et al., 2013). Here, we investigated the impact of environmental selection and dispersal processes on T4-like viral community assembly along an ~500-km transect with an environmental gradient from the estuary to open sea. Because most viruses in the ocean infect prokaryotes (Weinbauer, 2004; Baudoux et al., 2007; Payet and Suttle, 2008), we hypothesized that bacterial community composition (BCC) acts as an important environmental selection parameter in shaping VCC. To test this hypothesis, we used high-throughput sequencing to examine the composition and diversity of T4-like viruses and their potential bacterial hosts in the northern SCS.

Results

Samples were collected in the current study from the nSCS along a gradient extending from an estuarine environment to the continental shelf and on to the open sea (Fig. S1). Overall, three clusters of water samples were recognized based on temperature and salinity (Fig. S2, Table S1). Cluster 1 (sample A9-5 m) could be identified as the estuarine water mass (EWM) with relatively high temperature (28.1°C) and low salinity (31.19), whereas cluster 3 (comprising samples J2-50 m, I1-75 m, K3-75 m, K4-75 m and SEATS-75 m) could be identified as the sub-surface open sea water mass (ssOWM) and had the lowest water temperature (21.9–23.8°C) and highest salinity (34.22–34.36) among all samples. Cluster 2 samples showed a wider range of salinities (33.04–33.70) and temperatures (28.6–30.0°C) and could be separated into two sub-clusters based on trophic features. The first sub-cluster contained samples with a salinity <33.12 (samples J1-5 m, J2-5 m and J3-5 m), representative of the continental water mass (CWM; transitional region between eutrophic and oligotrophic zones), while the second sub-cluster consisted of samples from the surface open sea water mass (sOWM; oligotrophic region; samples J4-5 m, J4-25 m, I1-5 m, D-5 m, K2-5 m, K3-5 m, K4-5 m, and SEATS-5 m), which showed higher salinity (33.35–33.70).

The abundance of heterotrophic bacteria was highest in the eutrophic EWM sample, followed by the continental and open sea environment samples (Table S1). Among the autotrophic cyanobacteria, the highest concentration of Prochlorococcus was observed in the sOWM samples, followed by the ssOWM and costal CWM and EWM samples. Synechococcus were most abundant in samples from the continental and open sea environments but were less abundant than Prochlorococcus in the sOWM samples (Table S1). In addition, the abundance of picoeukaryotes was higher in ssOWM and EWM samples (Table S1).

Diversity among viral and bacterial communities

In total, 122 969 viral sequences and 750 658 bacterial sequences were generated after quality control in this study. We recovered 824 viral operational taxonomic units (vOTUs) and 1111 bacterial OTUs (bOTUs). Good’s coverage values from T4-like viral and bacterial sequences varied from 96.3% to 99.0% and 99.7% to 99.8% (Table S2) respectively. The rarefaction curve generated from the sequence data approached saturation.
sequences were grouped with phages belonging to the T-even and Pseudo T-even groups, whose hosts primarily inhabit animal guts and are considered essentially absent from the open sea (Fuhrman et al., 1993; Bano et al., 2004), despite being observed in the marine environment in previous studies (Filée et al., 2005).

In total, 17 bacterial phyla were identified across all samples. *Proteobacteria* was the most abundant phylum across all stations with the exception of sample J2-5m. *Bacteroidetes* and *Cyanobacteria* were highly abundant in samples from the open sea (Fig. S5A). At the class level, *α*- and *γ*-*Proteobacteria* and *Bacteroidia* were highly abundant in all samples. *β-Proteobacteria* were more abundant in samples from the estuarine environment than in any other samples. Samples collected from the open sea had a high abundance of *Oxyphotobacteria*, *Rhodothermia* and *Acidimicrobiia* (Fig. S5B), while *Phycisphaerae*, *Actinobacteria* and *Oxyphotobacteria* were enriched in CWM and EWM samples.

**Geographic patterns**

There was an obvious separation of viral communities among the samples, with community distribution corresponding to the water mass clusters (Fig. 3, Fig. S2). This finding was supported by the analysis of similarity (ANOSIM) global test (Global $R = 0.909$, $P = 0.001$). In addition, a vertical stratification between surface (sOWM) and sub-surface (ssOWM) communities was observed (Fig. 3). ANOSIM analysis showed that sample pairings CWM and sOWM, CWM and ssOWM and sOWM and ssOWM exhibited significant differences in viral community structure ($P < 0.05$) (Table 1). Furthermore, our results revealed a clear distance-decay pattern between viral community compositional similarity and geographic distance (decreased community similarity with increasing geographic distance) (Fig. 4A). This pattern was observed for the most abundant T4-like viral groups, i.e. Exo T-evens, Marine Group I and Marine Group IV (Fig. 4B–D). Exo T-evens and Marine Group I showed a significant relationship between community similarity and geographic distance, with the Exo T-evens group exhibiting the highest correlation, indicative of the strongest distance-decay pattern. Moreover, the Exo T-evens group made the greatest contribution to the dissimilarity between the CWM and sOWM, CWM and ssOWM and sOWM and ssOWM communities. Marine Groups I and IV were mainly responsible for the dissimilarity between the sOWM and ssOWM communities, as well as between the CWM and ssOWM communities, while Marine Group IV made a smaller contribution to the dissimilarity between the EWM and CWM communities (Fig. S6).

Bacterial community distribution was also corresponding to the water mass clusters (ANOSIM global test: global
$R = 0.498, P = 0.001$) (Fig. 3, Fig. S2). The vertical stratification of the BCC was less obvious than that of the VCC. In addition, the composition of the CWM communities differed significantly with the sOWM communities (ANOSIM pairwise tests: $R = 0.516, P < 0.05$) and ssOWM communities ($R = 0.538, P < 0.05$) (Table 1).

**Environmental selection and dispersal processes associated with the patterns of viral community**

Our results showed a significant correlation between the VCC and BCC (Spearman’s correlation, $P < 0.01$) (Table 2). Abiotic and abundance variables and geographic distance showed a significantly negative relationship with the T4-like viral community and with the Exo T-evens and Marine Group I sub-groups ($P < 0.01$) (Fig. 4; Table 2). Abiotic and abundance variables and the BCC had similar degrees of correlation (almost equivalent r-values) with the VCC, while geographic distance showed a relatively weak correlation (Table 2). Moreover, abiotic and abundance variables showed a significant correlation with the VCC of sOWM ($\rho = -0.481, P < 0.05$), while geographic distance was significantly correlated with the VCC of ssOWM ($\rho = -0.915, P < 0.001$) (Table 2).
To further explore the mechanisms shaping the viral biogeographic patterns, the relative influence of selective factors and spatial variables in forming viral community structure were analysed. Three abiotic (temperature, salinity and NO$_3^-$ + NO$_2^-$) and one abundance (heterotrophic bacterial abundance) environmental factors and two spatial factors (PCNM 1 and 2) were shown to make a significant contribution to VCC by canonical correlation analysis (CCA) (Fig. 5). Mantel tests further indicated significant relationships ($P < 0.05$) between the above four environmental variables and the VCC (Table S3). Pure abiotic and

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**Table 1.** Community comparison of T4-like virus and bacteria based on ANOSIM analysis with 999 permutations.

|          | EWM | CWM | sOWM | ssOWM |
|----------|-----|-----|------|-------|
| Virus    |     |     |      |       |
| EWM      | -   | -   | -    | -     |
| CWM      | 1   | -   | -    | -     |
| sOWM     | 1   | 0.987** | -   | -     |
| ssOWM    | 1   | 1*  | 0.775** | -   |
| Bacteria |     |     |      |       |
| EWM      | -   | -   | -    | -     |
| CWM      | 1   | -   | -    | -     |
| sOWM     | 0.938 | 0.516* | -   | -     |
| ssOWM    | 1   | 0.538* | 0.287* | -   |

*P < 0.05; **P < 0.01 and ***P < 0.001.
abundance factors ((E|S&B)), 9%) explained the same fraction of the variations as pure BCC (B|(E&S), 9.0%), and appeared to play a greater role than pure spatial factors ((S|(E&B)), 2.5%) (Fig. 6A and B). Shared environmental (abiotic and abundance and BCC) and dispersal (spatial parameters) processes (S∩B∩E) explained 2.5% of the variation in the VCC. In addition, shared abiotic and abundance parameters together with the BCC ((E∩B)|S) explained a large proportion of the variation (35.1%) in viral communities. Partial Mantel tests showed that changes in the VCC were significantly correlated with the BCC, abiotic and abundance and spatial variables in the environmental gradient from the estuary to the open sea (Fig. 6C).

Table 2. Spearman’s correlation analysis of relationships between VCC, BCC, geographic distance and Euclidean distance of all the abiotic and abundance variables.

|                         | Euclidean distance |                  | Bacterial community |                  | Geographic distance |                  |
|-------------------------|--------------------|------------------|---------------------|------------------|---------------------|------------------|
|                         | rho                | P                | rho                 | P                | rho                 | P                |
| T4-like viral community | -0.472             | <0.01            | 0.625               | <0.01            | -0.367              | <0.01            |
| CWM                     | -0.500             | 0.667            | 0.500               | 0.667            | -0.148              | 0.086            |
| sOWM                    | -0.481             | <0.05            | 0.297               | 0.190            | -0.264              | 0.174            |
| ssOWM                   | -0.212             | 0.556            | 0.818               | <0.01            | -0.915              | <0.01            |
| Exo T-evens             | -0.447             | <0.01            | 0.526               | <0.01            | -0.352              | <0.01            |
| Marine Group I          | -0.428             | <0.01            | 0.426               | <0.01            | -0.223              | <0.01            |
| Marine Group IV         | -0.422             | <0.01            | 0.332               | <0.01            | -0.148              | 0.086            |
Fig. 5. CCA was conducted to show abiotic (A) and abundance (B) and spatial (C) variables in governing the assembly of viral communities. HB\textsuperscript{a}, Heterotrophic bacterial abundance; Pro\textsuperscript{a}, Prochlorococcus abundance; Syn\textsuperscript{a}, Synechococcus abundance; Euka, picoeukaryotes abundance. [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 6. Variation in the viral community explained by abiotic and abundance (E), bacterial community (B) and spatial variables (S). (A) Variation partitioning analysis of viral community composition between bacterial community composition, abiotic and abundance and spatial variables. The variation explained by pure bacterial community composition, abiotic and abundance and spatial factors corresponds to the viral community without the effect of others by ANOVA permutation tests. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. The out circles represent 100% of the variation. (B) Mantel and partial Mantel tests to identify the correlations between the viral community structure and bacterial community composition and abiotic and abundance and spatial variables using Pearson’s coefficient. ‘\textsuperscript{1}’ indicates partial mantel test. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]
**Virus–bacterium interaction**

Co-occurrence network based on Spearman’s correlation coefficient analysis was constructed to deconvolute relationships between the predominant vOTUs and bOTUs (Fig. 7). The average path distance was 3.062, while the average clustering coefficient was 0.396. Most of the links in the network were virus-virus associations (77.73% of 494 links), with 14.78% of the links identified as virus–bacterium. Notably, positive connections dominated the interactions between vOTUs. All of the links among the Exo T-evens (22 links) and Marine Group I (3 links) and most of the links within Marine Group IV (8/9 links) were positive connections. There were no inter- and intra-links among the Estuary, Paddy and Lake Groups.

A total of 73 links were identified between virus and bacterium, including 50 positive correlations and 23 negative correlations. Most of the linked bOTUs were identified as α-Proteobacteria. In addition, there were 10 positive links between cyanobacteria and virus. A vOTU was frequently correlated to multiple bOTUs, which was often correlated with two or more vOTUs, resulting in one large interconnected cluster. There also had the correlation between one bOTU and several vOTUs. For example, one bOTU that was affiliated with the abundant family Flavobacteriaceae was both positively and negatively correlated with multiple vOTUs.

**Discussion**

Although the spatial distribution of viruses has been investigated in some marine environments, the mechanisms underlying the development of the complicated biogeographic patterns of viral community structure are still unclear (Chow and Suttle, 2015; Le Moine Bauer et al., 2017).
et al., 2018). In this present study, we investigated the biogeographic patterns of T4-like myoviruses in an ~500-km long subtropical estuary-sea environment and determined the relative importance of local environmental and dispersal processes on viral community assembly.

Contrasting biogeography of the T4-like myoviruses in the nSCS

We found a clear biogeographic distribution of T4-like viral groups along the transect from the estuary to open sea. Both Marine Groups I and IV showed a wide geographical distribution in the nSCS, supporting previous studies showing that these groups are abundant in various marine environments, such as the Arctic glaciers, the northeastern Gulf of Mexico and the Pacific Ocean (Filée et al., 2005; Bellas and Anesio, 2013). Marine Group I may prefer colder water with a higher salt content, as the sequences were more abundant in the sOWM and ssOWM samples than in other samples. Marine Group III was most abundant in the transitional continental shelf region than in coastal areas in the nSCS. The distribution of Marine Group II was similar to that of Marine Group III, suggesting that both sub-groups or their hosts may be adapted to coastal shelf environments rather than the relatively high-salinity oligotrophic open sea. Marine Group V was mainly found in the estuary water mass samples and was rare in sOWM and ssOWM samples, suggesting that Marine Group V is well adapted to the coastal shelf environments rather than the relatively low-salinity environment.

We found that T4-like viral community assembly was significantly associated with local environmental factors and spatial variables (Fig. 6; Table 2). On the one hand, the environmental variables (temperature, salinity, NO₃⁻ + NO₂⁻, heterotrophic bacterial abundance and bacterial community structure) strongly explained the cluster patterns in the viral communities (Fig. 5; Table 2, S3). Previous analysis has shown that temperature and salinity strongly affect VCC because they play an important role in the growth and survival of viruses (Guixa-Boixareu et al., 1996; Jiang et al., 2004; Mojica and Brussaard, 2014). Temperature can regulate infection dynamics and the sensitivity of viral lipid membranes or capsid protein, while the adsorption of viruses to host cells can be affected by salt concentration (Kukkaro and Bannister, 2009; Mojica and Brussaard, 2014). Surveys from the Tara Oceans expedition also demonstrated that viral community structure varied consistently with temperature and salinity on a global scale (Brum et al., 2013). Moreover, trophic status might be of greater importance to viral geography because viral population size and activity are highly dependent on host population size and activity (Middelboe, 2000; Suttle, 2000; Winter et al., 2004). This was supported by our findings showing that relatively high salinity and oligotrophic OWM and CWM samples grouped together but away from the low-salinity and eutrophic EWM samples (Fig. 3). In addition, BCC has been suggested as to be the most important
determining factor for the viral community as a result of specific virus–host interactions such as infection (Winter et al., 2004, 2005; Parada et al., 2008; Chow et al., 2014) (see below for detailed discussion).

On the other hand, spatial factors (dispersal processes) shape the viral community. In oceans, one of the dominant dispersal mechanisms for viruses is the movement of water masses (Frederickson et al., 2003; Winter et al., 2013; Chow and Suttle, 2015; Le Moine Bauer et al., 2018). Water mass transport usually contributes to a negative relationship between microbial compositional similarity and geographic distance (the distance-decay relationship) (Green and Bohannan, 2006; Hanson et al., 2012). In the nSCS, spatial variables appear to contribute significantly, although to a lesser extent than environmental selection, to viral community structure. Dispersal includes establishment, meaning that at least some reproduction in the new location rather than simply just being present (Hanson et al., 2012). Given the small size of viruses, there are few limitations to their global dispersal, yet host availability and environmental factors may increase limitations, either directly or indirectly, that restrict geographic range (Chow and Suttle, 2015).

The partial Mantel test corroborated this result by showing that environmental selection had a great effect than dispersal processes. This result was consistent with studies conducted in the Labrador Sea (Winter et al., 2013). However, viral communities in the Arctic Archipelago (~1000-km) showed a significant response to spatial factors rather than environmental variables (Winter et al., 2013). These differences may result from differing degrees of environmental heterogeneity and/or geographic scale (Chow and Suttle, 2015). Although samples collected in the current study covered a ~500-km transect, the degree of environmental heterogeneity in the nSCS was higher than that in the Arctic Archipelago, meaning that large environmental gradients can increase the influence of environmental selection on viral community assembly. This observation agrees with research carried out on marine bacteria, suggesting that the relative influence of dispersal processes might shift in response to the degree of environmental heterogeneity in community assembly (Logares et al., 2013; Mo et al., 2018).

Ecological role of the host in viral community assembly

In general, co-occurrence correlations between microbial taxa can be explained as competition or cooperation for nutrients, material and space (Deng et al., 2012). In our study, co-occurrence networks suggested complex interactions within and between viral and bacterial communities. Virus propagation is host-dependent and infection is usually species- or even strain-specific. Negative correlations between virus and bacteria indicate the presence of viral infection and lysis (Chow et al., 2014). Therefore, the host range of a vOTU may be suggested by negative correlations with bOTUs, with a greater number of negative connections indicative of a broad host range (such as the negative correlation between vOTU595 and three bOTUs). In the current study, bOTUs that were negatively correlated with T4-like viruses were affiliated with α- and β-Proteobacteria, Cyanobacteria, Bacteroidia and Actinobacteria, indicating that bacteria belonging to these taxa are the likely hosts of T4-like viruses in the nSCS. Positive correlations suggest co-occurrence as a result of similar preferred conditions or a commensal or a mutualistic relationship between organisms cooperating within the same niche (Barberan et al., 2012; Faust and Raes, 2012; Chow et al., 2014).

For example, the positive correlation between vOTU330 and Prochlorococcus might be a result of the viral lysis of bOTU9334 (negative correlation with vOTU330), since bOTU9334 showed 100% nucleotide sequence identity to Marinobacter sp. H4T4B5, which inhibits the growth of Prochlorococcus MIT9313 (Sher et al., 2011). An alternative explanation is that host lysis by viruses releases nutrients and stimulates the growth of uninfected hosts, including cyanobacteria and heterotrophic bacteria (Sher et al., 2011; Shelford and Suttle, 2018).

The positive associations (57.89%) within the viral community may be the result of mutualism between viruses in long-term co-evolutionary processes (Chow et al., 2014). Almost all of the intra-links for the Exo T-evens, Marine Group I and Marine Group IV were positive, showing that T4-like viruses affiliated with these groups may have a co-habitat or co-infection (Needham et al., 2013; Zhang et al., 2017). The vOTU1054 and vOTU1188 of Exo T-evens group, which were affiliated with Synechococcus phages S-CAM8 and S-SM2 respectively, were positively linked. Sullivan et al. (2003) demonstrated that phage S-SM2, which was isolated from Synechococcus WH7803, has a broad host range and can infect the original host of phage S-CAM8 (Synechococcus WH8017) (Sullivan et al., 2003). Synechococcus strains WH7803 and WH8017 are closely related and have similar growth conditions and ecological niches (Sullivan et al., 2006). These likely explain the positive link between vOTU1054 and vOTU1188 in our study. In comparison, negative correlations between viruses might indicate a competition or non-overlapping niches between viruses or their hosts (Barberan et al., 2012; Faust and Raes, 2012; Chow et al., 2014).

For example, vOTU219 and vOTU380 were affiliated with cyanophages S-TIM4 and P-SSM5 respectively, whose original hosts were Synechococcus WH8102 and Prochlorococcus NATL2A respectively. The negative correlation between vOTU219 and vOTU380 in our study
may correspond with the different niche preference of *Synechococcus* WH8102 and *Prochlorococcus* NATL2A.

**Conclusions**

The present study demonstrated the biogeographic and significant distance-decay patterns of the T4-like viral community in the nSCS, which is a good representative of an estuary–sea environment. We determined that T4-like viruses belonging to Marine Groups I and IV had a wide geographical distribution, those belonging to Marine Groups II and III were mainly present in lower-salinity continental environments, and that Marine Group V viruses preferred eutrophic coastal estuarine environments. Compared with spatial distance, environmental selection (e.g., temperature, salinity, nitrogen nutrient, bacterial abundance and community composition) had a greater impact on viral community structure. Among environmental selection factors, the bacterial community structure alone exerted almost the same degree of selective pressure as other abiotic and biotic variables in shaping T4-like viral community assembly. In addition, network analysis suggested that BCC may impact on viral community assembly and biogeography through diverse ecological interactions, such as lysis, co-infection and mutualism. Our data provide important ecological insights into marine viral biogeography, including community assembly mechanisms and interactions with host communities.

**Experimental procedures**

*Viral and bacterial sample collection and DNA extraction*

Eleven stations were visited during the cruise of the nSCS in September 2014. Water samples were retrieved from the surface (5 and 25 m) and subsurface (50 and 75 m) layers (Fig. S1). After collection, 5-L water samples were pre-filtered through a 20-μm sieve and then subjected to sequential tangential-flow filtration using a 0.22-μm-pore-size filter (Millipore Corp.) to obtain bacterial concentrates (50 ml) and a 30-kDa cartridge (Millipore Corp.) to concentrate viruses (50 ml). Total DNA was extracted from the viral concentrate using the phenol/chloroform/isoamylol method (Liu et al., 2017). Total DNA was extracted from the bacterial concentrate as described previously (Kan et al., 2008).

*Environmental parameters*

Temperature, salinity and depth were measured *in situ* using conductivity-temperature-depth oceanic profilers (SBE9/11 plus; Sea-Bird, USA). Temperature and salinity were used to cluster water masses using the pamk function of package fpc in R (Fig. S2) (R Development Core Team, 2015 (Li et al., 2018a). NO₃⁻ + NO₂⁻, SiO₂²⁻ and PO₄³⁻ were obtained by filtration using 0.45-μm cellulose acetate filters and measured using a colorimetric method. To estimate the abundance of microbes, replicate samples (1.98 ml) were collected and fixed using glutaraldehyde (0.5% (vol./vol.) final concentration) at 4°C for 15 min in the dark, before being frozen in liquid nitrogen. The abundance of autotrophic picoeukaryotes, *Synechococcus* and *Prochlorococcus* was determined by direct counting without staining using flow cytometer (Accuri C6; BD Biosciences) (Jiao et al., 2002). Heterotrophic bacterial abundance was also determined by flow cytometer (Accuri C6) after staining with SYBR Green I (Table S1) (Marie et al., 1999). All data analyses were performed using FlowJo vX.0.7 software (Tree Star, USA).

**Polymerase chain reactions, sequencing and phylogenetic analysis**

A fragment of the major capsid protein-encoding gene g23 was amplified from T4-type phages using degenerate primers MZIA1 bis and MZIA6, as described by Filée et al. (2005). The amplicons were sequenced using the 454 GS FLX platform. The sequences were quality screened using MOTHUR (v.1.41.1), and chimeric sequences were discarded (Schloss et al., 2009; Edgar, 2010). OTUs were identified with a 97% sequence similarity. Singleton OTUs that occurred only once identified by filter_otus_from_otu_table.py command within QIIME were removed and samples were subsampled to 2709 (minimum sample size) from the OTU table for downstream analyses. The most prevalent g23 OTUs, with a relative abundance >1% per sample (a total of 93 OTUs), were translated into amino acid sequences and aligned using MEGA7 (Kumar et al., 2016). A phylogenetic tree was constructed using the maximum likelihood method in RAxML, with 1000 bootstrap replicates (Stamatakis, 2014). The tree was viewed and graphically edited using iTOL (Letunic and Bork, 2016). Viral g23 sequences have been deposited in the National Centre for Biotechnology Information Sequence Read Archive (NCBI SRA) database under the accession numbers SRR11786657–SRR11786673 (BioProject accession number PRJNA632563; BioSample accession numbers SAMN14912226–SAMN14912242).

The conserved V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 338F and 806R (Mori et al., 2014; Li et al., 2018b). Resulting amplicons were sequenced using the Illumina MiSeq PE300 platform. Raw reads were trimmed using Trimmomatic and merged using FLASH (Magoc and Salzberg, 2011; Bolger et al., 2014). Thereafter, usearch61 was used to
identify and remove chimeric sequences (Edgar, 2010). The remaining sequences were clustered into OTUs based on 97% sequence similarity. bOTUs with a total community abundance of >0.001% were then selected for further analysis. The number of sequences from each sample was subsampled to the same amount (30 000). Classification was carried out using MOTHUR (v.1.41.1) with SILVA (v132) reference sequences and taxonomic outline. Bacterial 16S rRNA gene sequences have been submitted to the NCBI SRA database under the accession numbers SRR11794483–SRR11794499 (BioProject accession number PRJNA632681, BioSample accession numbers SAMN14917627–SAMN14917643). Details on the primers and sequence processing of viral and bacterial sequences are supplied in the Supporting Information.

**Community diversity and structure**

Rarefaction curves based on the identified vOTUs and bOTUs were estimated by PAST (v.3.18). The rarefied sequences were then used for Good's coverage and alpha diversity indices in QIIME. Non-metric multidimensional scaling (NMDS) analysis based on the Bray–Curtis similarity of VCC and BCC was performed to disentangle the difference between the samples. ANOSIM was used to test significant difference in bacterial and viral communities. Similarity percentage (SIMPER) analysis was used to conclude the contribution of major vOTUs to the observed dissimilarity between different oceanic water masses. NMDS, ANOSIM and SIMPER analyses were performed in PRIMER 6.0 (Clarke and Gorley, 2006).

**Relationships between VCC and deterministic and dispersal processes**

The Bray–Curtis similarity results for VCC and BCC as well as the Euclidean distance matrices for the abiotic and abundance parameters were transformed using algorithms of log$(x + 1)$ in PRIMER 6.0. Geographic distance was calculated based on the coordinates of the sampling stations using a spheroidal model of Earth (Winter et al., 2013). The relationships among geographic distance, Euclidean distance and Bray–Curtis similarity were analysed using Spearman’s rank correlations. CCA was used to connect the structure of viral communities with abiotic, abundance and spatial variables. The longest gradient lengths in the detrended correspondence analysis were >4, indicating that CCA is suitable for examining VCC. Prior to CCA analysis, the variables with a high variance inflation factor (>10) were eliminated to avoid collinearity among factors.

Variation partitioning analysis (VPA) was used to assess the relative importance of environmental and spatial parameters in forming viral community, which was widely used in ecological research to determine the relative importance of environmental selection versus dispersal processes for community structure (Legendre and Legendre, 2012; Winter et al., 2013; Mo et al., 2018). Bray–Curtis similarity is one of the most commonly used similarity quantification methods when it comes to ecological abundance data collected at different sampling locations (Legendre and Legendre, 2012; Ricotta and Podani, 2017). Principal coordinates analysis explores similarities or dissimilarities, such as Bray–Curtis similarity, and takes species identity into account, which is a better method of analysing bacterial community structure data for VPA (Mohammadi and Prasanna, 2003; Legendre and Legendre, 2012; Hamdan et al., 2013; Nagaraj et al., 2017; Hu et al., 2020). To avoid collinearity in the statistical calculation, principal coordinates axes with a cumulative 73.9% variation based on Bray–Curtis similarity were used to summarize the bacterial community structure (relative abundance data) for VPA. Abiotic and abundance variables, which were significantly (Mantel test, $P < 0.05$) correlated with viral community structure, were used. Spatial variables were generated using the principal coordinates of neighbour matrices analysis. The relative importance of all components was explained by pure abiotic and abundance environmental variables ($E$ ($E&B$)) (i.e. the exclusive abiotic and abundance variables excluding spatial variables and BCC), pure spatial variable (S($E&B$)), pure BCC (B($E&S$)) and the combined effects of spatial variables and BCC ($S \cdot B$)E (i.e. interaction component of spatial variables and BCC excluding abiotic and abundance variables), spatial and abiotic and abundance variables (($S \cup B$)E), BCC and abiotic and abundance variables (B(E$\cap$E)S), and the combined effects of spatial, BCC and abiotic and abundance variables ($S \cup B \cup E$) respectively. The Mantel and partial Mantel tests were conducted to verify the results obtained from VPA. All these statistical analyses were performed in R.

**Network analysis**

Potential interactions between viral and bacterial taxa were determined by modelling the community in network structure. Association networks were constructed using an online Molecular Ecological Network Analyses Pipeline (http://leg4.rcc.ou.edu/MENA) (Deng et al., 2012). Pairwise correlation matrices were generated for the viral and bOTUs. Similarity matrices were calculated based on Spearman’s rank correlation. Random matrix theory-based algorithm was used to determine the threshold of network (Deng et al., 2012; Zhang et al., 2017).
Cytoscape 3.2.1 was used to describe the network, showing nodes (vOTUs and bOTUs) linked by lines that denoted positive or negative correlations.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Fig. S1.** Location of the 11 sampling stations in the nSCS. Water samples were collected from surface and subsurface layers.

**Fig. S2.** (A) Distribution of salinity and temperature among the 17 samples in the nSCS. (B) Three clusters of water samples were identified based on the temperature and salinity. The two components (i.e. components 1 and 2) explained 100% of the point variability. Different water masses are indicated in green (EWM), grey (CWM), red (sOWM) and blue (ssOWM).

**Fig. S3.** Rarefaction curve of similarity-based operational taxonomic units (OTUs) at 97% similarity level. (A) Viral g23 and (B) bacterial 16S rRNA gene libraries from the nSCS.

**Fig. S4.** Frequency and abundance rank plot for viral (A-B) and bacterial (C-D) OTUs. A spline curve and scatter diagram were used to express the relationship between the frequency of OTUs occurrence and the number of OTUs and average concentration of OTUs to the community respectively. Each black dot represents an individual OTU.

**Fig. S5.** Bacterial community biodiversity and composition at the phylum (A) and class (B) level.

**Fig. S6.** SIMPER analysis of the dissimilarity among four viral oceanic water masses (cut-off of low contribution: 90%). Only dominant g23 OTUs were used.

**Table S1.** Location and environmental parameters of the 17 samples from the nSCS.

**Table S2.** Good’s coverage, richness (Chao I) and diversity (Shannon and Simpson) indices across all samples at the 97% similarity level.

**Table S3.** Mantel test for the correlation between viral community composition and abiotic and abundance environmental variables using Pearson’s coefficient. *P* < 0.05, **P** < 0.01 and ***P*** < 0.001.

**Table S4.** Mantel and partial Mantel tests for the correlation between the Exo T-even viral community and other variables. ‘|’ indicates partial mantel test.