Tide driven microbial dynamics through virus-host interactions in the estuarine ecosystem

Xiaowei Chen, Wei Wei, Jianning Wang, Hongbo Li, Jia Sun, Ruijie Ma, Nianzhi Jiao, Rui Zhang

State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Institute of Marine Microbes and Ecospheres, Xiamen University, Xiamen, 361102, PR China

College of the Environment and Ecology, Xiamen University, Xiamen, 361102, PR China

National Marine Environmental Monitoring Center, State Oceanic Administration, Dalian, 116023, PR China

Abstract

Microbes drive ecosystems and their viruses manipulate these processes, yet the importance of tidal functioning on the estuarine viruses and microbes remains poorly elucidated. Here, an integrative investigation on tidal patterns in viral and microbial communities and their inherent interactions over an entire spring-neap tidal cycle was conducted along a macrotidal subtropical estuary. The viral and microbial abundances oscillated significantly over the tidal cycle with relatively higher abundances observed at spring tide compared to neap tide. The distinct tidal dynamic patterns in bacterial production and community composition were tightly associated with the variations in viral infection, production and decay, revealing the tide-driven interactions between viruses and microbes. Concurrent with the higher viral decay but lower bacterial abundance and inhibited bacterial metabolism during the neap tide, lower gross viral production was coupled with a synchronous switching from viral lytic to lysogenic infection induced by the loss of viral infection efficiency and the transition from marine to freshwater bacterial populations triggered by tidal mixing. Our results highlighted the major tidal impact on the microbial dynamics through virus-host interactions, with cascading effects, neglected so far, on estuarine biogeochemical cycles.

Article Info

Article history:
Received 10 December 2018
Received in revised form 11 May 2019
Accepted 17 May 2019
Available online 20 May 2019

Keywords:
Estuary
Spring-neap tide
Virus
Microbe
Lysis-lysogeny switch

1. Introduction

Rhythmicities of environmental conditions generally set the reoccurring life events. Estuarine habitats are vital nodes of land-ocean interactions, particularly marked by strong periodic tidal fluctuations. The effect of gravitational forces exerted by the motion of celestial bodies generates the diurnal variability in tidal stages (e.g., high tide and low tide) and change in tidal amplitude over spring-neap cycle (Kowalik, 2004; Kvale, 2006). Estuaries, beach intertidal zones, salt marshes, and other nearshore systems suffer multiple forcing mechanisms associated with the tides, such as hydraulic head gradient-driven saltwater circulation, saltwater-freshwater mixing and tidal pumping (Heiss and Michael, 2014; Kowalik, 2004; Yu et al., 2014). Previous investigations have found that tide-induced mixing and advection greatly contributed to the biological processes such as the global phytoplankton growth-loss balance, the vertical migration of copepods, the semilunar spawning period of nekton and the grazing rate of pelagic and benthic herbivores (Findlay et al., 2013; Kowalik, 2004; Pernthaler, 2005; Sharples, 2007).

Microbes drive biogeochemical processes on a global scale (Falkowski et al., 2008). Lytic and lysogenic viral infections significantly affect the mortality, biomass and composition of microbial communities (Howard-Varona et al., 2017; Weinbauer, 2004; Zhang et al., 2007), fueling viral impact on microbial food web and microbial transformation of dissolved organic matter (Jiao et al., 2010; Suttle, 2005, 2007; Zhang et al., 2014). The viral lysis-lysogeny switch determines the release of progeny viral particles, which may decrease in infectivity and degrade when exposed to various physico-chemical and biological factors in the environment, such as ultraviolet light, salt stress and enzyme activity (Choi et al., 2010; Mojica and Brussard, 2014; Motlagh et al.,...
2. Materials and methods

2.1. Study sites and sampling

The JRE located in the northern South China Sea is a subtropical macrotidal estuary. Tides in the JRE are predominately semi-diurnal with a mean tidal range of 4.08 m, varying from 1.0 m to 6.42 m (Huang and Hong, 1999; Zuo et al., 2016). The estuary continuously receives large quantities of nutrient-rich freshwater input (1.17 × 10^10 m^3 per year) from the Jiulong River. The river is fed mainly by summer monsoon from April to July and there is no dam in the upstream to regulate the river (Huang and Hong, 1999; Li et al., 2011). Starting in 2012, we conducted long-term monthly observations at 15 stations in the JRE (Fig. 1A) and three stations along a gradient of hydrologic and biological features that were selected according to our previous investigations (Cai et al., 2016; Liu et al., 2017). During the monsoon season, selected stations were sampled every 2 days at a specified time points (10:45, 12:00 and 13:45 at stations S03, S05 and S07, respectively) from April 2—19, 2015 (Fig. 1B). The sampling followed an entire spring-neap tidal cycle, with the tidal range varying from 3.51 m (April 12) to 5.66 m (April 19). Based on the distinct differences in the daily variations of tidal range and salinity by analysis of variance (ANOVA, p < 0.05), April 4—6 (following the full-moon) and April 17—19 (following the new-moon) were classified as the spring tide period, and April 10—12 (following the one-quarter moon) was classified as the neap tide period, with the remaining days classified as the transition tide period (Fig. 1C).

Approximately 5 L of surface water sample (0.5 m depth) was collected into acid-cleaned polycarbonate bottles and was treated immediately for subsequent analysis. The samples for biological analysis were pre-filtered through 20 μm mesh filters to remove large particles and zooplankton. To determine the viral and microbial abundances by flow cytometer, 2 mL subsamples were fixed with glutaraldehyde to a final concentration of 0.5% at room temperature for 15 min in the dark. After the flash freezing in liquid nitrogen, samples were stored at −80 °C for later analysis.

2.2. Physical and chemical parameters

Data for tidal height and tidal range were collected from China Oceanic Information Network (http://www.nmdis.org.cn/). Temperature, salinity, pH and dissolved oxygen (DO) were measured using a YSI Professional Plus multi-parameter meter (YSI, Yellow Springs, OH, USA). Inorganic nutrients such as nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate (SiO_2^{4-}) were measured using an Auto Analysis III, AA3 instrument (Bran-Luebbe, Germany). Chlorophyll a (Chl a) concentration was determined by a high sensitive infrared kinetic double modulation fluorometer (FL5000, PSI, Czechia), corrected by high-performance liquid chromatography as described (Jiao et al., 2009).

2.3. Viral and microbial abundances

Viral abundance was determined by flow cytometer (Epics Altra II, Beckman Coulter, USA) with SYBR Green I stain (Molecular Probe, USA) according to the established protocol (Marie et al., 1999). Fluorescent beads (Molecular Probes) with a diameter of 1 μm were added as an internal standard. Autotrophic Synechococcus and picocyanobacteria were counted directly without staining by the flow cytometer (BD Accuri C6, USA). Heterotrophic bacterial abundance was also determined by flow cytometer (BD Accuri C6) after staining with SYBR Green I (Marie et al., 1999). All the flow cytometric data analyses were performed with the FlowJo vX.0.7 software (Tree Star, USA).
2.4. Bacterial production

Bacterial production (BP) was estimated by 3H-Leucine incorporation following the method described with some modifications (Kirchman, 2001). Briefly, triplicate 1.5 mL water samples and one trichloroacetic acid (TCA) killed control (1% final concentration) were inoculated with 3H-Leucine (specific activity, 16 Ci mmol⁻¹) to a final concentration of 20 nM. After 0.5–2 h incubation in the dark at in situ temperature, incubation was stopped by adding 5% TCA (final concentration) and centrifuged at 16,000×g for 10 min. The supernatant was extracted twice with 5% ice-cold TCA and rinsed with 80% ethanol. Later, radioactivity was measured with a liquid scintillation counter (280 TR, Waltham, USA) and BP rates were calculated as the average rates of the triplicate leucine incorporation rates corrected by the control.

2.5. Viral production and infection rate

To estimate viral production (VP) and infection rate, the viral reduction approach was used (Fig. S1) (Weinbauer et al., 2002, 2010; Winget et al., 2005). Approximately 600 mL water sample was filtered using tangential flow filtration (TFF) with a 0.22 μm-pore-size polyvinylidene difluoride cartridge ( Labscale, Millipore, USA) to obtain 50 mL bacteria concentrate and ca. 500 mL filtrate. The 50 mL bacteria concentrate was diluted with 250 mL virus-free water obtained from the 0.22 μm filter that was sequentially TFF ultrafiltered using a 30 kDa polysulfone cartridge (Millipore) (Cai et al., 2015; Weinbauer et al., 2002). The sample was then gently mixed and 200 mL of the mixture was aliquoted into four 50 mL sterile centrifuge tubes and incubated at in situ temperature in dry bath incubators (MK-20, Hangzhou Allsheng, China) under dark condition (Li et al., 2014). In two of these tubes, mitomycin C (the inducing agent of the lytic cycle) was added to a final concentration of 1 μg mL⁻¹ (Sigma-Aldrich), whereas the other two tubes were kept without addition as controls (Weinbauer et al., 2002). Subsamples of 1 mL in replicate for bacterial and viral enumeration were taken at 3 h intervals during the 15 h incubation. VP and viral infection were calculated using the online program VIPCAL (http://www.univie.ac.at/nuhag-php/vipcal/) (Luef et al., 2009). The lytic VP was assumed to be equal to the rate of viral accumulation in the control tubes with reduced natural viral abundance, and frequency of lytic infection (FIC) can be calculated as established method (Winter et al., 2004). The lysogenic VP and frequency of lysogenic infection (FLC) were calculated as the difference between viral increase in the tubes with mitomycin C treatments and the control tubes (Weinbauer et al., 2002). The lysogeny-to-lysis ratio was calculated as the ratio of FLC to FIC, to determine the switching index between the lysogenic and lytic viral infections.

2.6. Viral decay rate

Viral decay rate (VD) was measured according to Noble and Fuhrman (1997). Seawater was filtered by TFF with a 0.22 μm-pore-size cartridge to exclude bacteria and particles >0.22 μm, replicates of 50 mL filtrate were incubated at in situ temperature in the dark (Fig. S1). Subsamples of 1 mL in replicate were taken for bacterial and viral enumeration at 3 h intervals up to 15 h. The VD was calculated as the slope of the linear regression fitted to the natural logarithm of viral abundance plotted against time during 15 h incubation.
2.7. Bacterial community composition

For bacterial community composition analysis, 500 mL seawater from each station was collected onto 0.2 μm pore-size poly-carbonate filters (47 mm, Millipore) by vacuum filtration. Microbial DNA was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, USA) according to the manufacturer’s protocol. The V3–V4 region of bacterial 16S rRNA gene was amplified with the primer pair 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGTTATCTAAT) complemented with sample-specific barcodes. Sequencing libraries were generated using MiSeq Reagent Kit v3 for Illumina (Illumina, USA). Sequences that contained more than one ambiguous nucleotide that did not have a complete barcode and primer at one end, or that were shorter than 200 bp after removal of the barcode and primer sequences were eliminated. Chimera sequences were also identified and removed. After quality filtering, denoising and removal of potential chimeras, sequences were used to study the bacterial community composition. For OTU classification, reads were clustered at 97% similarity and taxonomy was assigned in Mothur (v.1.36.1) using the Silva 119 reference classification, reads were clustered at 97% similarity and taxonomy was assigned in Mothur (v.1.36.1) using the Silva 119 reference database (Quast et al., 2013; Schloss et al., 2009).

2.8. Virus-mediated bacterial mortality and carbon releasing

The virus-mediated bacterial mortality (VMM) was calculated by dividing lytic VP by burst sizes of 50 estimated from previous tidal measurement (Winget et al., 2011). The carbon content of bacterial standing stock was calculated by heterotrophic bacterial abundance and the average marine bacterial cell content of carbon (as a factor of 12.4 fg C cell−1)(Fukuda et al., 1998). The rate of lysed bacterial carbon (RLC) and the rate of lysed bacterial produced carbon (RLP) were then estimated from dividing VMM by the bacterial abundance and BP, respectively. To estimate the carbon released by viral lysis of bacteria, the VMM was multiplied by the average bacterial cell carbon content. The carbon not released by viral lysogeny was also estimated by a similar method based on lysogenic VP with the assumption that lysogenic VP would be totally transferred into lytic VP and cause the lysis of bacteria.

2.9. Statistical analysis

Shapiro-Wilk W tests for data normality were applied before analysis, and the data were logarithmically transformed to meet normality if necessary. Two-way ANOVA was used to determine the significant differences in environmental and biological parameters with respect to stations and spring-neap tidal periods. If significant differences were observed, the post hoc Tukey’s test was also performed. All statistical analyses described above were performed with SPSS 24.0 software (SPSS Inc., USA). Linear regression model was also used to characterize the relationship between abiotic and biotic factors using GraphPad Prism 7 (GraphPad, USA). Principal component analysis (PCA) for segregating sample grouping of physico–chemical parameters after normalizing the data, redundancy analysis (RDA) was performed to test whether the abundances and activities of viruses and microbes were related to environmental and biological variables using CANOCO 4.5 software (Microcomputer Power, USA). The significance of the axes was determined by Monte Carlo permutation tests.

3. Results

3.1. Tidal variability of the sampling environments

Three stations along the JRE were sampled over a spring-neap tidal cycle, and the environmental and biological variables are reported in Table S1. PCA biplot based on physico–chemical parameters distinguished the upper estuarine station S03 from the lower estuarine station S05 and coastal station S07, with S03 showed relatively low values for salinity, pH and DO but high nutrients (Fig. 1C and Table S1). Salinity, pH and DO at all stations showed a significant tidal signature that peaked at spring tide but reached minimum at neap tide, with a drastic variation at station S03 and moderate variation at stations S05 and S07 (ANOVA, p < 0.05; Fig. 2 and Table S2). An inverse tidal pattern in nutrients was observed at all stations with a stronger change amplitude at station S03, although it is not statistically significant (Fig. 2 and Table S2). Extremely low values of NO2− (0.17 μmol L−1) and PO43− (0.05 μmol L−1) were found at S03 at April 17 during the spring tide (Fig. 2E and G). In addition, Chl a concentration at S03 displayed a significant tidal variation with a higher value at spring tide (ANOVA, p < 0.001; Fig. 21).

3.2. Viral and microbial abundances revealed tidal variability

An apparent and similar tidal pattern among viral and microbial abundances was shared by three stations with a compatible amplitude (ANOVA, p < 0.01; Fig. 3 and Table S2). Viral abundance peaked during spring tide (8.48 ± 0.33 × 105 ml−1) and 8.13 ± 0.10 × 105 ml−1 at S03, S05 and S07, respectively, and then declined with tidal range to reach its minimum value near neap tide (Fig. 3A). The maximum of heterotrophic bacterial abundances was also recorded at spring tide (Fig. 3B). The virus-to-bacteria ratio during spring tide approximately doubled than the neap tide at all stations (Fig. S2A). The abundances of Synechococcus and picoeukaryotes at three stations significantly increased with the tidal range (ANOVA, p < 0.01; Fig. 3C and D). Furthermore, we observed that tidal variability of heterotrophic bacterial abundance was less pronounced than that of viral, Synechococcus and picoeukaryotic abundances, especially at station S03. RDA analysis revealed the concentrations of viruses, heterotrophic bacteria, Synechococcus and picoeukaryotes were positively correlated with DO, pH and salinity but negatively correlated with nutrient levels (pseudo-F statistic, p < 0.05; Fig. 5A and Table S3).

3.3. Tidal variability in bacterial production, viral infection and decay

The rates of BP, VP, VIF and FLC were measured to investigate the dynamics of virus–host interactions. The highest BP at station S03 (6.86 ± 0.07 μg C L−1 d−1) occurred when spring tide appeared and the lowest value (3.11 ± 0.22 μg C L−1 d−1) was recorded during neap tide, showing a more distinct tide-associated change than the lower estuarine station S05 and the coastal station S07 (Fig. 3E). Estuarine stations S03 and S05 shared a similar spring-neap tidal pattern for VP with the lowest values occurred during spring tide, significantly increasing with the decrease in tidal range (Figs. 3F and 6A). Station S03 showed a more intense tidal change in both lytic and lysogenic viral infection rates. A FIC peak value (22.42 ± 4.77%) was recorded at April 17 during spring tide, and two FLC peak values were observed at April 4 (18.82 ± 3.89%) and April 17 (16.48 ± 2.66%), following spring tide (Fig. 3G and H). The dynamics of lytic and lysogenic VP at station S03 were similar to the changes in FIC and FLC, resulting in higher total viral production occurred with the higher tidal range (Fig. S2). The lysogeny-to-lysis ratio at three stations shared a similar tidal pattern that a significant higher value was usually recorded near neap tide (ANOVA, p < 0.05; Fig. 3I), showing a significantly negative correlation with tidal range at S03 and S05 (Fig. 6B). RDA analysis revealed the salinity, pH, DO, NO3−, PO43− and heterotrophic bacterial abundance were significantly related to the dynamics of BP, VP, VIF, FIC, FLC and
the lysogeny-to-lysis ratio during spring-neap cycle (pseudo-F statistic, \( p < 0.05 \); Fig. 5B and Table S3). The tide-associated lysogeny-to-lysis ratio had a strongly negative relationship with BP but was positively coupled to VD.

3.4. Tidal trends in bacterial community composition

Bacterial community composition was represented by 13 most abundant populations along with other bacterial members at class level according to their relative sequence abundances (Fig. 4A). Different bacterial groups displayed distinct distributions and tidal variations across three stations. For example, Betaproteobacteria, the typical freshwater population (Paver et al., 2018; Riemann et al., 2008), was more abundant at the upper estuarine station S03, coupled with the maximum relative abundance occurring during neap tide, but Bacteroidetes showed the inverse trend (Fig. 4A). Spearman’s rank correlation indicated that variations in certain bacterial populations were tightly associated with tidal range, as well as viral, heterotrophic bacterial abundances, BP, lytic and lysogenic viral infections, particularly at station S03 (Fig. 4B). For instance, when the high tidal range turned the hydrologic environment of S03 into a coastal-like feature during spring tide, the relative abundances of Bacteroidetes, Cyanobacteria and Actinobacteria were increased compared to the decreases in Betaproteobacteria and Verrucomicrobia, coupled with increases in viral and heterotrophic bacterial abundances but decrease in the lysogeny-to-lysis ratio.

3.5. Virus-mediated bacterial mortality and carbon releasing showed tidal specialization

At station S03 during spring tide, VMM was \( 26.52 \pm 17.37 \times 10^7 \) cells L\(^{-1}\) d\(^{-1}\) together with approximately 30\% d\(^{-1}\) of RLC and 70\% d\(^{-1}\) of RLP, compared to ca. fourfold lower values of VMM, RLC and RLP at neap tide (Table 1). Hence, more carbon was released by viral lysis during spring tide \( (3.29 \pm 2.15 \mu g L^{-1} d^{-1}) \) relative to the neap tide \( (0.74 \pm 0.002 \mu g L^{-1} d^{-1}) \) at S03. The amount of carbon not released by viral lysogeny referred to the reduction in lysate carbon due to the establishment of lysogeny rather than lysis. At S03, the amount of carbon not released by viral lysogeny during spring tide \( (3.24 \pm 1.83 \mu g L^{-1} d^{-1}) \) was comparable with the carbon released by lysis, but this amount during neap tide \( (1.88 \pm 0.43 \mu g L^{-1} d^{-1}) \) was ca. 2–3 times higher than lysis-released carbon. In fact, the ratio of the amount of carbon released by viral lysis and not released by lysogeny was significantly positively correlated with tidal range at all stations, indicating that organic carbon tended to be released into the environment from viral lysis rather than stored as bacterial biomass from neap to spring tide shifting (Fig. 6C).

4. Discussion

Spring-neap tidal dynamics of physico-chemical variables were observed at all stations, with the amplitude of the change decreased along the estuary (from the upper estuarine station S03 to...
to coastal station S07), and perfectly matched the dilution effect pattern, wherein eutrophic fresh water was diluted by oligotrophic offshore seawater (Fig. 2). Although the samples among three stations were collected at different diurnal tidal phase (e.g., high tide and low tide; Fig. 1B), viral and microbial abundances at all stations shared similar tidal patterns and amplitudes which oscillated more in relation to the spring-neap tide than diurnal tide (Fig. 3). These results suggested the sensitivity of viruses and microbes to spring-neap tidal dynamic, potentially confirming the primary tidal influence exerted by spring-neap cycle over diurnal tide (Cadier et al., 2017; Heiss and Michael, 2014). Future field work covers more than one entire spring-neap tide along the estuarine habitat, coupled with the corresponding diurnal sampling within each day, might help to distinguish the impacts of spring-neap tide and diurnal tide on microbial ecosystem.

The tidal trend in Synechococcus and picoeukaryotic abundances that increased with tidal range can be predicted to match the previous results that the abundances of estuarine cyanobacteria and picoeukaryote generally increase in relation to the spring-neap tide than diurnal tide (Fig. 3). These results suggested the sensitivity of viruses and microbes to spring-neap tidal dynamic, potentially confirming the primary tidal influence exerted by spring-neap cycle over diurnal tide (Cadier et al., 2017; Heiss and Michael, 2014). Future field work covers more than one entire spring-neap tide along the estuarine habitat, coupled with the corresponding diurnal sampling within each day, might help to distinguish the impacts of spring-neap tide and diurnal tide on microbial ecosystem.

Our results revealed that lysogenic viral infection and the lysogeny-to-lysis ratio were negatively related to bacterial abundance and production (Fig. 5B). This is consistent with previous finding that lytic viral infection prefers the metabolically active bacteria to produce more viral progeny, whereas lysogeny favors lower bacterial abundance or activity (Maurice et al., 2013; Payet and Suttle, 2013). Despite the presence of more riverine runoff-derived nutrients during neap tide at S03, lower BP significantly contributed to lower bacterial abundance ($R^2 = 0.695, p = 0.005$;
linear regression), suggesting the potential inhibition effect of bacterial metabolism at neap tide (Shen et al., 2018). These results can be explained by that bacteria are sensitive to changes in ionic strength, and the drastic variation in salinity during tidal cycle can trigger the shift in physiological stress on bacterial metabolic processes (Bettarel et al., 2011b; Kukkar and Bamford, 2009; Wei et al., 2019). Freshwater bacteria were previously revealed to be greatly inhibited by seawater (Troussellier et al., 2002), whereas marine bacteria showed relatively high adaptability to variation in salinity (Forsyth et al., 1971). The freshwater-bacteria-dominated microbial community at neap tide suffered from high osmotic pressure, which restricted their metabolic activity to use energy-consuming mechanisms to maintain the osmotic balance in their cells instead of using this energy for cell division (Bettarel et al., 2011b). During spring tide, metabolism in the marine-bacteria-dominated microbial community was stimulated by efficient substrate utilization, increasing the bacterial abundance and subsequent lytic viral production and viral abundance (Fig. 3).

Viral production and the counterbalancing loss from viral decay jointly determine the net balance of viral abundance over tidal cycle. Similar to their host, the realized niche of viruses would be constrained by salinity since salt stress may directly affect their survival and successful infection (Wei et al., 2019), potentially through decrease in capsid pressure and consequent reduction in DNA injection efficiency (Cordova et al., 2003; Evilevitch et al., 2008). Viruses have been found to require adequate ionic strength (e.g., Na$^+$ and Mg$^{2+}$) to maintain structural stability and remain successful infectivity to host (Keynan et al., 1974; Mojica and Brussaard, 2014), demonstrating that a higher salt concentration usually promotes viral adsorption and subsequent infection (Bettarel et al., 2011a; Mei et al., 2015). These can explain the significantly negative relationship between viral decay rate and tidal range at estuarine stations S03 and S05 (Fig. 6A), which reflects higher viral losses induced by the infective inefficiency and high particle decomposition resulting from low salinity at neap tide (Mojica and Brussaard, 2014; Wei et al., 2019). In turn, lower viral decay coupled with higher lytic viral activity led to higher net production of viral particles at spring tide (Fig. 3 and Fig. S2),
determining the budget for viral abundance over tidal cycle with higher values recorded at spring tide but lower during neap tide.

There was a strong negative association between the lysogeny-to-lysis ratio and tidal range at S03 and S05, with a significant higher lysogeny-to-lysis ratio in response to lower levels of bacterial productivity and abundance but higher viral decay at neap tide.

Fig. 5. Redundancy analysis correlation (RDA) triplot reveals (A) the tidal dynamics in viral abundance and microbial abundance (response variables, in red) in relation to environmental variables (explanatory variables, in black) and (B) the tidal dynamics in the bacterial production, viral production, infection and decay rate (response variables, in red) in relation to environmental and biological variables (explanatory variables, in black). All explanatory variables in the triplot are significant (see Table S3). Sample numbers 1 to 9, 10 to 18 and 19 to 27 are the samples from stations S03, S05 and S07, respectively. The symbols with different color and shapes mean the sampled station and the tidal periods. DO, dissolved oxygen; Sal, salinity; NO2, NO3; SiO2; VA, viral abundance; HBA, heterotrophic bacterial abundance; VBR, virus-to-bacteria ratio; Syn, Synechococcus abundance; Peuk, picoeukaryotic abundance; BP, bacterial production; VP, viral production; VD, viral decay rate; FIC, frequency of lytic infection; FLC, frequency of lysogenic infection and LysoLysis, the lysogeny-to-lysis ratio. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 6. Linear relationships between tidal range and (A) the viral decay rate, (B) the lysogeny-to-lysis ratio and (C) the ratio of carbon released by lysis and not released by lysogeny. The colored best-fit lines with 95% confidence intervals from linear regression are shown and the lines with a significance level of \( p < 0.05 \) are bold on the graph.

Table 1

| Station | Tidal periods | VMM (10^7 cells L^-1 d^-1) | Carbon in BSS (\mu g L^-1) | Gross bacterial carbon (\mu g L^-1 d^-1) | RLC (% d^-1) | RLP (% d^-1) | Carbon released by lysis (\mu g L^-1 d^-1) | Carbon not released by lysogeny (\mu g L^-1 d^-1) |
|---------|---------------|-----------------------------|-----------------------------|------------------------------------------|-------------|------------|-------------------------------------------|---------------------------------------------|
| S03     | Spring        | 26.52 ± 17.37               | 11.14 ± 0.76                | 5.56 ± 1.21                             | 30.62 ± 22.58 | 70.62 ± 68.96 | 3.29 ± 2.15                              | 3.24 ± 1.83                                 |
|         | Transition    | 9.64 ± 1.54                 | 10.01 ± 0.66                | 3.39 ± 0.31                             | 11.93 ± 1.63 | 35.82 ± 8.64 | 1.20 ± 0.19                              | 2.33 ± 0.56                                 |
|         | Neap          | 5.99 ± 0.02                 | 9.73 ± 0.24                 | 4.03 ± 1.13                             | 7.64 ± 0.16 | 19.20 ± 5.37 | 0.74 ± 0.02                              | 1.88 ± 0.43                                 |
| S05     | Spring        | 13.98 ± 6.25                | 11.62 ± 0.97                | 5.69 ± 4.18                             | 14.71 ± 6.34 | 39.78 ± 17.81 | 1.73 ± 0.77                              | 1.17 ± 0.77                                 |
|         | Transition    | 8.68 ± 5.03                 | 10.08 ± 1.29                | 8.66 ± 9.44                             | 10.62 ± 6.31 | 22.32 ± 15.67 | 1.08 ± 0.62                              | 1.86 ± 1.11                                 |
|         | Neap          | 9.99 ± 3.01                 | 10.13 ± 0.52                | 6.76 ± 1.10                             | 12.15 ± 3.06 | 18.11 ± 2.58 | 1.24 ± 0.37                              | 2.24 ± 0.16                                 |
| S07     | Spring        | 5.09 ± 1.46                 | 15.59 ± 1.21                | 7.74 ± 3.13                             | 4.02 ± 0.93 | 9.47 ± 4.77 | 0.63 ± 0.18                              | 0.49 ± 0.16                                 |
|         | Transition    | 4.78 ± 3.07                 | 12.78 ± 2.08                | 4.20 ± 0.83                             | 4.55 ± 2.88 | 13.16 ± 7.30 | 0.60 ± 0.38                              | 0.80 ± 0.28                                 |
|         | Neap          | 5.787 ± 3.73                | 11.98 ± 0.55                | 7.79 ± 5.26                             | 6.09 ± 4.15 | 14.54 ± 15.77 | 0.72 ± 0.46                              | 0.69 ± 0.07                                 |
| Total   |               | 10.93 ± 9.82                | 11.69 ± 2.12                | 6.00 ± 3.81                             | 12.24 ± 11.98 | 29.52 ± 32.29 | 1.36 ± 1.22                              | 1.64 ± 1.21                                 |

BSS, bacterial standing stock; RLC, the rate of lysed bacterial carbon; RLP, the rate of lysed bacterial produced carbon. The standard deviation is given after the mean value.
Previous studies have indicated that viruses prefer to integrate within bacteria, favouring a lysogenic life cycle, to avoid further destruction when facing high viral decay (Mojica and Brussaard, 2014; Weinbauer, 2004). Additionally, viral lytic-lysogenic switch was generally constrained by bacterial metabolism that greatly impacted by surrounding environmental conditions (Maurice et al., 2010b), owing to viruses rely heavily on the hosts being healthy enough to complete their lytic cycle (e.g., the synthesis of offspring particle constituents) (Mei et al., 2015). For example, the marine temperate phage fHSIC was found to enter the lysogenic life cycle due to a reduction in its host’s growth rate at low salinity (Williamson and Paul, 2006). Indeed, host HSIC-1a cells growing at low salinity were found to produced ca. two orders of magnitude fewer phages than those growing at high salinity (Long et al., 2007). The low metabolic activities of microbes under high osmotic stress from spring to neap tide shifting may limit their susceptibility to lytic viral infection, causing the viral infection switching from lytic to lysogenic cycle and thus the higher values of the lysogeny-to-lysis ratio at neap tide.

Lysogeny is generally considered as the favorable viral survival strategy during episodes of low energy resources (Paul, 2008; Weinbauer, 2004). Indeed, the lysogeny-to-lysis ratio was significantly negative with heterotrophic bacterial abundance over spring-neap tidal cycle in the JRE ($R^2 = 0.308, p < 0.01$; linear regression), suggesting that the high density of microbes at spring tide did not drive viral lytic to lysogenic switching (Knowles et al., 2016). Ultimately, the harsh environmental conditions facing drastic tidal forcing resulted in lower bacterial activities and tended to drive the viral infection switching towards lysogeny. Unlike seasonal patterns in viral lysis and lysogeny were usually driven by the trophic status (Brum et al., 2016; Payet and Suttle, 2013), tidal stirring generating fast changing in surrounding habitat would trigger a highly variable amplitude in the host bacteria’s ecophysiological parameters, which might greatly influence the patterns in lysis-lysogeny switch over tidal cycle (Maurice et al., 2013, 2010a). The tidal shift in viral life strategies in conjunction with change in high viral decay, led to the lower viral lytic activities and bacterial mortality, which would help both virus and host survive at neap tide (Paul, 2008).

The tidal transition in bacterial community composition may also affect the viral infection pattern since host phylogeny has been found to be tightly related to the viral life strategies (Keshri et al., 2017; Kim and Bae, 2018; Maurice et al., 2010b). Tidal disturbances might act as a filter for bacterial community adaptions, resulting in the “loss” of some species but the “activation” of others among freshwater and seawater bacterial populations (Chauhan et al., 2009). Spearman rank correlation revealed that lysogenic viral infection and the lysogeny-to-lysis ratio at station S03 generally increased along with the increases in certain bacterial populations such as Betaproteobacteria and Verrucomicrobia that were previously confirmed as the typical freshwater species (Paver et al., 2018; Riemann et al., 2008), but showed negative correlations with Bacteroidetes and Cyanobacteria (Fig. 4B). These results suggested that viral lytic and lysogenic properties may be contributed partially by the change in viral infection ability induced by tidal shift in freshwater and marine microbial communities. The coexistence of freshwater and marine viruses has been found in the JRE (Cai et al., 2016; Liu et al., 2017). Previous studies have also
revealed that the freshwater viral community might be able to efficiently infect marine hosts, whereas the freshwater microbes did not act as good hosts for the marine viruses (Bonilla-Findji et al., 2009; Xu et al., 2014). Consequently, the reduced infection efficiency due to the marine viruses to freshwater bacterial members would also be a potential factor causing the higher level of lysogeny during neap tide than during spring tide.

4.1. Implications to estuarine carbon cycle

Tidal dynamics of viral lysis and lysogeny were highly variable at the upper estuarine station S03, resulting in higher carbon released by lysis during spring tide than neap tide (Table 1). This high viral lysis at spring tide enhanced the transfer of microbial biomass into the pool of dissolved organic matter, and diverted organic matter flow away from higher trophic levels, thus accelerating the transformation of carbon from particulate (e.g., living organisms) to dissolved states (Fuhrman, 1999; Jiao et al., 2010). The ratio of carbon released by viral lysis and not released by lysogeny significantly increased with tidal range at all stations (Fig. 6C), indicating lysogeny would lower the efficiency of viral shunt and keep more carbon flux within microbial biomass at neap tide, channeling most of the carbon to higher trophic levels in the food web (Wommack and Colwell, 2000). Totally, we found lysogeny reduce 1.64 ± 1.21 µg L−1 d−1 carbon into environmental carbon pool in JRE (Table 1), which was higher than the amount released by lysis (1.36 ± 1.22 µg L−1 d−1). Overall, our findings indicated that the differential virus-mediated carbon flux induced by lysis-lysogeny switch and by tidal shifting over spring-neap cycle, neglected so far, must be accounted in the modelling of the microbial food web and biogeochemical processes in estuarine-coastal systems (Sheyn et al., 2018).

5. Conclusions

Taken together, this study presented a comprehensive assessment of estuarine viral and microbial dynamics over an entire spring-neap tidal cycle (as summarized in Fig. 7). We demonstrated that:

- An apparent spring-neap tidal pattern in viral and microbial abundances was recorded, with relatively higher abundances observed at spring tide compared to neap tide.
- Tidal dynamics in viral and microbial communities were shaped by viral lysis-lysogeny switch induced by tidal mixing, with higher level of lysogeny at neap tide compared to the higher level of lysis at spring tide.
- The switched viral life strategies during spring-neap tidal cycle would cause an intense influence on microbial food web and carbon cycle in the estuarine ecosystems.

Author contributions

RZ and WW initiated and planned the work, XC, WW, JW, HL, JS and RM carried out sampling and performed research, XC and WW analyzed data and XC and RZ wrote the manuscript with input from all authors.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank Chengda Li, Jiezhen Xie and Tingwei Luo for their assistance with the sampling. This work was supported by the projects of National Natural Science Foundation of China (31570172 and 418611440018) and Public Science and Technology Research Fund Projects for Ocean Research (201505003-3). Hongbo Li was supported by The Visiting Fellowship of the State Key Laboratory of Marine Environmental Science at Xiamen University. Rui Zhang was partially supported by Qingdao National Laboratory for Marine Science and Technology (QNLMM20160R0P0303) and GCMAC1507. Xiaowei Chen and Ruijie Ma were supported by The PhD Fellowship of the State Key Laboratory of Marine Environmental Science at Xiamen University. The authors thank the editor and anonymous reviewers for their time, effort and valuable contributions to this manuscript.

Appendix A. Supplementary data

Supplementary data to this chapter can be found online at https://doi.org/10.1016/j.watres.2019.05.051.

References

Abu-Bakar, A., Ahmadian, R., Falconer, R.A., 2017. Modelling the transport and decay processes of microbial tracers in a macro-tidal estuary. Water Res. 123, 802–824. https://doi.org/10.1016/j.watres.2017.07.007.

Almeida, M.A., Cunha, M.A., Alc nata, F., 2001. Loss of estuarine bacteria by viral infection and predation in microcosm conditions. Microb. Ecol. 42, 562–571. https://doi.org/10.1007/s00248-001-0020-1.

Anderson, S.R., Dios-Cass, Q.P., Harvey, E.L., 2018. Short-term estimates of phyto-plankton growth and mortality in a tidal estuary. Limnol. Oceanogr. 36. https://doi.org/10.1034/j.1043-970X.1999.00464.x.

Ayliward, F.O., Boeuf, D., Mende, D.R., Wood-Charlton, E.M., Vislova, A., Eppley, J.M., Romano, A.E., DeLong, E.F., 2017. Diel cycling and long-term persistence of viruses in the ocean’s euphotic zone. Proc. Natl. Acad. Sci. U.S.A. 114, 11446–11451. https://doi.org/10.1073/pnas.1714821114.

Bauer, J.E., Cal, W.-J., Raymond, P.A., Bianchi, T.S., Hops, T.S., Hopkins, C.S., Regnier, P.A.G., 2013. The changing carbon cycle of the coastal ocean. Nature 504, 61–70. https://doi.org/10.1038/nature12857.

Bettaré, Y., Bouvier, T., Agis, M., Bouvier, C., Van Chu, T., Combe, M., Mari, X., Nghiêm, M.N., Nguyen, T.T., Phan, T.T., Pringault, O., Rochelle-Newall, E., Torrèton, J.-P., Tran, H.Q., 2011a. Viral distribution and life strategies in the bang dang estuary, vietnam. Microb. Ecol. 62, 143–154. https://doi.org/10.1007/s00248-011-9835-6.

Bettaré, Y., Bouvier, T., Bouvier, C., Carré, C., Desnues, A., Domaizon, I., Jacquet, S., Robin, A., Sinne-Ngando, T., 2011b. Ecological traits of planktonic viruses and prokaryotes along a full-salinity gradient. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 76, 360–372. https://doi.org/10.1111/j.1574-6941.2011.01054.x.

Bettaré, Y., Dolan, J.R., Hornak, K., Lenée, R., Martin, M., Pedrotti, M.L., Rochelle-Newall, E., Sinnek, K., Sinne-Ngando, T., 2002. Strong, weak, and missing links in a microbial community of the N.W. Mediterranean Sea. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 42, 451–462. https://doi.org/10.1016/S0168-6496(02)00283-5.

Bonilla-Findji, O., Rochelle-Newall, E., Weinbauer, M.G., Pizay, M.D., Kerros, M.E., 2013. The changing carbon cycle of the coastal ocean. Nature 504, 61–70. https://doi.org/10.1038/nature12857.

Brum, J.R., Hurwitz, B.L., Schobel, G., Ducklow, H.W., Sullivan, M.B., 2016. Seasonal time bombs: dominant temperate viruses affect Southern Ocean microbial dynamics. ISME J. 10, 437–449. https://doi.org/10.1038/ismej.2015.125.

Cadier, M., Gorgues, T., Belhevue, S., Sourisseau, M., Memery, L., 2017. Tidal cycle control of biogeochemical and ecological properties of a macrotidal ecosystem. Geophys. Res. Lett. 44, 8453–8462. https://doi.org/10.1002/2017GL074173.

Cai, L., Yang, Y., Jiao, N., Zhang, R., 2015. Evaluation of tangential flow filtration for the concentration and separation of bacteria and viruses in contrasting marine environments. PLoS One 10, e0136741. https://doi.org/10.1371/journal.pone.0136741.

Cai, L., Zhang, R., He, Y., Feng, X., Jiao, N., 2016. Metagenomic analysis of virus-iplankton of the subtropical Jiulong River estuary, China. Viruses 8, 35. https://doi.org/10.3390/v8020035.

Celussi, M., Paoli, A., Bernardi Aubry, F., Bastianini, M., Del Negro, P., 2008. Diel microbial variations at a coastal Northern Adriatic station affected by Po River outflows, 76, 36–44. https://doi.org/10.1016/j.ecss.200705.038.

Chauhan, A., Cherrier, J., Williams, H.N., 2009. Impact of sideways and bottom-up control factors on bacterial community succession over a tidal cycle. Proc. Natl. Acad. Sci. U.S.A. 106, 4301–4306. https://doi.org/10.1073/
Heiss, J.W., Michael, H.A., 2014. Saltwater-freshwater mixing dynamics in a sandy beach aquifer over tidal, spring-neap, and seasonal cycles. Water Resour. Res. 46, 2713–2729. https://doi.org/10.1002/wrcr.20352.

Kvale, E.P., 2006. The origin of neap–spring tidal cycles, 235, 5–18. https://doi.org/10.1038/nrg1506.00101.

Li, G., Gao, K., Yuan, D., Zheng, Y., Yang, G., 2011. Relational diagram of photosynthetic carbon fixation with environmental changes in the Jidong River estuary of the South China Sea, with special reference to the effects of solar UV radiation. Mar. Pollut. Bull. 62, 1852–1858. https://doi.org/10.1016/j.marpolbul.2011.02.050.

Li, Y., Luo, T., Sun, J., Cai, L., Liang, Y., Jiao, N., Zhang, R., 2014. Lytic viral infection of bacterioplankton in deep waters of the western Pacific Ocean. Biogeosciences 11, 2531–2542. https://doi.org/10.5194/bg-11-2531-2014.

Liu, L., Cai, L., Zhang, R., 2017. Co-existence of freshwater and marine T4-like myoviruses in a typical subtropical estuary. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 93, 2720–2732. https://doi.org/10.1093/femsec/fi197.

Long, A., Patterson, S.S., Pais, J.H., 2007. Macroarray analysis of gene expression in a marine pseudotemperate bacteriophage. Aquat. Microb. Ecol. 49, 1–14. https://doi.org/10.3354/ame031145.

Luef, B., Lef, P., Feduzzi, P., 2009. Online program “vical” for calculating lytic viral production and lysogenic cells based on a viral reduction approach. Environ. Microbiol. 11, 78–85. https://doi.org/10.1111/j.1462-2920.2008.01008.x.

Marie, D., Pentrez, y., Vaulot, D., Brussaard, C., 1999. Enumeration of photoplankton, bacteria, and viruses in marine samples. Curr. Protoc. Cytom. https://doi.org/10.1002/0471142688.v011a017.

Mei, Y., He, C., Huang, Y., Liu, Y., Zhang, C., Chen, X., Shen, P., 2015. Salinity regulation strength of viral capsids. Biophys. J. 85, 70–82. https://doi.org/10.1016/j.bpj.2008.06.051.

Maurice, C.F., Mouillot, D., Bettarel, Y., De Wit, R., Sarmento, H., Bouvier, T., 2010b. Microbial food web dynamics in a shallow tidal estuary. Aquat. Microb. Ecol. 31, 161. https://doi.org/10.3354/ame02386.

Mie, Y., He, C., Huang, Y., Liu, Y., Zhang, C., Chen, X., Shen, P., 2015. Salinity regulation of the interaction of haloviruses SNJ with its host and alteration of the halovirus replication strategy to adapt to the variable ecosystem. PLoS One 10, e0123874. https://doi.org/10.1371/journal.pone.0123874.

Momen, F., Billen, G., Servais, P., 2003. Mortality rates of autotrophic and fucal bacterioplankton in natural aquatic systems. Water Res. 37, 4151–4158. https://doi.org/10.1016/S0043-1354(03)00349-9.

Moja, K.D.A., Brussaard, C.P.D., 2014. Factors affecting virus dynamics and microbial host-virus interactions in marine environments. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 89, 495–515. https://doi.org/10.1093/femsec/fiu193.

Molter, A.M., Bhatbarjeej, A.S., Goel, R., 2015. Microbiological study of bacteriophage induction in the presence of chemical stress factors in enhanced biological phosphorus removal (EBP). Water Res. 81, 1–14. https://doi.org/10.1016/j.watres.2014.05.023.

Noble, R.T., Fuhrman, J.A., 1997. Virus decay and its causes in coastal waters. Appl. Environ. Microbiol. 63, 77–83.

Oladepe, O.A., 2011. Diet fluctuations in the abundance and community diversity of coastal bacterioplankton assemblies over a tidal cycle. Microb. Ecol. 63, 96–102. https://doi.org/10.1007/s00248-011-9940-6.

Paul, J.S., 2008. Prophages in marine bacteria: dangerous molecular time bombs or mechanisms for rapid bacterial evolution? mSystems 3. https://doi.org/10.1128/mSystems.00232-18.

Payet, J.P., Suttle, C.A., 2013. To kill or not to kill: the balance between lytic and lysogenic viral infection is driven by trophic status. Limnol. Oceanogr. 58, 1234–1241. https://doi.org/10.4319/lo.2013.58.6.1234.
2008. The native bacterioplankton community in the central Baltic sea is influenced by freshwater bacterial species. Appl. Environ. Microbiol. 74, 503–515. https://doi.org/10.1128/AEM.15983-07.

Santos, A.L., Mendes, C., Gomes, N.C.M., Henriques, I., Correia, A., Almeida, A., Cunha, A., 2009. Short-term variability of abundance, diversity and activity of estuarine bacterioplankton and bacterioplankton. J. Plankton Res. 31, 1545–1555. https://doi.org/10.1093/plankt/fbp063.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stresor, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Sharples, J., 2007. Potential impacts of the spring-neap tidal cycle on shelf sea primary production. J. Plankton Res. 30, 183–197. https://doi.org/10.1093/plankt/fbm088.

Shen, D., Jürgens, K., Beier, S., 2018. Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient. Environ. Microbiol. 20, 1170–1184. https://doi.org/10.1111/1462-2920.14059.

Sheyn, U., Rosenwasser, S., Lehahn, Y., Barak-Gavish, N., Rotkopf, R., Bidle, K.D., Koren, I., Schatz, D., Vardi, A., 2018. Expression profiles of host and virus during a coccolithophore bloom provides insights into the role of viral infection in promoting carbon export. ISME J. 12, 704–713. https://doi.org/10.1038/s41396-017-0004-x.

Suttle, C.A., 2007. Marine viruses. Nature 447, 365–366. https://doi.org/10.1038/nature05410.

Suttle, C.A., 2004. Marine viruses — major players in the global ecosystem. Nat. Rev. Microbiol. 5, 801–812. https://doi.org/10.1038/nrmicro1750.

Suttle, C.A., Chen, F., 1992. Mechanisms and rates of decay of marine viruses in seawater. Appl. Environ. Microbiol. 58, 3721–3729.

Thingstad, T., Lignell, R., 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat. Microb. Ecol. 13, 19–27.

Troussellier, M., Schäfer, H., Batailler, N., Bernard, L., Courties, C., Leharon, P., Muyzer, G., Servais, P., Vives-Rego, J., 2002. Bacterial activity and genetic richness along an estuarine gradient (Rhone River plume, France). Aquat. Microb. Ecol. 24, 13–24. https://doi.org/10.3354/ame028013.

Wei, W., Wang, N., Cai, L., Zhang, C., Jiao, N., Zhang, R., 2019. Impacts of freshwater and seawater mixing on the production and decay of viroplankton in a subtropical estuary. Microb. Ecol. 340 https://doi.org/10.1002/2018MC000801.

Wei, W., Wang, N., Cai, L., Zhang, C., Jiao, N., Zhang, R., 2019. Impacts of freshwater and seawater mixing on the production and decay of viroplankton in a subtropical estuary. Microb. Ecol. 340 https://doi.org/10.1002/2018MC000801.

Weinbauer, M.G., Rowe, J., Wilhelm, S., 2010. Determining rates of virus production in aquatic systems by the virus reduction approach. In: Manual of Aquatic Viral Ecology. American Society of Limnology and Oceanography, pp. 1–8. https://doi.org/10.4319/mato.2010.978-0-9845591-0-7.2.

Weinbauer, M.G., 2004. Ecology of prokaryotic viruses. FEMS Microbiol. Rev. 28, 127–181. https://doi.org/10.1016/j.femsre.2003.08.001.

Weinbauer, M.G., Winter, C., Hofle, M.G., 2002. Reconsidering transmission electron microscopy based estimates of viral infection of bacterio-plankton using conversion factors derived from natural communities. Aquat. Microb. Ecol. 27, 103–110. https://doi.org/10.3354/am027103.

Williamson, S.J., Paul, J.H., 2006. Environmental factors that influence the transition from hyogonic to lytic existence in the phclISic/Listonella pelagia marine phage-host system. Microb. Ecol. 52, 217–225. https://doi.org/10.1007/s00248-006-9113-5.

Winteg, D.M., Helton, R.R., Williamson, K.E., Bench, S.R., Williamson, S.J., Wommack, K.E., 2011. Repeating patterns of viroplankton production within an estuarine ecosystem. Proc. Natl. Acad. Sci. U.S.A. 108, 11506–11511. https://doi.org/10.1073/pnas.110907108.

Winteg, D.M., Williamson, K.E., Helton, R.R., Wommack, K.E., 2005. Tangential flow diafiltration: an improved technique for estimation of viroplankton production. Aquat. Microb. Ecol. 41, 221–232.

Winteg, D.M., Wommack, K.E., 2009. Diel and daily fluctuations in viroplankton production in coastal ecosystems. Environ. Microbiol. 11, 2904–2914. https://doi.org/10.1111/j.1462-2920.2009.02038.x.

Winter, C., Bouvier, T., Weinbauer, M.G., Thingstad, T.F., 2010. Trade-offs between competition and defense specialists among unicellular planktonic organisms: the “killing the winner” hypothesis revisited. Microbiol. Mol. Biol. Rev. 74, 42–57. https://doi.org/10.1128/MMBR.00034-09.

Winter, C., Henrdl, G.J., Weinbauer, M.G., 2004. Diel cycles in viral infection of bacterioplankton in the North Sea. Aquat. Microb. Ecol. 35, 207–216.

Wommack, K.E., Colwell, R.R., 2000. Viroplankton: viruses in aquatic ecosystems. Microbiol. Mol. Biol. Rev. 64, 69–114.

Xu, J., Sun, M., Shi, Z., Harrison, P.J., Liu, H., 2014. Response of bacterial metabolic activity to riverine dissolved organic carbon and exogenous viruses in estuarine and coastal waters: implications for CO2 emission. PLoS One 9, e102490. https://doi.org/10.1371/journal.pone.0102490.

Yoshida, T., Nishimura, Y., Watahi, H., Haruki, N., Morimoto, D., Kaneko, H., Honda, T., Yamamoto, K., Hingamp, P., Sako, Y., Goto, S., Ogata, H., 2018. Locality and diel cycling of viral production revealed by a 24 h time course cross-omics analysis in a coastal region of Japan. ISME J. 12, 1287–1295. https://doi.org/10.1038/s41396-018-0052-x.

Yu, Q., Wang, Y., Gao, J., Gao, S., Flemming, B., 2014. Turbidity maximum formation in a well-mixed macrotidal estuary: the role of tidal pumping. J. Geophys. Res. Oceans 119, 7705–7724. https://doi.org/10.1002/2014JC010228.

Zhang, R., Wei, W., Cai, L., 2014. The fate and biogeochemical cycling of viral elements. Nat. Rev. Microbiol. 12, 850–851. https://doi.org/10.1038/nrmicro3384.

Zhang, R., Weinbauer, M.G., Qian, P.-Y., 2007. Viruses and flagellates sustain apparent richness and reduce biomass accumulation of bacterioplankton in coastal marine waters. Environ. Microbiol. 9, 3008–3018. https://doi.org/10.1111/j.1462-2920.2007.01410.x.

Zhang, X., Shi, Z., Ye, F., Zeng, Y., Huang, X., 2013. Picophytoplankton abundance and distribution in three contrasting periods in the Pearl River Estuary, South China. Mar. Freshw. Res. 64, 692. https://doi.org/10.1017/MFR12303.

Zuo, S., Han, Z., Huang, Y., Han, J., Xie, M., 2016. Distributions of surficial sediments and its response to dynamic actions in the Xiamen Bay sea area, China. Acta Oceanol. Sin. 35, 9–18. https://doi.org/10.13334/j.1184-3052.s2016.02.004.