

Factors determining variations in viral abundance and viral production in a tropical estuary influenced by monsoonal cycles

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Abstract

Viruses are the most abundant, dynamic, and ubiquitous components in the marine and estuarine environments. They outnumber prokaryotes in their abundance by at least one order of magnitude, but their relationship varies across environments. The present study examined the environmental factors (temperature, salinity, inorganic nutrients, and host abundance) that influence abundance and distribution of viruses in a tropical monsoonal estuary (Cochin estuary-CE) in India. Water samples were collected daily for one year from two stations with different hydrological settings in the CE. Virioplankton abundance (VA) fluctuated daily with no definite pattern, but synchronized with the variations in prokaryotic abundance. On a seasonal scale, the abundance of virus and prokaryotes were higher during the summer months (pre-monsoon period) compared to monsoon and post-monsoon. The temporal dynamics of virioplankton was influenced by prokaryotic abundance (PA), viral production (VP), temperature, salinity and nutrient concentrations. The viral to prokaryote ratio (VPR) ranged from 1- 44. There were significant ($p < 0.001$) temporal and spatial variations in VA, PA, VP and VPR. The non-parametric distance-based linear regression analysis indicated viable prokaryotic abundance as the most important predictor variable for viral abundance. Other determining factors such as salinity, phosphate, and nitrate might have an indirect influence on the viral pool by altering host dynamics either by decreasing susceptibility to infection or by modifying characteristics of viral proliferation. Elevated densities of virus and prokaryotes during pre-monsoon season suggested an active and important participation of viruses in the dynamics of the microbial communities in this estuary during dry pre-monsoon season.

Keywords: Viruses; Prokaryotes; Virus to Prokaryote ratio; Viral Production, Cochin estuary

Introduction

Viruses are abundant and ubiquitous components of the aquatic ecosystems. They play crucial roles in the regulation of carbon and nutrient fluxes, govern bacterial diversity and diversification, mediate lateral gene transfer and have direct implications on the global climate (Suttle, 2007). Typically, viral abundance is in the range of 10^4 - 10^7 viruses mL^{-1} in marine environments and their abundance decreases with increase in depth and distance from the shore (Paul et al., 1993). In fact, many intrinsic and extrinsic factors affect the distribution and abundance of viruses. Intrinsic factors include viral factors such as virus life cycle, burst size, host range, and capsid size and host intrinsic factors include host size, abundance, and physiology (Suttle, 2016). Extrinsic factors such as temperature, salinity, pH and nutrient concentrations can have a direct effect on the virus abundance and persistence (Suttle, 2016). Previous studies on viral distribution in aquatic environments revealed positive correlations with bacterioplankton distribution (Hewson et al., 2001) and chlorophyll *a* (Maranger and Bird, 1995). Virioplankton abundance is seasonally driven by primary and secondary productivity of the ecosystem. High production rates of viruses resulting from the lysis of host cells and increases with the productivity of the system (Maranger and Bird, 1995). Although viral abundance and infection rates have been estimated to be high in eutrophic systems, some studies suggest that on a large spatial scale, there is no direct link between virioplankton abundance and trophic conditions (Corinaldesi et al., 2003).

The virus-to-prokaryote ratio (VPR) is used as a proxy to study the relationship between the viruses and prokaryotes in the environments (Wommack and Colwell, 2000). Usually high VPR is considered as a feature of high and progressing viral oscillations in the system, whereas a low VPR is suggestive of high viral decay rates or low viral abundance. VPR ratio provides an insight in to the distribution pattern of viruses and relationship between bacteria and virus in an ecosystem. However, environmental factors play important roles in regulating virus dynamics and host-virus interactions.

While the distribution of virioplankton in freshwater and marine environments is well documented (Corinaldesi et al., 2003; Wommack and Colwell 2000), there are only a few reports on the dynamics of virus from estuaries from temperate (Almeida et al., 2001; Wommack et al., 1992), subtropical (Auguet et al., 2009; Hewson et al., 2001), and tropical regions (Parvathi et al., 2015; Jasna et al., 2017). Cochin estuary is the second largest and most productive tropical estuarine system along the southwest coast of India. It has two openings to the Arabian Sea at Azhikode and Cochin. The estuary is about 80 km long, a few hundred meters to 4 km wide with a depth ranging from 1.5 to 7.0 m, and has a tidal amplitude of 1 m. The estuary is immensely influenced by monsoons with an apparently fresh water-dominated system during the southwest monsoon (SWM) (June-September). Typically, monsoonal rain begins in the study domain during the first week of June and peaks during mid-June to August (Qasim, 2003). During the monsoon, heavy rainfall (40–50 cm in a few hours) occurs in the region (Qasim, 2003) resulting in near-zero salinity in the estuary (Jyothibabu et al., 2006). The estuary progressively changes to partially mixed during post monsoon (PM) (November-January) and to well mixed during pre-monsoon (PRM) (February-May) (Qasim 2003).

Previous studies on viral dynamics showed that the distribution of virioplankton was determined by the abundance of bacterioplankton and Chlorophyll *a* in Cochin estuary (Parvathi et al., 2015). The seasonal variations in viral abundance were mostly dependent on prokaryotic distribution, which in turn were directly or indirectly influenced by seasonal fluctuations in salinity (Parvathi et al., 2015; Jasna et al., 2017). However, there is a lack of understanding of factors that

induce short-term variations in virioplankton abundance. In this study, we report the variations in viral abundance (VA), prokaryotic abundance (PA), and viral production (VP), along with other physicochemical parameters (temperature, salinity, and inorganic nutrients) based on 365 days of observations in a tropical estuary (Cochin estuary) influenced by monsoonal rains.

Materials and methodology

2.1 Study site and sampling

Samples were collected daily from two stations (Figure. 1) in the Cochin estuary for 365 days during 2013-2014. The station S1 was located at the Cochin inlet and represented a highly dynamic environment, whereas S2 was located on the northern arm of the estuary, 3 km away from S1. Water samples were collected from 0.5 m depth using 5 L niskin sampler (Hydro-Bios, Kiel-Holtenau, Germany). The water samples collected were kept in the iceboxes and brought to the laboratory within an hour of collection.

2.2 Physicochemical parameters

A portable conductivity, temperature and depth (CTD) profiler (Seabird19 plus V2, SBE Scientific, Washington) was used to record vertical temperature, and salinity (accuracy $\pm 0.001^\circ\text{C}$ for temperature and ± 0.001 S/m for conductivity). Physico-chemical parameters were measured daily. The dissolved oxygen (DO) was also estimated by Winkler's method. Dissolved inorganic nutrients such as ammonia (NH_4), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), and silicate (SiO_4) were analysed spectrophotometrically by following standard procedures (Grasshoff et al., 1999). Chlorophyll *a* (Chl *a*) was estimated using standard procedure (Parsons et al., 1984).

2.3 Biological parameters

2.3.1 Viral abundance, Prokaryotic abundance, and Total viable counts (TVC)

The viral abundance and prokaryotic abundance were estimated after filtration of water samples on to a Al_2O_3 coated anodisc filter (0.02 μm pore-size, Whatman USA), stained using SYBR green I fluorescent dye and enumerated under an epifluorescence microscope (Olympus BX 41, Japan) (Patel et al., 2007). VPR was determined as the ratio of abundance of viruses to prokaryotes. The physiologically active bacteria (TVC) was estimated after mixing the samples yeast extract and antibiotic cocktail (nalidixic acid, pipemidic acid, piromidic acid and cephalixin) as previously described (Joux and Lebaron, 1997).

2.3.2 Viral Production (VP)

Viral production was estimated at fortnightly intervals in both stations. In order to estimate VP, the dilution technique by Wilhelm and colleagues was employed (Wilhelm et al., 2002). The first order regression of viral abundance versus time in triplicate incubation after correcting for the loss of the prokaryotic hosts between experimental samples and natural samples were used for calculating viral production using the formula, $\text{VP} = m \times (\text{P}/\text{p})$ where 'm' is the slope of the regression line, 'p' is the concentration of prokaryotes after dilution and 'P' is the concentration of prokaryotes prior to dilution (Hewson and Fuhrman, 2007). Viral mediated mortality of prokaryotes (VMM) was estimated by dividing the viral production estimate and the burst size as determined (average burst size was 50) by transmission electron microscopy. The fraction of bacterial abundance lysed (% BA lysed) by viruses per day was estimated from the bacterial abundance and viral mediated mortality. Viral turnover rates were determined by dividing viral abundance by viral production rates (Helton et al., 2005; Winget et al., 2005).

2.4 Statistical Analysis

The correlation between different parameters such as VA, PA, VP, TVC, VPR, and other physicochemical parameters was performed using Pearson correlation. To determine whether the seasonal and spatial variation were significant, analysis of variance (one way ANOVA) was performed using SPSS software (version 15). The interrelationships between the biological components and their environmental variables were analysed using Principal component analysis (PCA). To determine the relative importance of predictor variable for VA and VP, distance based linear modeling (DistLM) analysis using PAST software (version 3) was used (McArdle and Anderson, 2001).

3. Results and Discussion

3.1 Temporal variations in physico-chemical parameters.

The estuary was freshwater dominated during MON (June-September). During MON, the salinity at the inlet (S1) was very low (5.59 ± 6.62 psu) when the temperature was $27 \pm 0.99^\circ\text{C}$. During MON, due to heavy rainfall and freshwater influx from the adjoining rivers, the salinity in the CE decreased drastically. The salinity started to build up in the estuary (16.86 ± 8.26 psu) due to the retrieval of monsoonal rains in PM (October- January), and reached its maximum (24.48 ± 6.02 psu) during PRM (February-May). The distribution of environmental parameters during the study period showed seasonal fluctuations (Figure 2 and Table 1). The temperature in CE did not exceed 8°C on an annual scale. The temperature ranged from 25.02 to 32.71°C at S1 and 25.12 to 33.14°C at S2. The variation in the water temperature may be due to the influence of season (Saraladevi et al., 1983). The salinity, however displayed variations ranging from 0.23 - 34.46 psu at S1 and 0.13 - 32.48 psu at S2 respectively. The seasonal variation in salinity was significant ($p < 0.05$) which is evident from both stations in the present study and comparable with the previous reports from CE (Madhupratap, 1987; Jyothibabu et al., 2006). Both salinity and temperature displayed significant seasonal variations ($p < 0.05$) at both stations.

The DO ranged from 0.09 - 9.8 mg L⁻¹ during the study period of one year at the two stations. The water column was well oxygenated during MON (2.50 - 8.03) but was less saturated during PRM (2.53 - 4.58). Spatially, DO was higher at S2 (5.24 ± 1.01 mg L⁻¹) compared to S1 (4.96 ± 1.04 mg L⁻¹). All the inorganic nutrients were high (NO_2 , 0.92 ± 1.36 μM ; NO_3 , 16.71 ± 11.08 μM ; PO_4 , 1.80 ± 1.13 μM ; and SiO_4 , 43.10 ± 33.10 μM) in both the stations. The silicate concentration was high (0.39 to 138.63 μM) during MON, which decreased during PM (30.67 ± 25.67 μM) and PRM (22.30 ± 16.07 μM) (Figure 2). The nitrate (NO_3) concentration was also highest during MON (24.91 ± 7.73 μM) followed by PM (14.22 ± 11.85 μM), and PRM (12.76 ± 8.66 μM), probably due to high river discharge during MON (Table 1). At all the stations, the concentration of NO_2 decreased considerably during PM and PRM (Figure 2). On the contrary the concentration of PO_4 was low and comparable during all the seasons. The lowest phosphate concentration was found during MON (1.19 ± 0.18 μM), followed by PM (1.31 ± 0.78 μM) and PRM (2.10 ± 1.15 μM). The phosphate concentration was comparatively higher in PRM. Desorption of phosphate from the suspended particles occur due to high salinity resulting in higher values in PRM (Reddy and Sankaranarayanan, 1972; Martin et al., 2008). In short, physiochemical parameters showed significant variations in CE (Table 1 and 2). The high levels of nutrients such as nitrate and silicate are observed throughout the study period. This is mainly contributed by several rivers that empty into the system especially during MON. Additionally, there are several non-point sources such as agricultural and aquaculture along the banks of the Cochin backwaters that contribute to the

nutrient budget of the system (Qasim, 2003; Saraladevi et al., 1983; Jyothibabu et al., 2006). The Chl *a* (0.07 to 27.01 mg m⁻³) and pheophytin (2.13 to 22.18 mg m⁻³) concentrations were high during the study period. Overall, Chl *a* showed significant temporal variations, being highest during PRM as reported previously (Madhu et al., 2007). The overall trend in distribution of physicochemical parameters showed significant seasonal variation ($p < 0.05$), in concurrence with previous reports from CE (Qasim, 2003; Jyothibabu et al., 2006; Surya et al., 2015; Parvathi et al., 2015).

3.2 Temporal variations in viral abundance (VA), prokaryotic abundance (PA), virus- to-prokaryote ratio (VPR), and viral production (VP)

Viral abundance displayed significant ($p < 0.05$) temporal variations at both the sites (Figure 3, Table 2). Over an annual scale, the variations in viral abundance was higher ($1.04\text{--}49.15 \times 10^6$ VLPs mL⁻¹) compared to variations in prokaryotic abundance ($0.28 - 10.92 \times 10^6$ cells mL⁻¹). However, there was no particular pattern in the daily fluctuations of VA and PA at both the stations. The VA was found to be significantly ($p < 0.001$) high at S1 ($1.32\text{--}39.64 \times 10^6$ VLPs mL⁻¹) when compared to S2 ($1.14 - 30 \times 10^6$ VLPs mL⁻¹). But, PA was higher at S2 ($0.28 - 3.64 \times 10^6$ cells mL⁻¹) compared to S1 ($0.23\text{--}4.98 \times 10^6$ cells mL⁻¹). The virus-to-prokaryote ratio (VPR) ranged from 1.09 to 44 (Figure 3). Spatially, VPR was significantly higher ($p < 0.05$) at S1 (12.15 ± 7.29), compared to S2 (8.13 ± 6.83) respectively (Table 2). The high VPR values at both the stations suggests a tight coupling between PA and VA. The VPR values were comparable with our previous reports from CE (5-30) (Jasna et al., 2017; Parvathi et al., 2015). VPR values were also comparable with values from other environments such as the Chesapeake Bay (12.6–25.6), Lake Geneva (56.6 ± 5.6), and temperate lakes in France (2-42) (Parvathi et al., 2013; Pradeep Ram et al., 2015; Wommack et al., 1992). Viral production (VP) measured at S1 and S2 on a fortnightly basis ranged from $0.17\text{--}24.48 \times 10^9$ VLPs mL⁻¹ h⁻¹ during the study period (Figure 4).

Seasonally, a pronounced pattern in VA and PA was observed with highest abundance during PRM and lowest during MON. Day-to-day variations were high during the PRM when compared to the MON and PM. The maximum abundance was recorded during the warmer months (April-May). VA increased by one order of magnitude from MON ($5.30 \pm 1.19 \times 10^6$ VLPs mL⁻¹) to PRM ($17.76 \pm 9.28 \times 10^6$ VLPs mL⁻¹). This seasonal pattern was also observed in prokaryotic abundance with highest abundance during PRM ($2.13 \pm 1.52 \times 10^6$ cells mL⁻¹) and least during MON ($0.51 \pm 0.12 \times 10^6$ cells mL⁻¹) (Table 1, Figure 3). This is interesting as both prokaryotic hosts and viral abundance were higher during PRM indicating active viral-host interactions. The oscillations in VPR was high during PRM (2.4 to 43.67) followed by PM (1.36 to 33.94) and MON (1.09 to 22.72) (Figure 3). There were significant seasonal variations in VA, PA and VPR ($p < 0.05$) (Table 2). The microbial density across aquatic ecosystems seems to influence VA and thereby the VPR values (Knowles et al., 2016; Wigington et al., 2016). Seasonal variations and terrestrial nutrient inputs are expected to change VPR in every ecosystem.

Similarly, high VP rates were recorded during PRM ($15.18 \pm 5.44 \times 10^9$ mL⁻¹ h⁻¹) and lowest during MON ($2.36 \pm 2.09 \times 10^9$ mL⁻¹ h⁻¹). There were significant temporal variations in VP between the two stations studied ($p < 0.05$) (Table 2). VMM was generally high and varied from 0.37 to 68.6%. There was significant variation in VMM with season ($p < 0.05$). VMM was higher during PRM (50.28 ± 12.34) compared to PM (23.91 ± 13.87) and MON (7.88 ± 5.12) (Figure 5). There was no specific pattern in viral lysis rates but the lysis rates were significantly ($p < 0.001$) higher during PRM (66%) compared to MON (<2%). The carbon released through viral mediated

bacterial lysis also showed significant seasonal variations. The rates ranged from 1.3 to 267 $\mu\text{CL}^{-1}\text{d}^{-1}$. The rates were higher during PRM ($209.91 \pm 48.88 \mu\text{CL}^{-1}\text{d}^{-1}$) when compared to MON ($37.88 \pm 32.44 \mu\text{CL}^{-1}\text{d}^{-1}$). The results of this study are comparable with previous studies from CE (Jasna et al., 2018). Viral turnover time (VTT) ranged from 0.3 to 15 d^{-1} and was high during MON (Figure 5). These temporal variations in viral parameters could be attributed to host factors as well as physico-chemical factors in the CE. Virus infect prokaryotes and an equilibrium exist between cell growth and cell lysis (Thomas et al., 2011). Previous studies from CE suggested the dependence of viral dynamics on bacterial population and physico-chemical factors such as salinity and light (Parvathi et al., 2013). Trophic status of the system is an important factor influencing the viral production and distribution (Maranger and Bird, 1995; Hewson et al., 2001; Danovaro et al., 2003). Eutrophic environments support a high standing stock of bacteria compared to oligotrophic environments indicating that a higher number of hosts are available for viral infection in eutrophic conditions (Danovaro et al., 2003). Although several studies suggest that viral abundance increases with increased nutrient availability (Maranger and Bird, 1995; Hewson et al., 2001; Danovaro et al., 2003), others show lack of such a relationship (Corinaldesi et al., 2003). The abundance patterns influencing the seasonal variations in microbial mortality have been recorded in several aquatic environments (Tsai et al., 2008; Ortmann et al., 2011; Tsai et al., 2015). However, though CE is characterised by excess nutrients, it does not always lead to a substantial increase in the rate of phytoplankton production. This is particularly because high concentration of nutrients alone is not conducive to a substantial increase in phytoplankton production (Qasim, 1973). Physico-chemical factors such as salinity influence the distribution of prokaryotes which in turn influence the viral distribution and activity. Seasonally, salinity is reported to be the second most important predictor factor for viral abundance after prokaryotic abundance in this estuary (Jasna et al., 2017). Due to seasonal changes brought in by monsoons and tidal influx, this estuary undergoes extensive transformation from a well-mixed estuary during MON, to a highly stratified estuary during PRM. Variations in salinity influence viral activity indirectly influencing the host activity or directly by inactivation of viruses or by altering the integrity of capsid receptors which in turn inhibit the binding of viruses to their hosts (Kukkaro and Bamford, 2009; Wells and Deming, 2006).

3.3 Statistical analysis

The potential relationships among physico-chemical variables and biological components such as prokaryotic abundance (PA), viral abundance (VA), viral production (VP) and total viable count (TVC) were tested by Karl Pearson's correlation analysis (Table 3). The VA, VP, PA, and TVC showed significant positive correlation with salinity, and temperature ($p < 0.001$), whereas no correlation was observed for DO, NO_3 , PO_4 , and SiO_4 (Figure 3). VA and PA positively correlated with temperature. Warm waters favour the growth of bacteria and thereby support the abundance of viruses, especially in summer. Other factors, such as salinity and nitrate might have indirectly influenced the viral pool by altering host dynamics either by decreasing susceptibility to infection or by modifying characteristics of viral proliferation (Finke et al., 2017). The bacterial enzymatic activity (such as protease and nuclease) enhanced with temperature, which would in turn increase viral inactivation and viral decay (Noble and Fuhrman, 1997). High temperature also influences UV-induced virus decay (Noble and Fuhrman, 1997) and grazing (González and Suttle, 1993).

One way ANOVA demonstrated the significant spatial and temporal variations. PCA was performed to demonstrate the factors determining the variations in VA, PA, VPR, and VP. The biplots derived from the PCA analysis depicted the dependence of VA and VPR on PA and

environmental parameters like Salinity, Temperature, DO, NO₃, PO₄, SiO₄ and Chl *a* (Figure 6). The two major principal coordinates could explain 81% variation at S1 and 83% at S2. The effects of physico-chemical and biological parameters for explaining VA was demonstrated while using non-parametric distance-based linear regression model analysis (DistLM) (Table 4). DistLM analysis showed that the most important determining factor/predictor variable for the variations in VA was viable prokaryotic abundance (TVC) as they are the most abundant hosts for viruses in CE. The other important predictor variables for VA were salinity, temperature, and nitrate ($p = 0.001$). In the case of VP, most important predictor variables were salinity, temperature, VA, and NO₃. The viral dynamics may be influenced by environmental and biological factors that modify infectivity, degrade or remove virus particles, adsorption, and proliferation within the host cell, e.g. temperature, UV, nutrients, host physiology (Wommack and Colwell, 2000; Mojica and Brussaard, 2014). Salinity is often reported to be related to viral abundance (Jiang and Paul 1994; Auguet et al., 2005; Parvathi et al., 2015; Jasna et al., 2017; Marine et al., 2013). In the present study, the viral abundance was higher in PRM, when both salinity and temperature were high. The previous reports from CE suggested salinity as a major factor that determine the zone-wise distribution of viruses directly or indirectly through controlling the bacterial distribution. During MON, the abundance of virus and bacteria decreases due to the gradual dilution of estuarine waters through riverine influx or by alteration of viral integrity because of salinity or by other factors (Jiang and Paul, 1994). Elsewhere, it is reported that on a seasonal cycle, the highest abundance of viruses were in summer when the host abundance and metabolic activity were higher (Jiang and Paul, 1994; Williamson et al., 2002; Auguet et al., 2005). In the present study, the viral abundance was higher in PRM, when both salinity and temperature were high. The previous reports from CE supported salinity as a major factor that determines the zone-wise distribution of viruses directly or indirectly through controlling the bacterial distribution (Jasna et al., 2015).

4. Conclusion

Viruses are important biological agents that control the marine microbial mortality, productivity and biogeochemical cycles. This study provides accurate estimates of viral and bacterial abundance on a daily basis for a period of one year at two locations in the CE and draws conclusive evidence on factors influencing their daily distribution and variations. VA, VPR and VP was found to be directly influenced by prokaryote variables, which in turn varied with different physicochemical factors such as salinity, nitrate, and phosphate. Over the entire data set prokaryotic abundance was, by far, the most important predictor of viral abundance. There were no definite pattern of daily variations for both VA and PA. But, the seasonal pattern were very conclusive. High viral abundance, high viral production and high viral induced prokaryotic mortality indicated that virioplankton play significant roles in the biogeochemical cycles in the tropical monsoonal estuary. The viral induced prokaryotic mortality released significant amount of carbon in the estuary particularly during PRM. We conclude that prokaryotic abundance is the most important biological factor and environmental factors like salinity, nitrate and phosphate positively impact viral abundance and production in this monsoonal estuary. This would enable us to incorporate these factors in ecological models involving viruses for the studying food web dynamics

Author Contributions

JV, AVJ, AS and AAJ have collected and analysed samples. MD have performed analysis of chemical parameters. MNV performed analysis of chlorophyll *a*. JV and PA has collected the samples, performed statistical analysis of the data and preparation of the manuscript.

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Legends

Table: 1 mean and range (min-max) of environmental and biological parameters. S1, and S2 are the two sampling stations in the CE. Temp-Temperature, DO – Dissolved Oxygen, , NO₃ – Nitrate, PO₄ - Phosphate, SiO₄ – Silicate, PA- Prokaryotic abundance, TVC-Total Viable Bacteria, VA-Viral abundance, VP-Viral Production, VPR-Virus to prokaryotes ratio

Table: 2 Comparison of physicochemical and biological parameters based on One-way Analysis of Variance. Temp-Temperature, DO–Dissolved Oxygen, NO₃ – Nitrate, PO₄ - Phosphate, SiO₄ – Silicate, PA- Prokaryotic abundance, TVC-Total Viable Bacteria, VA-Viral abundance, VP-Viral Production, VPR-Virus to prokaryotes ratio. * indicates *P<0.05, ** P<0.01 and *** p<0.001 level (N=365).

Table:3 Pearson Correlation coefficient between virus, prokaryotic abundances, and environmental parameters for the whole data set N=730, Significant correlations are P<0.05, ** P<0.01 and *** p<0.001.Temp-Temperature, DO – Dissolved Oxygen, NO₂ – Nitrite, NO₃ – Nitrate, PO₄ - Phosphate, SiO₄ – Silicate, PA- Prokaryotic abundance, TVC-Total Viable Bacteria, VA-Viral abundance, VP-Viral Production, VPR-Virus to prokaryotes ratio.

Table: 4 DistLM (non-parametric distance-based linear regression analysis) for understanding the effects of physico-chemical parameters on biological variables explaining VA and VP. Bold values indicate significant * *p*-values (*p* = 0.001). The abbreviations used are DO: Dissolved oxygen, NO₃: Nitrate, PO₄: Phosphate, VA: Viral abundance, PA: Prokaryotic abundance, Chl *a*: Chlorophyll *a*. F^P: Pseudo F-values, P_c: *p*-values, SS (Trace): portion of sum of squares relative to the analyzed predictor variable and prop: proportion of variation explained by the explanatory variables

Figure: 1 Station map showing sampling locations in CE. Red circle representing sampling location in 2013-2014 at S1 and S2 in the CE.

Figure: 2 Daily variations of physicochemical parameters such as (a) Temp-Temperature (b) salinity, (c) DO – Dissolved Oxygen (d) NO₃ – Nitrate (e) PO₄ – Phosphate and (f) SiO₄ – Silicate. S1 and S2 are the two sampling stations which are represented by red and blue colours respectively. The black dotted line separating the seasonal variation in MON, PM and PRM.

Figure: 3 Daily variations of biological parameters such as (a) VA-Viral abundance (b) PA-Prokaryotic and (c) VPR-Virus to prokaryotes ratio. S1 and S2 are the two sampling stations which are represented by red and blue colours respectively. The black dotted line separating the seasonal variation in MON, PM and PRM.

Figure: 4 Viral Production in S1 and S2 with standard deviation, and the dotted line separating the seasonal variation in MON, PM and PRM.

Figure 5 Monthly variations of (a and b) Viral-mediated mortality (VMM %), (c and d) Viral turnover time (VTT) (d⁻¹), (e and f) % of BA lysed, and (g and h) Carbon released (µg/C/L/day) during S1 and S2 separately.

Figure: 6 A) Principal component analysis (PCA) biplot representing the distribution of biological parameters and interrelationship of physicochemical and biological parameters during S1 (Circle) and S2 (box) respectively. Salinity, Temp-Temperature, DO – Dissolved Oxygen, NO₂ – Nitrite, NO₃ – Nitrate, PO₄ - Phosphate, SiO₄ – Silicate, PA- Prokaryotic abundance, TVC-Total Viable Bacteria, VA-Viral abundance, VP-Viral Production, VPR-Virus to prokaryotes ratio. The three colours differentiate the seasonal variations.

Table: 1

parameters	Spatial		Seasonal		
	S1	S2	MON	PM	PRM
Salinity	17.72(0.43-33.9)	14.97 (0.27-31.18)	5.76 (0.23-25.78)	18.60 (2.61-31.73)	23.24 (7.21-33.9)
Temperature (°C)	29.58 (25.52-31.68)	29.68 (24.58-32.71)	28.10 (25.52-29.78)	29.85 (28.15-32.48)	30.87 (26.91-32.71)
DO (mg/L)	4.96 (2.47-9.01)	4.04 (2.26-8.16)	5.06 (2.49-8.03)	4.76 (1.15-44.87)	4.44 (2.55-9.01)
NO ₃ (µM)	13.67 (0.13-52.77)	21.21 (0.10-55)	0.54 (3.04-53.98)	0.43 (1.15-44.89)	1.823 (0.14-55)
PO ₄ (µM)	1.4 (0.01-5.01)	2.49 (0.08-4.94)	23.16 (0.21-5.01)	12.76 (0.07-3.87)	14.21 (0.01-3.97)
SiO ₄ (µM)	37.76 (1.55-151.41)	49.68 (0.39-121)	2.09 (5.37-147.73)	1.31 (1.62-151.41)	2.09 (0.39-84.24)
Chl a (mg m ⁻³)	1.9 (0.07-13.9)	3.89 (0.18-20.19)	2.56 (0.18-27.01)	2.21 (0.07-15.9)	4.03 (0.17- 27.01)
PA (10 ⁶ Cells/mL)	1.08 (0.23-4.51)	2.42 (0.28-3.64)	76.11 (0.23-2.14)	30.67 (0.34-2.59)	22.29 (0.32-4.51)
TVC (10 ⁶ Cells/mL)	0.54 (0.11-1.79)	0.57 (0.13-1.83)	0.90 (0.11-0.59)	1.32 (0.24-1.96)	2.134 (0.16-1.24)
VA (10 ⁶ VLPs/mL)	11.74 (0.13-3.96)	15.64 (0.11-2.99)	7.66 (0.13-1.61)	10.42 (0.29-2.62)	17.76 (0.11-3.96)
VP (10 ⁹ VLPs/mL)	7.46 (0.11-24.48)	7.14 (0.17-23.56)	2.36 (0.02-6.34)	6.55 (1.03-10.92)	16.79 (6.81-24.48)
VPR	12.15±7.27	8.13±4.83	9.46 (1.09-22.72)	10.17 (1.12-33.94)	10.86 (2.45-43.67)

Table: 2

Parameters	S1	S2	Station	Season	Interaction
Salinity	175.879**	169.127**	19.155**	586.46**	5.102**
Temperature	304.85**	45.837**	3.463*	203.774**	13.597**
DO	21.207**	3.734*	168.076**	44.796**	6.09**
NO ₂	72.747**	45.823**	32.542**	164.154**	14.445**
NO ₃	87.146**	118.411**	76.309**	151.538**	53.396**
PO ₄	11.76**	17.468**	142.573**	78.657**	11.918**
SiO ₄	156.684**	193.605**	26.362**	611.388**	9.125**
PA	48.118**	79.498**	457.569**	232.944**	59.205**
VA	112.985**	73.511**	165.488**	331.805**	13.964**
VPR	15.309**	1.426	50.956**	6.228**	11.347**
TVC	118.23**	126.432**	78.23**	98.23**	4.18**

Table: 3

	VA	PA	VPR	TVC	VP	Salinity	TEMP	DO	NO ₃	PO ₄
VA										
PA	0.20***									
VPR	0.35***	-0.02								
TVC	0.16***	0.20***	0.02							
VP	0.46***	0.12***	0.30***	0.06						
Salinity	0.20***	0.31***	0.05	0.48***	0.58***					
Temp	0.09**	0.32***	-0.06	0.46***	0.54***	0.48***				
DO	-0.04	-0.15***	0.02	-0.17***	-0.08**	-0.21***	-0.22***			
NO ₃	-0.10**	-0.14***	-0.06	-0.32***	-0.51***	-0.62***	-0.41***	0.12***		
PO ₄	-0.01	0.12***	-0.08*	0.01	-0.07*	-0.15***	0.14***	-0.19***	0.18***	
SiO ₄	-0.05	-0.15***	-0.01	-0.36***	-0.31***	-0.65***	-0.46***	0.24***	0.63***	0.09**

Table: 4

Variable (VA)	SS(trace)	F ^P	Pc	Prop.
Salinity	1914.7	57.93	0.001*	0.72476
Temperature	1128.5	16.405	0.001*	0.42716
DO	515.75	5.3369	0.02	0.19523
NO ₃	1850.5	51.443	0.001*	0.70045
PO ₄	288.01	2.6919	0.094	0.10902
PA	522.21	5.4201	0.016	0.19767
TVC	555.32	5.8552	0.001*	0.2102
Chlorophyll <i>a</i>	262.08	2.4228	0.105	9.92E-02
Variable (VP)				
Salinity	1917.4	58.225	0.001*	0.72577
Temperature	1119.1	16.168	0.001*	0.42359
DO	522.5	5.4239	0.014	0.19778
NO ₂	407.64	4.0141	0.028	0.1543
NO ₃	1822.2	48.909	0.001*	0.68974
PO ₄	264.27	2.4453	0.106	0.10003
PA	502.66	5.1695	0.019	0.19027
VA	1696.44	27.8759	0.001*	0.26362
TVC	417.17	4.1255	0.035	0.15791
Chlorophyll <i>a</i>	242.28	2.2213	0.124	9.17E-02

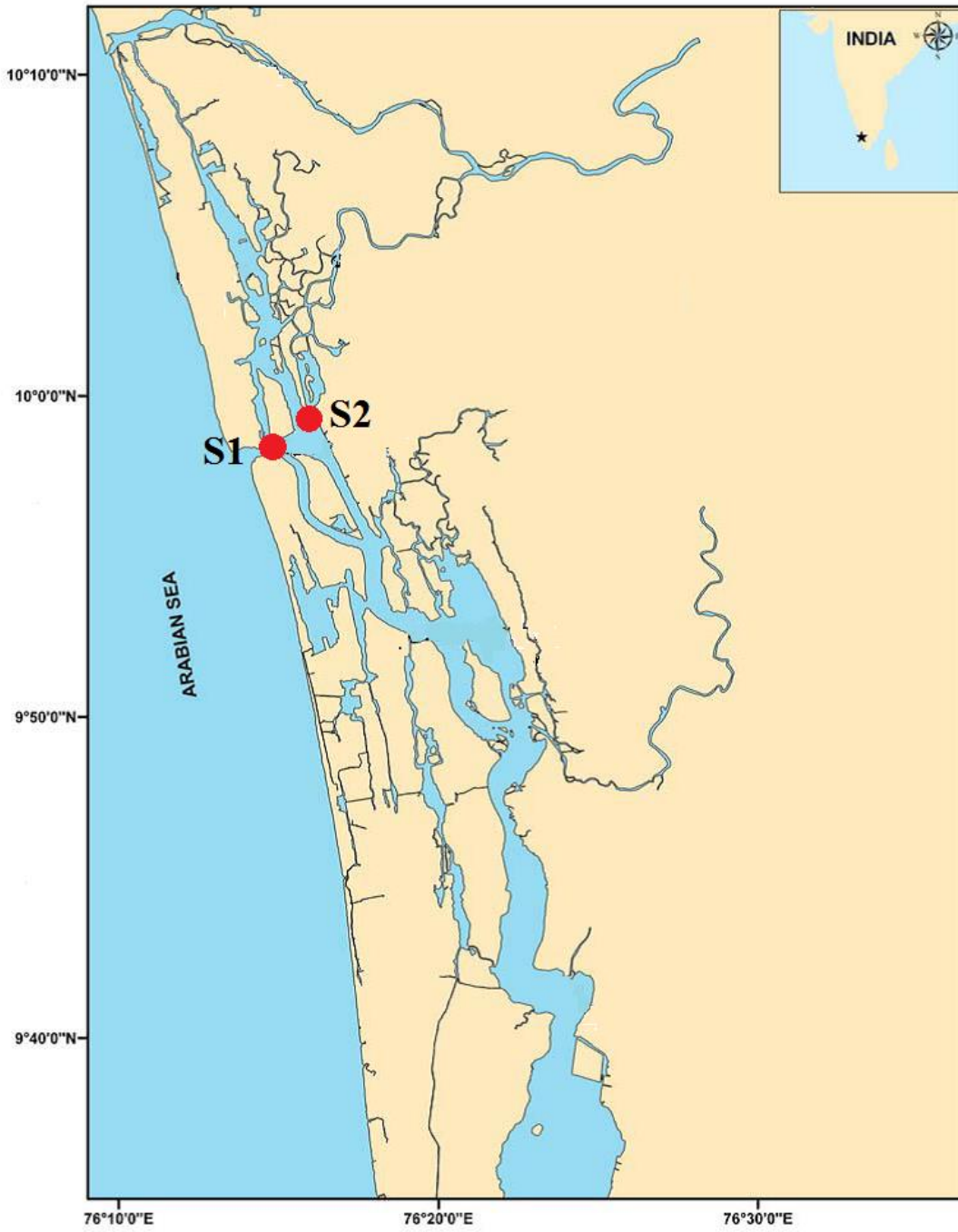


Figure: 1

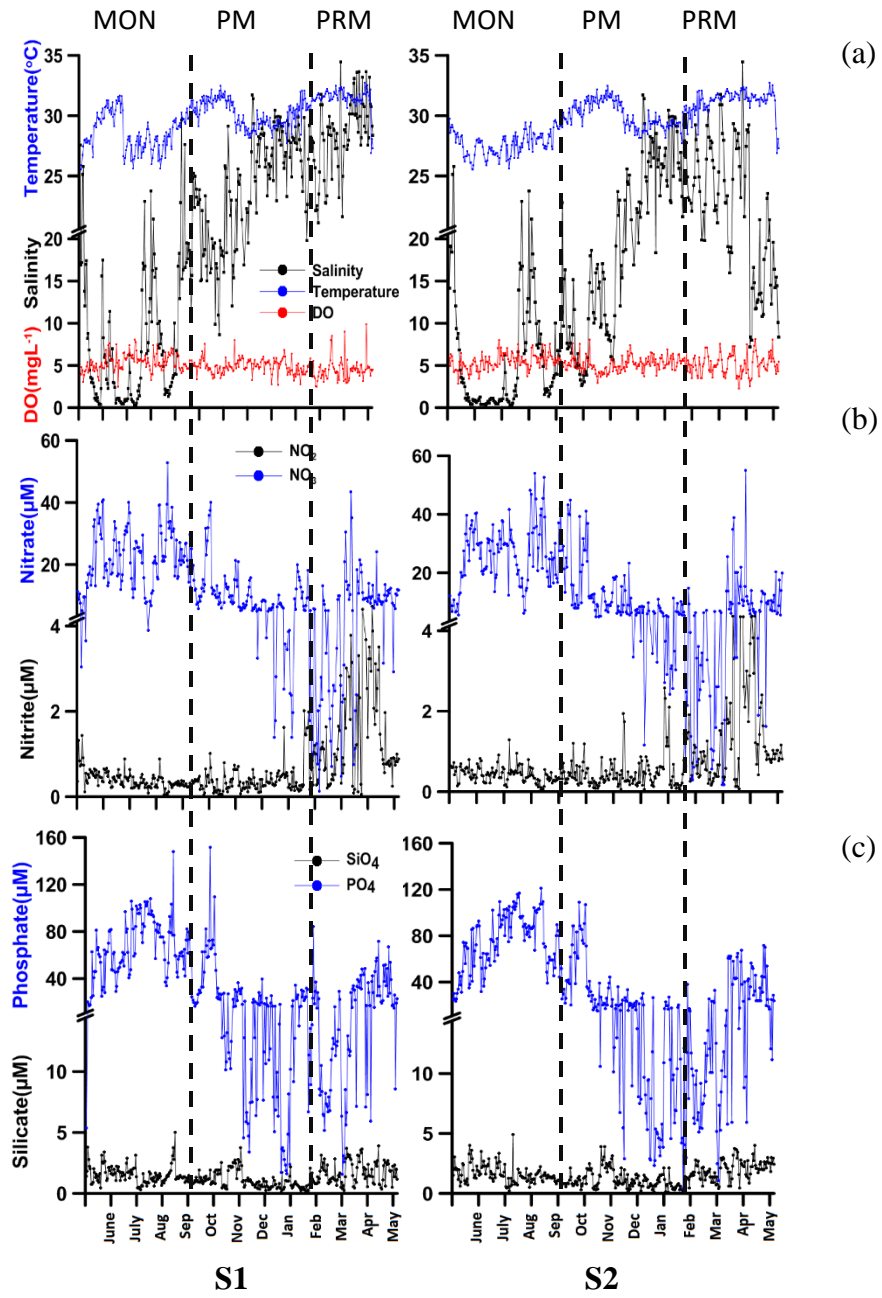


Figure: 2

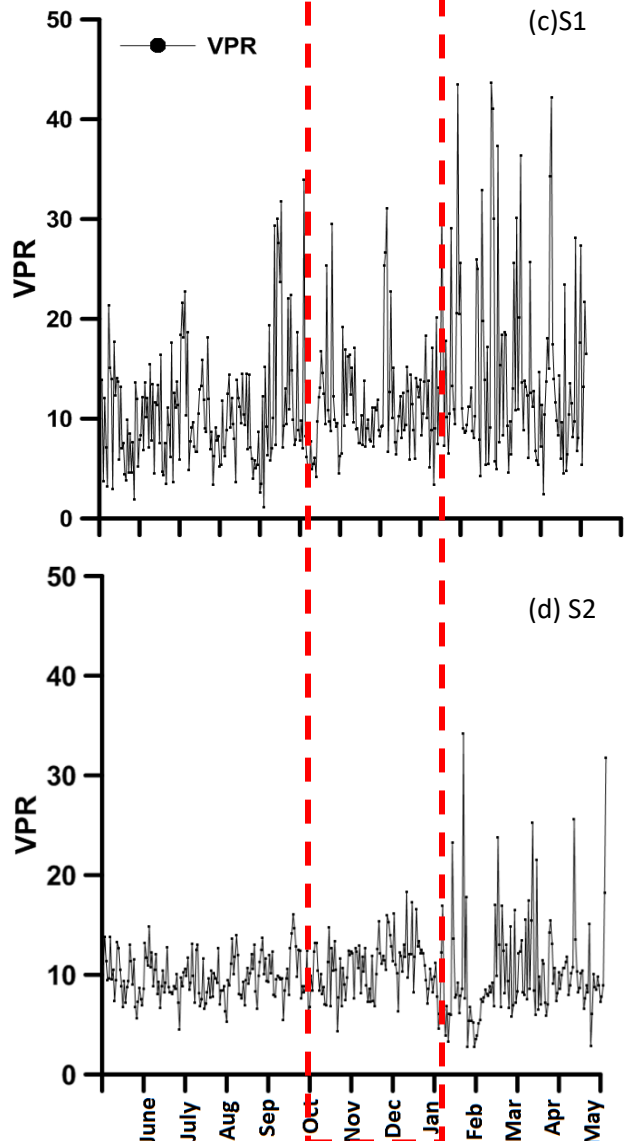
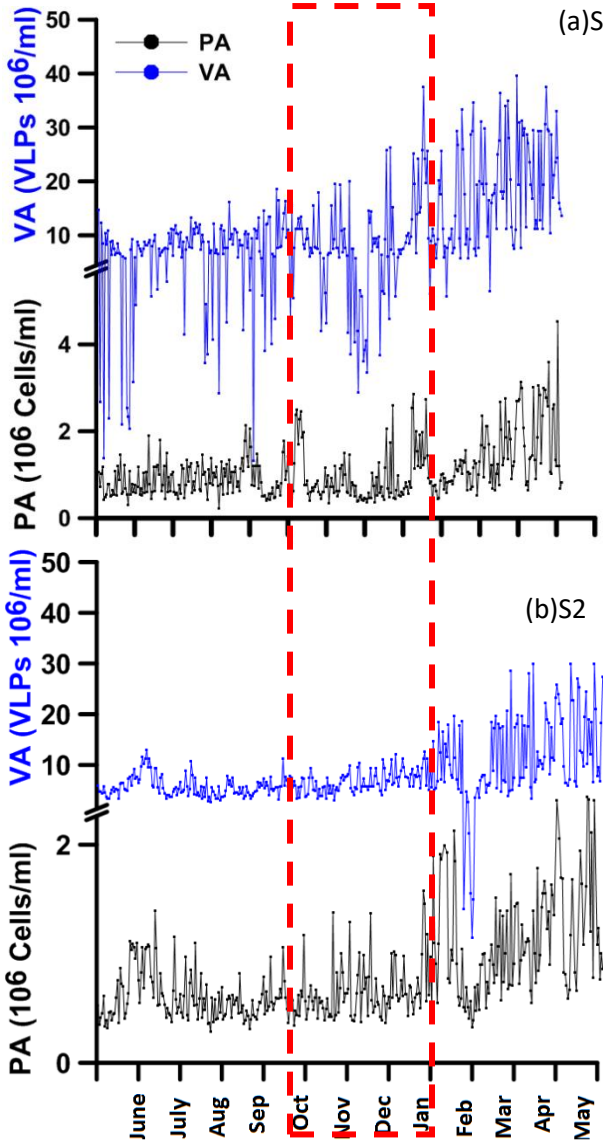


Figure: 3

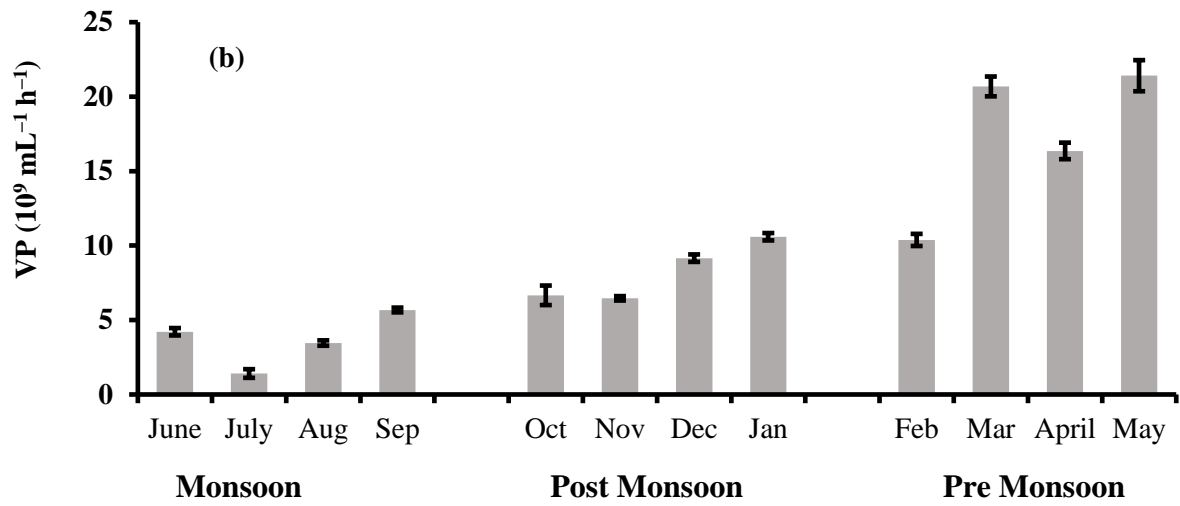
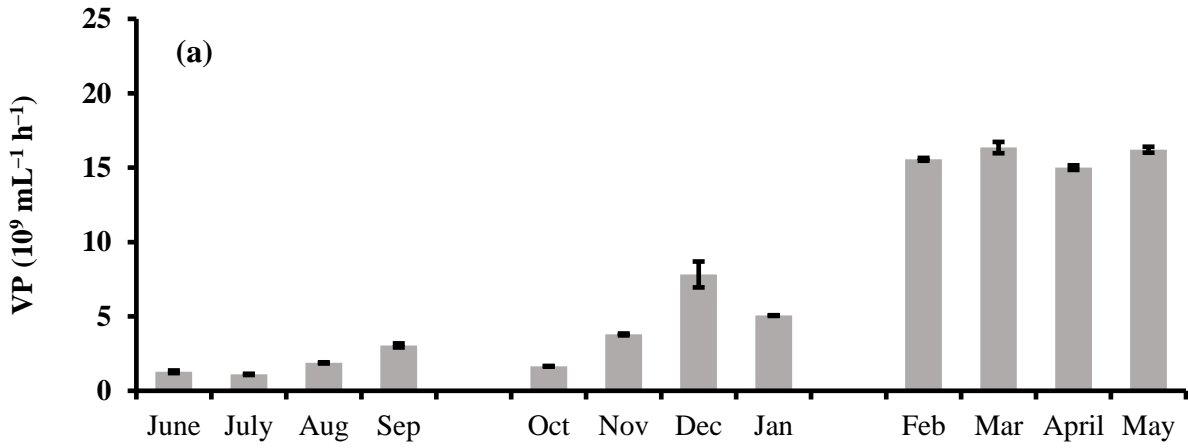


Figure: 4

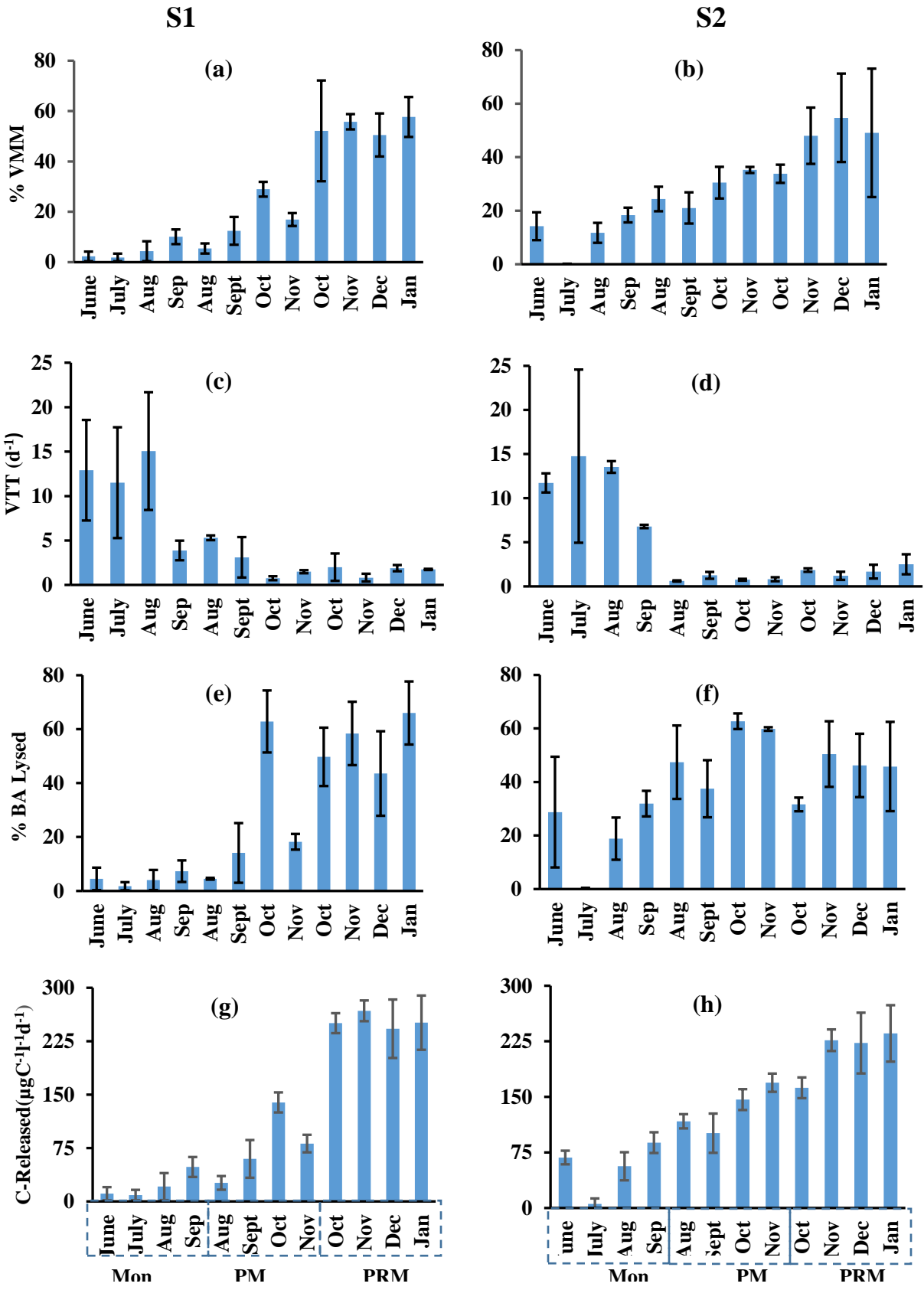


Figure: 5

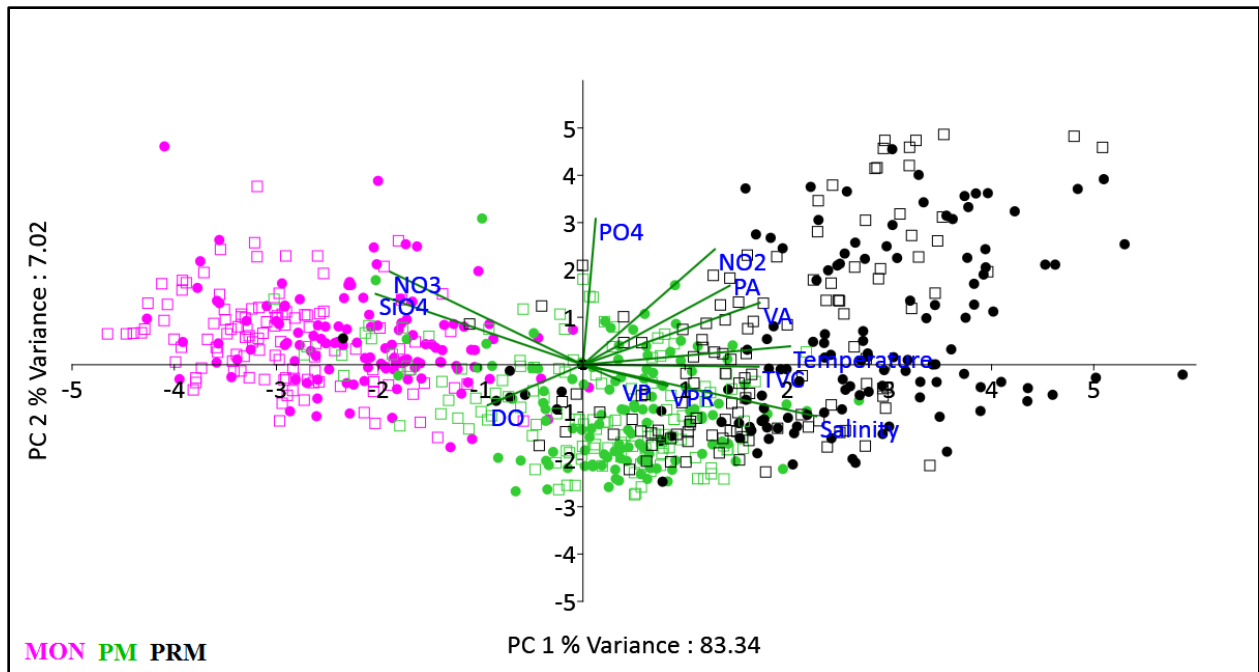


Figure: 6