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Diurnal variations in bacterial and viral production in Cochin estuary, India

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ABSTRACT

Microbes play a central role in the decomposition and remineralization of organic matter and recycling of nutrients in aquatic environments. In this study, we examined the influence of physical, chemical and biological parameters on the rate of bacterial and viral production with respect to primary production over a diurnal period in Cochin estuary. Time-series measurements were made every 2 hours for 12 hours (6 AM -6 PM) during periods of low and high salinities. The light intensity as photosynthetically active radiation (PAR), temperature, salinity, nutrients like $\text{NO}_3\text{-N}$, $\text{SiO}_4\text{-Si}$, $\text{PO}_4\text{-P}$ and chlorophyll *a* (Chl *a*) were measured along with bacterial production (BP), viral production (VP) and net primary production (NPP). NPP showed a strong positive correlation with light and Chl *a* ($r^2 = 0.56$ and 0.47 respectively), while VP showed a strong positive correlation with light, salinity and Chl *a* ($r^2 = 0.37, 0.58$ and 0.37 respectively), and negative correlation with BP ($r^2 = -0.39$) at $P \leq 0.05$. We observed a diurnal pattern in bacterial production but did not have any significant correlation with light. Similar diurnal pattern was seen in VP, the peak of which was in succession with BP, suggesting that virus-mediated lysis plays an important role in loss processes of bacteria in Cochin estuary. The results of our study highlight the light and physico-chemical dependent diurnal variation in virioplankton production in a tropical estuarine ecosystem.

Key words: *Photosynthetically active radiation, bacterial production, viral production, net primary production, Chl a, nutrients, Cochin estuary*

INTRODUCTION

Bacteria and viruses dominate the aquatic environment in abundance and diversity, and play important roles in oxidizing the organic matter and regenerating the inorganic nutrients. Aquatic viruses have drawn considerable research interest owing to their significance in the dynamics of aquatic environments (Bergh et al. 1989; Fuhrman and Suttle 1993, Jacquet 2010, Breitbart, 2012). Viruses are often more abundant than bacteria and cause mortality of their bacterial and phytoplankton hosts. Viruses influence the diversity of autotrophic and heterotrophic microbial communities by controlling the abundance of the most abundant hosts ('killing the winner' hypothesis) (Jacquet et al. 2002; Weinbauer and Rassoulzadegan 2004; Wommack and Colwell 2000) and or *via* the transfer of genes between hosts directly by transformation or indirectly by transduction during the events of lytic induction (Jiang and Paul 1994; Weinbauer and Rassoulzadegan 2004; Wommack and Colwell 2000). Viruses can cause up to 10-60% of the bacterial mortality (Jacquet et al. 200; Weinbauer and Rassoulzadegan 2004), up to 70% cyanobacterial mortality (Proctor and Fuhrman 1990) and 10-70 % phytoplankton mortality in the aquatic systems (Jacquet et al. 2002; Nagasaki 2008). This in turn affects the biogeochemical cycling of nutrients and dissolved organic matter (DOM) pool in the ecosystem (Bratbak et al. 1992; Sime- Ngando et al. 2008; Winget and Wommack 2009).

Light is the most important physical factor that influences both autotrophic processes and the short-term variations in primary production are a consequence of the diurnal light cycles. The heterotrophic prokaryotes also display diel cycles as a consequence of bacterial activity on the released photosynthetic extracellular organic material (Gasol et al. 1998; Kuipers et al. 2000). The UV-range of sunlight spectrum has a profound influence on microbial production either directly or indirectly. Studies have shown that the relationship between the visible light or PAR and the bacterial activity could be very complex; direct, indirect or none (Sommaruga et al. 1997; Winter et al. 2010). Sunlight – induced UV damage is the most prominent viral decay factor (Helbling et al. 1995; Noble and Fuhrman 1997). However, the susceptibilities of viruses to damage from sunlight vary, since some virus decay more rapidly than others (Suttle and Chen 1992; Wilhelm et al. 1998; Wommack et al. 1996). In effect, viruses should display a strong variation in surface waters due to sunlight-mediated viral decay even though the viral abundance show only moderate variations at the scale of hours to days (Wommack and Colwell 2000). Other physical factors such as salinity, turbulence, temperature and water movements influence short term variations in natural communities (Jacquet et al. 2010; Li et al. 2010; Winget and Wommack 2009). The microbial communities are

also influenced by population dynamics and biotic interactions in lakes (Jugnia et al. 2006; Lee and Fuhrman 1991) and marine systems (Noble and Fuhrman 1998; Suttle and Chen 1992; Weinbauer and Suttle 1999). Since viruses are obligate parasites, close relationships exist between viruses and its bacterial and phytoplankton hosts (Maranger and Bird 1995). In fact, it is possible that the activity of phytoplankton, bacteria and virus change at shorter time scales of hours (Gasol et al. 1998; Maranger and Bird 1995; Winget and Wommack 2009; Winter et al. 2004).

Short term variations in VP in marine environments have been reported by several investigators (Bettarel et al. 2002; Parada et al. 2008; Winget and Wommack 2009; Winter et al. 2004), but no such studies have been conducted to the best of our knowledge in tropical estuaries. Diurnal variations in primary productivity with significant correlation with light intensity have been reported from Cochin estuary (Qasim et al. 1969). Another previous study has shown that bacterial production (BP) is independent of primary production (PP) in this ecosystem (Thottathil et al. 2008). In the present study, we hypothesized that there could be short-term oscillations in viral production (VP) and BP with changes in environmental factors in Cochin estuary. In order to substantiate the above hypothesis and to understand the response of biological production to physico-chemical parameters, we measured net primary production (NPP), Chlorophyll *a*, BP and VP and other physico-chemical parameters such as temperature, salinity, suspended particulate matter (SPM), dissolved oxygen, pH and nutrients (nitrate, phosphate and silicate) at fixed time intervals during light hours and during periods of low and high salinity.

MATERIALS AND METHODS

Study site

Cochin estuary (Lat 9° 40' & 10° 12'N and Long 76° 10' & 76° 30'E) is one of the extensive estuarine systems along the south west coast of India. It is a tropical estuary with 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 tidal amplitude of 1 m. The characteristics of the estuary are largely influenced by the South-West and the North East monsoons. The estuary is essentially fresh water during the South West monsoon (June-September), but since the North East monsoon does not bring significant rains and hence the river discharge is low in November-January. The atmosphere is characterized by intermittent cloud cover due to northeast monsoon from November-January. Six major rivers discharge about 2.0×10^{10} m³ of fresh water into the estuary annually (Srinivas et al. 2003) and the water inflow declines post South West monsoon inducing a

salinity gradient in the estuary (Martin et al., 2008). In December, the estuary is partially stratified with respect to salinity with heavy siltation (Qasim 2003). In the present study, the sampling station was at a fixed point (Lat 9°58 '35.55''N and Long 76°16 '18.0''E) in the lower estuary, 2 km away from the Cochin inlet (Fig. 1) with a maximum tidal amplitude of 1 m and an average depth of 4 m.

Sampling procedure

The sampling was carried out for two days in the month of July (LSI on 22.07.09 and LSII on 30.07.2009) and three days in the month of December (HSI on 5.12.2009, HSII on 11.12.2009 and HSIII on 17.12.2009). LS and HS denote periods of low and high salinity. Observations were made at every two hours for 12 hours from a small power boat. The experiment was restricted to 12 hours with the assumption that autotrophic activities occur from sunrise to sunset in the tropics. Samples were collected from the photic layer (0.5 m) and the light intensity was measured as PAR using a LICOR Quantum meter (QSL-2101, Biospherical Instruments Inc. USA). At every 2 hour intervals, 6-8 readings were recorded and the mean PAR was determined. The samples were collected from 0.5 m depth since maximum gross productivity is reported at this depth during June-January in Cochin estuary (Qasim et al. 1969). Light attenuation coefficient (k) was derived from secchi depth using the formula, $k = 1.7/D$, where D is the secchi disc depth. Water samples were collected using 5L Niskin bottles (Hydro-Bios, Germany) for estimating dissolved oxygen (DO), BP, VP, NPP, dissolved nutrients (nitrate, phosphate and silicate) and Chl a .

Estimation of microbial production

Water samples collected in polycarbonate bottles in triplicates were transported to the laboratory and analyzed within thirty minutes. Bacterial production was estimated by incorporating ^3H -thymidine (^3H -TdR) with a specific activity of 18 Ci/mmol (BARC, Mumbai, India) at a rate of 10 nM as described by Fuhrman and Azam (1982). Briefly, a 30 ml water sample along with trichloroacetic acid (TCA) killed control (1% final concentration) was incubated for 1 h in dark at ambient temperature. The sample was filtered through 0.22 μm (Millipore, USA) membrane filter, extracted twice with 3 ml 5% ice-cold TCA and once with 3 ml 80% ethanol. The dried filters were put in scintillation vials and 0.5 ml of ethyl acetate was added to dissolve the filters followed by 5 ml of scintillation cocktail (SRL chemicals, India). The radioactivity was measured using a liquid scintillation counter (LS 6500 Multi Purpose Scintillation counter, Beckman coulter, USA). The disintegration values per minute (dpm) after correcting for blank were converted to moles TdR of

bacterial cells (2×10^{18} cells mol^{-1}) (Bell 1993; Pradeep Ram 2007) and bacterial carbon (20 fg C cell⁻¹).

Alternatively, after the initial incubation with ³H-TdR for bacterial production, the samples were processed for the estimation of viral production as previously described (Noble and Steward 2001). Five-milliliter l of the sample was filtered through a 0.2 μm syringe-tip filter (Acrodisc, Gelman) to remove the bacterial cells and the filtrate was treated sequentially with a nuclease cocktail (DNase I, RNase I and micrococcal nuclease) to remove the dissolved DNA followed by 5M NaOH and 30% TCA to distinguish ³H incorporated DNA resulting from metabolism of ³H-TdR by the cell. After hydrolysis, the macromolecules were precipitated with cold 5% TCA, washed and filtered through 0.2 μm membrane filter (Millipore, USA). The dried filter was processed for scintillation counting as described above for bacterial production. The mole TdR values were converted to viruses/L/h (viral production rates are expressed as particles/volume/time) using a conversion factor of 6×10^{20} viruses per mole TdR (Fuhrman and Noble 1995).

Estimation of microbial abundance

Water samples were stained using SYBR green I fluorescent dye (Invitrogen, CA, USA) as previously described (Noble and Fuhrman 1998). Briefly, 1 ml of the sample was filtered through a 0.02 μm pore-size (25mm diameter) anodisc filter backed by 0.8 μm cellulose mixed ester membrane (Millipore) under 20 Kpa vacuum. The anodisc filter was placed on the SYBR green solution (diluted 1:10) and incubated in dark for 15 min. After incubation, the filter was placed over a cover slip containing 30 μl antifading solution [50% glycerol, 50% phosphate buffered saline (pH 7.5), 0.1% p-phenylenediamine] on a microscopic slide. The slides were used either immediately for counting or stored 20°C until enumeration. For each filter, 10-20 fields were selected randomly and a total of >200 viruses and >200 bacteria were counted at 1000 \times with an Olympus BX 41 epifluorescence microscope under blue excitation.

Chemical and biological parameters

All the carboys, filtering devices, glasswares and tubings were acid washed (10% HCl) and rinsed (thrice) with deionized water prior to use. The water samples collected were stored in iceboxes and brought to the laboratory within an hour of collection. Samples for dissolved oxygen (DO) collected in 125 ml glass bottles were fixed by adding magnesium chloride and alkaline potassium hydroxide. In the laboratory, DO was analyzed titrimetrically using Winkler's method. Dissolved nutrients (nitrate, phosphate and silicate) were analyzed following standard procedures (Grasshoff 1999) using

a spectrophotometer (Shimadzu, UV-2550, Japan). Appropriate standards were used in each measurement.

Primary productivity was measured by the *in-situ* ^{14}C assimilation method (Lohrenz 1993). Samples were filled into four 250 ml Nalgene bottles in duplicate (two light and two dark bottles) after sieving through 200 μm nylon net piece to remove zooplanktonic organisms. Each bottle was inoculated with 1 ml labeled $\text{NaH}^{14}\text{CO}_3$ with an activity of $5\mu\text{Ci ml}^{-1}$ (BARC, Mumbai, India) and suspended at 0.5 m depth. One set was incubated in situ for 12 hrs (6 AM-6 PM) for overall NPP and other bottles were filled, inoculated and retrieved at every two hour interval from 6 AM to 6 PM (6 AM-8 AM, 8 AM-10 AM, 10 AM-12 PM, 12 PM-2 PM, 2 PM-4 PM, 4 PM-6 PM). On retrieval, water samples were immediately passed through a GF/F filter paper (0.7 μm) under gentle pressure (< 50 mm Hg) in diffused light. The excess dissolved inorganic carbon (DI^{14}C) was removed by exposing the filter paper to fumes of concentrated hydrochloric acid for one minute. The filters were then placed in scintillation vials and 5 ml scintillation cocktail (was added and the radioactivity was measured in a liquid scintillation counter. (The production rate was calculated based on 12 h photoperiod for overall NPP (expressed as $\mu\text{g C L}^{-1} \text{d}^{-1}$) and NPP at each time interval was expressed in $\mu\text{g C L}^{-1} \text{h}^{-1}$).

Chlorophyll *a* (Chl *a*) concentration was measured as a proxy for phytoplankton biomass and pheophytin values were used as an indicator of herbivorous zooplankton grazing. Chl *a* and pheophytin (acidified with 0.1 N HCl) concentrations were determined by filtering water samples (500 ml) through 0.7 μm , 47 mm dia GF/F filters (Whatman, USA), followed by extraction with 90 % acetone for 24 h in the dark at 0°C and measuring the extinction coefficient in a fluorometer (Model 7200-000, Turner Designs Instruments, Trilogy, USA) (Parsons et al. 1984). Suspended particulate matter was determined gravimetrically from the material retained in the 0.45 μm filter paper (Millipore, USA) as previously described (APHA 1985).

Statistical analysis

Statistical analysis was performed using SPSS 1.6 software (Statistical package for the social sciences, 1.6 version software, IBM, India). Pearson correlation was performed to analyze relation between various measured variables (two-tailed significance assumed at $p < 0.05$ and < 0.01).

RESULTS AND DISCUSSION

Diurnal variations in bacterial and viral productions

A short term study was conducted to understand the effect of PAR and other physical factors on viral, bacterial and primary production in Cochin estuary during July and December 2009. The observations were made on two days during the month of July (LSI and LSII) and three days in the month of December (HSI, HSII and HSIII). On LSI, BP was low at 6 AM ($2.3 \mu\text{g C L}^{-1}\text{h}^{-1}$) and increased to $13.4 \mu\text{g C L}^{-1}\text{h}^{-1}$ by 10 AM and further it was reduced to $5.3 \mu\text{g C L}^{-1}\text{h}^{-1}$ by 12 noon (Fig. 2h). Similarly on LSII, bacterial production showed a diurnal rhythm with 4.5, 7.7 and $6.2 \mu\text{g C L}^{-1}\text{h}^{-1}$ at 6 AM, 12 noon and 6 PM respectively. The BP peak was seen at 10 AM on LSI and at 12 noon on LSII. In December, BP values were low compared to that of July. On all the three days in December, the BP peak was seen at 10 AM (3.26 , 3.52 and $3.2 \mu\text{g C L}^{-1}\text{h}^{-1}$ on HSI, HSII and HSIII respectively) and there was a drop in BP (1.66 , 1.11 and $1.85 \mu\text{g C L}^{-1}\text{h}^{-1}$ on HSI, HSII and HSIII respectively) at 12 PM. The BP values were on a rise during the late evening hours on most of the days.

Viral production also showed a diurnal rhythm in July and December but there was a sharp decline in the production during early afternoon. On LSI, viral production increased from 6 AM to 12 noon (0.8×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ to 5.0×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$), which was followed by a sharp decline to 0.9×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ by 2 PM (Fig. 2j). Viral production then slowly increased to 1.7×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ by 6 PM on LSI. Similarly on the LSII viral production increased from 0.5×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ at 6 AM to 1.3×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ by 10 AM on sunny day, which further dropped to 0.5×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ by 12 noon. However, the production showed a slight increase to 0.70×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ by 6 PM. Similar trend was seen in VP on all the three days of observation in the month of December. VP values were higher than that of values recorded in July. The trend in VP was similar to the observations in the month of July. Highest VP was seen at 12 PM (6.19 , 5.9 and 8.26×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ on HSI, HSII and HSIII respectively) followed by a sudden decline in VP at 2 PM (2.52 , 3.75 and 3.37×10^{10} viruses $\text{L}^{-1}\text{h}^{-1}$ on HSI, HSII and HSIII respectively).

The bacterial abundance showed an average value of $1.93 \pm 0.38 \times 10^9$ cells L^{-1} in July and $1.96 \pm 0.17 \times 10^9$ cells L^{-1} in December whereas the average viral abundance was $1.98 \pm 0.41 \times 10^{10}$ cells L^{-1} in July and $1.16 \pm 0.15 \times 10^{10}$ cells L^{-1} in December (Table. 1). The viral to bacterial ratio ranged from 5-22 in July to 2-16 in December. There was no definite diurnal pattern on a short term for the bacterial and viral abundance. The bacterial abundance did not seem to have influenced by physical,

chemical or biological factors. On the contrary, the viral abundance showed fluctuations in response to bacterial production and salinity.

Diurnal variations in PAR and other physical factors

The PAR was low during early morning and late afternoon hours with maximum peak at either 10 AM or 12 PM during the observation period. On an average, PAR was high in December ($472.65 \pm 107.87 \mu\text{E m}^{-2} \text{ s}^{-1}$) than in July ($268.9 \pm 102.87 \mu\text{E m}^{-2} \text{ s}^{-1}$) (table 1). In July, the light intensity ranged from 25.4- 861.1 $\mu\text{E m}^{-2} \text{ s}^{-1}$ while it was 1.24-1079.2 $\mu\text{E m}^{-2} \text{ s}^{-1}$ in December (Fig. 2a). The Cochin estuary was characterized by high turbidity during the high and low saline period with high attenuation coefficient (average $4.34 \pm 0.45 \text{ m}$ during low saline and $3.44 \pm 0.42 \text{ m}$ during high saline period) and high SPM ($50.06 \pm 13.4 \text{ mg L}^{-1}$ and $28.86 \pm 11.25 \text{ mg L}^{-1}$ during low and high saline period respectively) (Table. 1). Attenuation coefficients (k) did not vary much which ranged between 3.3 - 8.5 m^{-1} and 2 - 8.5 m^{-1} in July and December respectively whereas the SPM during July was slightly higher ranging from 30- 88 mg L^{-1} and 18- 46 mg L^{-1} during December (Fig. 2b).

Salinity is an important physical factor influencing organic production in Cochin estuary (Madhu et al., 2010). The lowest salinity in the range of 0 –2 ppt was recorded in July while December was characterized by high salinity in the range of 17-27 ppt (Fig. 2c). Generally, changes in temperature are not well marked in Cochin estuary. Temperature during July ranged from 26.6 to 29.8 °C and from 28.0-30.3°C in December. The pH was around neutral in July (7.0-7.4) but was found to be slightly higher in December (7.2-8.3). The pH was highest in the range 8.17-8.27 on HSIII (Fig. 2d).

Diurnal variations in chemical factors

The DO ranged from 4.9 - 6.5 mg L^{-1} in July and from 4.3- 10.4 mg L^{-1} during December. The $\text{NO}_3 - \text{N}$ was high during July and low in December. During July $\text{NO}_3 - \text{N}$ ranged from 5.5 - 26.9 μM and during December the range was between 1.7 - 16.9 μM . $\text{PO}_4 - \text{P}$ was generally high and showed a diurnal rhythm with maximum values at 2 PM on both days in July but lacked any trend on all the three days in December (Table. 1). $\text{SiO}_4 - \text{Si}$ was high on all days under study but did not show any particular rhythm. The values ranged from 20.0- 72.8 μM in July whereas in December, the values ranged from 19.2-75.0 μM (Table. 1).

Diurnal variations in primary production and other biological factors

There was a diurnal rhythm in phytoplankton production with maximum NPP recorded in concurrence or soon after maximum PAR on all days. NPP over a day (6 AM-6 PM) in July ranged from 219.2 - 279.7 $\mu\text{g C L}^{-1} \text{d}^{-1}$ and from 115.2 - 171.8 $\mu\text{g C L}^{-1} \text{d}^{-1}$ in December (Fig 2f). NPP was also determined at every 2 hr time interval in a day to determine the maximum primary productivity peak. The maximum NPP was recorded between 12 to 2 PM. The NPP was 94.3 $\mu\text{g C L}^{-1} \text{h}^{-1}$ on LSI and 141.4 $\mu\text{g C L}^{-1} \text{h}^{-1}$ on LSII in July whereas it was 94.3, 102 and 76 $\mu\text{g C L}^{-1} \text{h}^{-1}$ on HSI, HSII and HSIII respectively in December.

Chlorophyll *a* peaked during early afternoon and declined towards the late afternoon (Fig. 2e). The values in July ranged from 0.7 - 5.5 $\mu\text{g L}^{-1}$ while in December from 2.07 to 11.02 $\mu\text{g L}^{-1}$. Phaeophytin was maximum and minimum during the morning and noon hours respectively on all the days of observation and the values ranged from 1.15- 7.65 $\mu\text{g L}^{-1}$ in July and 0.32-1.68 $\mu\text{g L}^{-1}$ in December. Phaeophytin concentration increased during the evening hours when chlorophyll values were on the decline.

Factors correlating with biological production

Viral production rates in Cochin estuary showed significant positive correlation (at $P \leq 0.05$) with salinity ($r^2 = 0.56$), light ($r^2 = 0.37$), SPM ($r^2 = 0.42$), pH (0.48) and chl *a* ($r^2 = 0.37$) (Table. 2) and a significant negative correlation with BP ($r^2 = -0.39$), $\text{NO}_3\text{-N}$ ($r^2 = -0.62$) and $\text{PO}_4\text{-P}$ ($r^2 = -0.56$) at $P \leq 0.05$. The BP rates showed significant positive correlation only with nutrients $\text{NO}_3\text{-N}$ ($r^2 = 0.47$) and $\text{PO}_4\text{-P}$ ($r^2 = 0.69$) at $P \leq 0.05$ and significant negative correlation with salinity ($r^2 = -0.63$ at $P \leq 0.05$). However, NPP showed strong positive correlation with light ($r^2 = 0.66$ at $P \leq 0.05$) and Chl *a* ($r^2 = 0.46$ at $P \leq 0.05$).

The results of our study suggest a definite diurnal pattern in viral production with a single peak at around noon and further reduction by early afternoon. A simple reason for VP following the light pattern is the damage to viral particles or loss of infectivity of virus induced by solar radiation. Previous studies have also shown that viruses are extremely sensitive to solar radiation (Noble and Fuhrman 1997; Suttle and Chen 1992; Wilhelm et al. 1998; Wommack et al. 1996). The high UV radiation in the early afternoon would have led to either the loss of infectivity or infection of host cells to avoid UV-induced damage. The effect of PAR on VP is largely unknown unlike the effect of UV radiation (Winter et al., 2004), but it is evident that the viruses underwent a rapid repair as PAR

decreased in the evening (Wilhelm et al. 1998). Another explanation for the diurnal pattern of VP is that viruses infecting autotrophic hosts are more active during the day exhibiting this pattern. To support this argument, the VP rates seemed to be tightly coupled with the algal biomass (Chl *a*) indicating a higher number of algal viruses. This also implies that higher the algal biomass, greater is the available resources for the viruses to proliferate. It is possible that in the case of eutrophic environments, when the conditions are favorable for the bacteria and phytoplankton, they become more susceptible to viral lysis than in oligotrophic environments. Earlier studies have showed that though there are significant variations in VP rates, that viral production lack a definite diel pattern (observations made at 4 hrs interval) of infection and /or lysis like in Chesapeake bay (Jiang and Paul 1994; Winget and Wommack 2009). But diurnal observations at shorter intervals have not been attempted before and hence the diurnal pattern with a single peak in productivity was not noticed in earlier studies. Since there are noticeable diurnal changes in the host abundance, the viral activity also shows definite pattern of activity during the light hours in a day.

In our study the viral production was in the order of 10^{10} viruses $L^{-1}h^{-1}$ in both July and December. The viral production reported from other waters were in the range of 10^6 to 10^9 viruses $L^{-1}h^{-1}$ (Poorvin et al. 2004; Weinbauer and Rassoulzadegan 2004; Winget and Wommack 2009; Winter et al 2004). The effect of viruses is probably larger in eutrophic waters than oligotrophic waters (Steward et al. 1996). A high viral production rates indicate that viruses are important agents responsible for the control of bacterial and algal population through cell lysis in Cochin estuary. But the VP rates were lower during the low saline period than the high saline period. A possible explanation for this is that in the presence of high SPM during low saline period (due to riverine input), viruses attach to sediment particles and become unavailable for infection. VP rates were higher when the suspended load was lower in December. Another possible reason for this is the effect of tides. The VP patterns did not show any relation with high tides except on one occasion (data not shown).

There was a diurnal pattern in bacterial production but did not show any significant correlation with light. Diel variation in bacterioplankton productivity, especially in autotrophic bacteria has been well documented (Bettarel 2002; Jugnia et al. 2006). VP and BP peaks were observed at different times of the day with VP peaks closely following BP peaks, which obviously was due to the mortality of bacterial hosts by viruses. Selective attack of viruses on fastest-growing bacteria could be responsible for the observation of viral peak occurring after the bacterial peaks (Maranger et al. 1994). A delay between the production of viruses and bacteria has been previously reported

(Weinbauer and Suttle 1999). The results suggest that the viral production depends on the growth of the host and are implicated in the reduction of bacterial (Fuhrman and Schwalbach 2003; Middelboe et al. 2001; Winter et al. 2001) and phytoplankton communities (Jacquet et al. 2002; Martinez et al. 2007). Moreover, bacteria growing in areas of elevated phytoplankton concentrations are more susceptible to lysis induced by viral infection than those growing in nutrient-deficient environments (Maranger and Bird 1995). The bacterial production rates determined in our study are similar to that previously reported from Cochin estuary (Thottathil et al. 2008) and from similar estuarine environments in India (Pradeep Ram 2007; Ramaiah et al. 1995).

There was a diurnal rhythm in NPP and Chl *a* with a single peak associated with variations in PAR similar to previous observations on primary productivity with daily illumination in Cochin estuary (Qazim et al. 1969). This diurnal variation in phytoplankton production could be due to endogenous photosynthetic rhythm (Kerr et al., 1985). Among the various factors physical factors, light availability is the most significant factor that controls phytoplankton productivity in estuarine environments (Cole and Cloern 1984). The light penetration in the estuary is by and large reduced because of high turbidity which has been indicated as a significant factor controlling primary production (Cloern 1987). But even though the SPM was lower in December, NPP was less in December than in July. The probable reason behind this is the activity of viruses removing a significant portion algal production through cell lysis. The primary productivity was affected indirectly by light limitation and probably due to lysis by virus. Though there was no significant correlation between NPP and VP, a significant correlation was seen between VP and Chl *a*. Strong relationships have been reported between VP and phytoplankters, often quantified using Chl *a* (Maranger and Bird 1995) but a direct relationship between VP and NPP has not been addressed in the literature extensively.

Being a tropical estuary, seasonal changes in temperature are not remarkable in Cochin estuary with meager 3-4°C annual variation. Statistical analysis of our data indicated that bacterial and viral activities were not limited or affected by temperature in this stable temperature regime of Cochin estuary (data not shown).

Nutrients are available in surplus in Cochin estuary and hence do not limit phytoplankton production in Cochin estuary at any time of the year (Balachandran et al. 2005). This is also evident from the data obtained in our study (Table. 1). Silicate in the estuary is always high and is associated with freshwater influx and land runoff (Martin et al., 2008). The system is also characterized by a high N:P ratio throughout the year indicating excess of N in the estuary (Martin et al., 2008). There was no

correlation for any of nutrients with NPP. However it should not be overlooked that different phytoplankton species have different nutrient requirements, but high concentrations of nutrients alone is not conducive to a substantial increase in phytoplankton productivity (Bergh et al. 1989). It is known that inorganic nutrients, composition and bioavailability of organic matter and predation are the major factors affecting the spatiotemporal variability of prokaryotic production. Though the impact of nutrient supply is less pronounced in eutrophic systems, the bacterial production and viral abundance were positively influenced by nitrate and phosphate. The viral production was independent of nutrient concentrations though nitrate and phosphate have been reported to increase viral production in oligotrophic waters (Motegi and Nagata 2007).

Conclusion

The present study highlights the short term variations in bacterial and viral production in a tropical eutrophic estuary. We demonstrate for the first time that there are diurnal patterns in bacterial, viral and phytoplankton production with single peak occurring at different times of the day. Our study is the first to report the viral production from Indian waters. The patterns in diurnal variations in VP could be attributed to various processes such as the changes in viral activity due to viral-host interactions, viral-host contacts, host biomass and the influence of a host of environmental factors including light and salinity. These processes are likely to work in tandem to create observed patterns of diurnal variations/ rhythm. The study indicates that viral attack could be responsible for large scale mortality of planktonic populations in this ecosystem. Our study suggests that viral production rates could be coupled with bacterial or phytoplankton mediated processes. Further studies are on to determine the diversity of viral communities in Cochin estuary and the extent to which these communities shape its biology.

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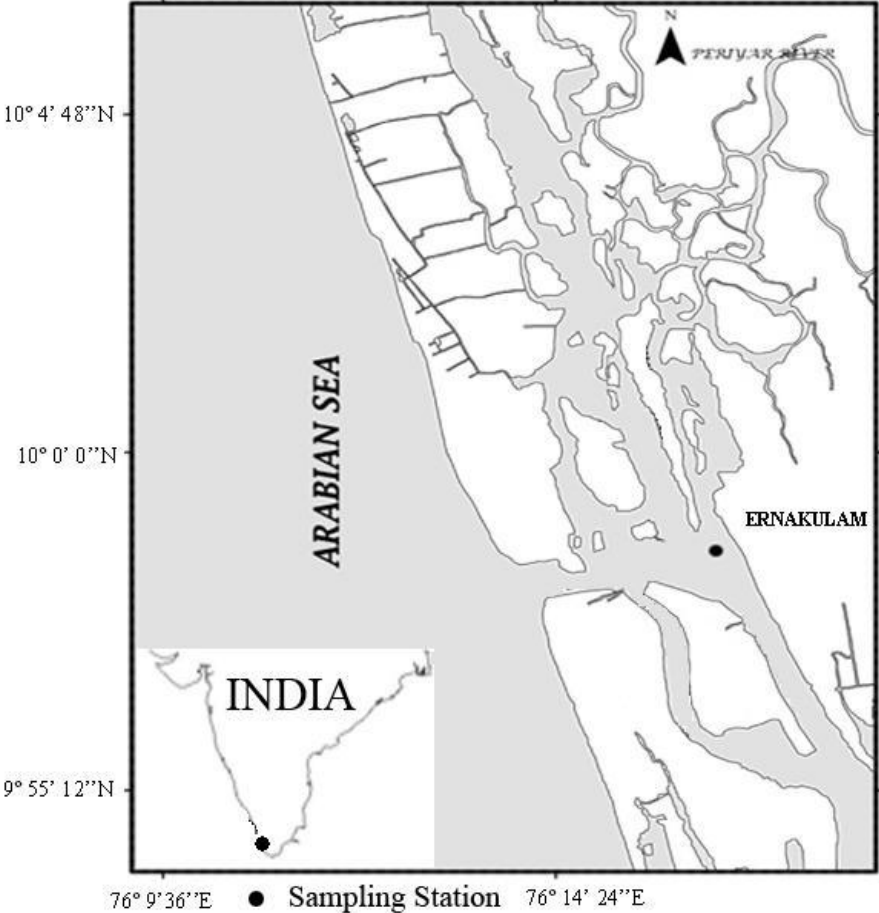
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Figure 1. Map indicating the sampling site (9°58 '35.55"N and 76°16 '18.0"E) in the Cochin estuary



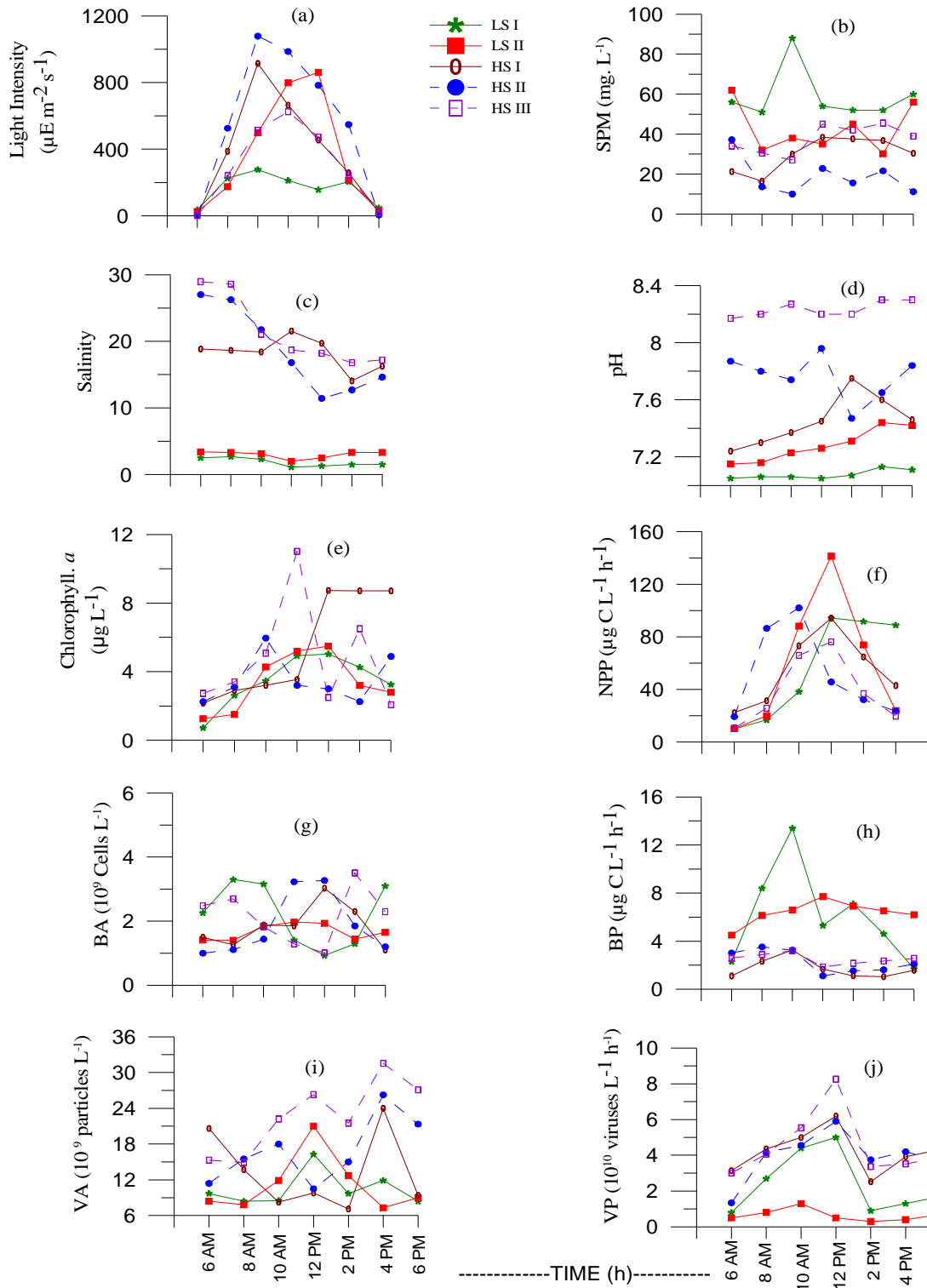
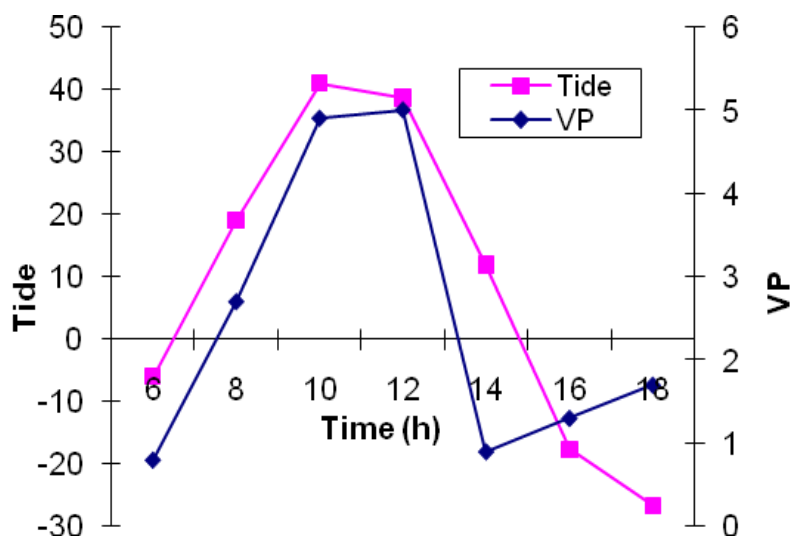


Figure 2. Graphs showing diurnal variations in physical, chemical and biological parameters like (a) Light (b) Suspended particulate matter-SPM (c) Salinity (d) pH (e) Chlorophyll a (f) Net primary productivity-NPP (g) Bacterial abundance-BA (h) Bacterial production-BP (i) Viral abundance-VA (j) Viral productivity-VP

Figure 3. Figure showing the coincidence of tide and viral production during the LSI. The highest high tide coincided with the VP peak.



Parameters	LOW SALINE		HIGH SALINE	
	Average	Standard deviation	Average	Standard deviation
Light-PAR ($\mu\text{E m}^{-2} \text{ s}^{-1}$)	268.91	145.48	417.12	131.93
Attenuation Coefficient (m)	4.34	0.64	3.44	0.52
Suspended Particulate Matter (mg L^{-1})	50.06	10.62	28.86	9.44
Temperature ($^{\circ} \text{C}$)	28.3	0.83	28.9	0.61
Salinity	1.10	1.04	19.41	1.71
pH	7.18	0.14	8.23	0.22
Dissolved Oxygen (mg L^{-1})	5.52	0.36	6.52	1.14
Nitrate ($\mu\text{M L}^{-1}$)	16.74	3.34	7.38	5.01
Phosphate($\mu\text{M L}^{-1}$)	9.8	1.31	2.39	0.68
Silicate($\mu\text{M L}^{-1}$)	50.9	8.0	53.77	6.17
Chlorophyll. a ($\mu\text{g L}^{-1}$)	3.43	0.1	4.57	2.43
Pheophytin .a ($\mu\text{g L}^{-1}$)	1.36	1.33	2.43	1.74
Net Primary Productivity ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	58.13	2.1	48.48	8.25
Bacterial Production ($\mu\text{g C L}^{-1} \text{ h}^{-1}$)	6.24	0.18	2.18	0.41
Viral Production ($10^{10} \text{ viruses L}^{-1} \text{ h}^{-1}$)	1.52	1.24	4.22	0.29
Bacterial Abundance ($10^9 \text{ cells L}^{-1}$)	1.93	0.38	1.96	0.17
Viral Abundance ($10^{10} \text{ particles L}^{-1}$)	1.98	0.41	1.16	0.15

Table 1: Showing average values and standard deviation of different parameters measured in the study during low and high saline periods. Average values were calculated by averaging the values obtained at different time intervals for each day during the low saline and high saline periods separately.

	ATN	SPM	SAL	PH	DO	NO3	PO4	CHL	NPP	BP	VP	BA	VA
LIGHT	-0.48**	-0.27	0.16	0.12	0.03	-0.06	0.02	0.23	0.65**	-0.03	0.37*	0.13	0.08
ATN	1	0.32	-0.09	-0.28	-0.302	-0.241	.018	-0.24	-0.23	0.28	-0.03	0.01	-0.8
SPM		1	0.40*	0.45**	0.25	-0.54**	-0.38*	0.15	-0.24	-0.15	0.42*	0.04	-0.29
SAL			1	0.77**	0.22	-0.64*	-0.77*	0.15	-0.05	-0.63**	0.58**	-0.06	0.36*
PH				1	0.68**	-0.54**	-0.54**	0.24	-0.14	-0.49**	0.48**	0.09	0.62**
DO					1	-0.35*	-0.24	0.26	-0.19	-0.3	0.21	0.16	0.58**
NO3						1	0.61**	-0.35*	-0.06	0.047**	-0.31	0.003	-0.30
PO4							1	-0.03	0.24	0.69**	-0.26	-0.003	-0.31
CHL								1	0.35*	-0.16	0.37*	-0.01	0.29
NPP									1	0.23	0.14	-0.21	0.04
BSP										1	-0.40*	0.03	-0.36*
VSP											1	0.09	0.40*
BA												1	-0.01

Table 2: Pearson correlation coefficients among various parameters as determined using SPSS software. The significant correlations are indicated as ** for significance at the 0.01 level (2-tailed): P=0.418, and *for significance at the 0.05 level (2-tailed): P= 0.325