

Variation of bacterial metabolic rates and organic matter in the monsoon-affected tropical estuary (Godavari, India)

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Abstract

Bacteria play a central role in the oxidation of organic matter. In tropical estuaries, bacterial metabolic rates and carbon modification are regulated by physicochemical changes influenced by the annual monsoon-mediated river run-off. The present study is the first report on the effect of monsoon influenced river discharge magnitude on bacterial metabolic rates and organic matter variation in the tropical Godavari estuary and adjacent coastal waters. Concentrations, nature, and sources of particulate organic carbon and nitrogen were investigated by elemental Analyser-Isotopic Ratio Mass Spectrometer (EA-IRMS) whereas concentrations of dissolved organic carbon (DOC) and nitrogen (DON) were determined by a high-temperature catalytic oxidation in surface waters of Godavari estuary, East coast of India. Bacterial abundance and metabolic rates were measured, and pCO₂ levels were calculated for surface water samples. The maxima obtained in bacterial respiration rate (994 μg C L⁻¹d⁻¹) was associated with maximum concentrations and terrigenous nature of organic matter with high C: N ratio (~12). The highly depleted δ¹³C_{POC} (-32.9 ± 1.4 ‰) associated with Chl-a maxima suggested its contribution from fresh water planktons. Significant positive correlation of Bacterial productivity (BP), bacterial growth efficiency (BGE) and bacterial respiration (BR) with particulate organic matter associated with low DOC: POC ratio could imply a heterotrophic preference for an abundant form of organic matter. We, moreover, uncovered that terrigenous organic matter is modified efficiently in the estuarine transect by heterotrophic bacteria under favorable physicochemical conditions like low salinity and high nutrient content. Furthermore, the majority (76%) of BGE values were <8%, and average pCO₂ levels were ~14 times greater than the atmospheric levels. Overall, this study reported that the Godavari estuary is net heterotrophic and hence significantly contribute to global carbon cycle biogeochemistry.

Key words: Bacterial metabolic rates, organic matter, pCO₂ levels, river discharge, Godavari estuary.

Introduction

Due to changing climatic conditions and increase in atmospheric CO₂ concentrations, carbon cycle has received much attention. Attempts have been made to understand the underlying mechanisms involved in carbon modification and transformation. Heterotrophic bacteria radically modify and mineralize the organic carbon, thus introducing it to the aquatic food web and making it available to the higher planktons (Pomeroy 1974; Azam et al. 1983). During the bacterial mineralization, part of organic carbon gets converted to biomass (production), and rest gets respired as inorganic carbon i.e. CO₂

(Hopkin and Smith 2005). Respiration of organic carbon in the estuarine system represents a return of CO₂ to the atmosphere previously fixed by the terrestrial systems (Richey et al. 2002; Cole et al. 2007). In aquatic systems, modification of organic matter by bacteria has been reported to be affected by temperature (Lopez and Moran 2007; Rivkin and Ledgenger 2001), salinity pattern (David et al. 2000; Crump et al. 2004), light availability, pH variation, turbidity (Roland and Cole 1999), quality of the organic matter (Reinthaler et al. 2005; Apple et al. 2006; Ylla et al. 2012) and bacterial community composition (Logue et al. 2016). These factors have a significant impact on bacterial mineralization of organic carbon, particularly in estuaries. Estuaries are natural reactors for modification, mineralization and transformation of organic carbon resulting into ecological and biogeochemical gradation within the region. (Sarma et al. 2011, 2012; Bhavya et al. 2015). Particularly in monsoonal estuaries, terrestrial organic carbon load depends on the intensity of monsoon, which in turn brings variations in river discharge patterns. Whereas, nature and sources of organic carbon vary based on catchment vegetation, physicochemical control, and river discharge pattern, which in turn brings changes in bacterial modification and metabolic rates.

In spite of knowing the bacterial contribution in carbon cycling and biogeochemical processes, studies on measurement of their metabolic rates on changing nature and source of organic matter and relation to surface water pCO₂ levels are scarce. Some studies related to bacterial metabolic rates have been carried out in higher latitudes (Benner et al. 1995; Maranger et al. 2005; Vazquez Dominguez et al. 2007). Models and observations predicting the bacterial metabolic rates in changing climate scenario are mostly derived from data collected in temperate regions (Pollard and Ducklow 2011; Amado et al. 2013). Few studies have reported the bacterial metabolic rates for tropical and subtropical estuarine and coastal areas (Ram et al. 2003; Lee et al. 2007; Thottathil et al. 2008; Yuan et al. 2010). But, observations of river discharge pattern, magnitude, associated organic matter and its impact on bacterial metabolic rates remains unaddressed. Therefore given present and future climatic changes, it is imperative to understand the factors that influence the mineralization of terrestrially derived organic carbon and bacterial metabolic rates.

The present study was carried out in tropical Godavari estuary with an attempt to: (i) investigate the variations in bacterial metabolic rates (Respiration and production rate, growth efficiency and carbon demand) with respect to river discharge magnitude (ii) understand the impact of river discharged organic matter on bacterial metabolic rates and variation. (iii) estimate the pCO₂ levels thereby understanding the nature of the estuary.

Materials and Methods

Study area

River Godavari is among the 60 largest rivers in the world with a major fluvial contribution to the Bay

of Bengal. The catchment area of the river is 3, 12,812 km² spread in various states of India. It ranks 34th and 32nd regarding the catchment area and water discharge, respectively (Ludwig et al. 1996; Gaillardet et al. 1999). The river flows eastwards over peninsular India for about 1480 km and drains into Bay of Bengal in the state of Andhra Pradesh. The Godavari basin climate is generally dry having a higher temperature during the months of April to June (summer season) and relatively low temperature during winter (December to February). The Godavari catchment receives about 82% of the annual rainfall during the summer monsoon and the rest in the winter monsoon (CPCB 1995) with an average rainfall of 1512 mm y⁻¹. Godavari river brings a huge amount of organic matter, about 22% of which is estimated to be lost in the main channel (Balakrishna and Probst 2005). After Rajamundry, the river flow is regulated by a century-old “Low Dam” at Dowalaiswaram. After the dam, the river bifurcates into two major distributaries, eastward flowing major Gautami-Godavari, and southward flowing minor Vasistha-Godavari. The present study was conducted from the major tributary (Figure 1). Maximum depth of the estuary is ~15 m with an average depth of ~5 m.

The freshwater discharge in the Godavari estuary is mainly controlled by the variations in the monsoonal rainfall over the Indian subcontinent, which is regulated by a century old dam at Dowalaiswaram. Excess water from the dam reservoir is released when the threshold level (14.02 m) is reached, which occurs soon after the monsoon begin. During the present study period (the year 2010), Godavari river discharged from mid- May and continued until December with maxima during August-September (Figure 2). Prior to discharge (January to May), a source of water to the estuary was due to the tidal currents. An intrusion of sea water was noticed up to ~80 km from the mouth of the estuary to the upper estuary (Sridevi 2013, Ph.D. thesis). Based on the river discharge magnitude during the year 2010, the study period was classified into three periods. No discharge period (0- <100 m³s⁻¹; January to June. 2010), Peak discharge period (>100-20000 m³s⁻¹; July to September 2010) and moderate discharge period (>100-< 2000 m³s⁻¹; October to December 2010). Discharge magnitude was negligible during May and June (<100 m³s⁻¹) hence these two months are considered under no discharge period. Surface water sampling was carried out at six stations during 29-30 March (No discharge period), 18-19 August (Peak discharge period) and 15-16 November (Moderate discharge period) representing all three discharge periods. Based on the salinity gradient during no discharge period (4 months average), study region was further divided spatially into upper estuary; station 1 (12.5±6 psu), middle estuary; station 2-3 (24.5±5.8 psu), lower estuary, station 4 (29.6 ±4.5 psu) and coastal region 5-6 (33.6±1 psu) (Figure 1).

Sampling and analysis

Discharge data for Godavari River was collected from the dam authorities at Rajamundry. Surface seawater samples were collected by mechanized boat at six stations (Figure 1) using a clean bucket and

transferred in 2 L plastic bottles cleaned with soap and acid (1N Hydrochloric acid) solution. All the samples were maintained in an ice box till the further analysis. Laboratory experiments were conducted to measure bacterial metabolic rates. In situ temperature and salinity was measured by casting a Conductivity, Temperature and Depth profiler (CTD) SBE19 plus (Seabird Electronics, USA). Analysis of dissolved inorganic nutrients like dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP) and dissolved inorganic silicate (DSi) were carried out using auto analyser (Skalar, San++, Netherlands) following Grashoff et al. (1992). For Chl-a measurement, 2 L water sample was filtered through GF/F filter (Whatman) and phytoplankton biomass retained on filter was extracted after 12 h incubation with N, N-dimethylformamide (DMF) at 4°C in the dark, and then analysed spectrofluorimetrically (Varian Eclipse Fluorescence spectrophotometer, USA) following Suzuki and Ishimaru (1990). Total suspended matter (TSM) was measured based on weight difference of the material retained on 0.22 µm pore size polycarbonate filters after passing 1 L of the water sample. As the planktons and other microscopic organisms contribute to TSM, to retain these microscopic particles and to achieve high particle retention, 0.22 µm filters were used. Filters were repeatedly rinsed with distilled water at the end of filtration. The samples for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were analysed by a high-temperature catalytic oxidation method, (Benner and Hedges 1993; Spyres et al. 2000) using Total Organic Carbon analyser -VCSH with ASI-V auto sampler and TNM-1- Shimadzu. For measuring particulate organic carbon (POC) and particulate organic nitrogen (PON) and their respective stable isotope ratios, 2 L water sample was filtered through GF/F filters which were pre-combusted at 450°C for 5 h. After acid fumigation treatment, elemental and isotopic ratio measurements were made on a single filter using Elemental Analyser-Isotopic ratio mass spectrometer (EA-IRMS, Delta V- Finnigan, Germany). The detection limit of this method for determination of organic carbon and total nitrogen was <1µg g⁻¹. The IAEA standards (IAEA-600 Caffeine, IAEA-CH-3 Cellulose, IAEA N-1 Ammonium sulfate, IAEA NO-3, Potassium nitrate) and laboratory standards such as L-glutamic acid and L-alanine were used for calibration as internal standards. Based on repeated analysis of standards, precision for the content of organic carbon and total nitrogen were ±0.05 and ±0.005 % (relative standard deviation), respectively. The long-term precision (9 years) of the instrument is ±0.2 ‰ for both carbon and nitrogen isotopes. The isotopic ratios were reported in per mil notation, and results were expressed relative to conventional standards, i.e. Pee Dee Belemnite (PDB) and atmospheric N₂ for carbon and nitrogen respectively and represented as δ notation, as given below.

$\delta^{13}\text{C} = [(X_{\text{samp}} - X_{\text{std}}) / X_{\text{std}}] \times 1000 \text{ ‰}$ Where, X_{samp} represents ¹³C/¹²C of a sample, and X_{std} denotes ¹³C/¹²C of standard for carbon.

Bacterial metabolic rates measurement

For bacterial metabolic rates measurement, a 2L water sample was filtered through a 0.7 μm GF/F glass fiber. Filtration removed essentially all the bigger planktons like phytoplankton and zooplanktons. Bacterial respiration (BR) rate was measured in filtered water incubated at room temperature as the difference in dissolved oxygen at 0 h and 24 h (Biddanda and Cotner 2002; Ram et al. 2007) assuming the respiratory quotient of 0.86 (Bell and Ahlgren 1987), although this technique has its share of potential ambiguities. Dissolved Oxygen (DO) was analysed using Winkler's titration method of Carritt and Carpenter (1966) using automated titration unit (Metrohm-Switzerland). Winkler technique is most commonly used technique to estimate BR offering high sensitivity (Carignan et al. 1998), although there is a disadvantage of preventing continuous monitoring of oxygen concentrations. Simultaneously filtered water was siphoned out in a 500 ml Nalgene bottles (duplicates), wrapped in a black cloth and incubated at room temperature for checking bacterial abundance (BA). Samples were collected at 0h and 24h from incubated Nalgene bottles and preserved at 4 °C with 5% formalin until analysis. BA was measured after staining cells with 4'-6'-Diamidino-2-phenylindole (DAPI) stain (Porter and Feig 1980) and counting using epifluorescence microscope (Olympus BX-51, Japan). Bacterial productivity (BP) rates were calculated by converting the increase in cell number with time (Hagstrom et al. 1979; Toolan 2001) and was converted to carbon units using a conversion factor of 20×10^{-15} g C cell⁻¹ (Hobbie et al. 1977; Lee and Fuhrman 1987). These BP rates might be conservative estimates as removal of bacterial cells can occur by filtration, removal of particle attached bacterial cells, by viral lysis, and by grazers that manage to get through the filters. Bacterial growth efficiency (BGE) was calculated as $\text{BGE \%} = [\text{BP} / (\text{BP} + \text{BR}) \times 100]$. Bacterial carbon demand (BCD) was calculated as $\text{BCD} = \text{BP} + \text{BR}$. Bacterial respiration rate, productivity rate, and carbon demand were expressed as $\mu\text{g C L}^{-1}\text{d}^{-1}$ and BA as number $\times 10^8$ cells L⁻¹. The pCO₂ was computed using measured salinity, temperature, pH and alkalinity using CO₂ SYS program (Lewis and Wallace 1998) and represented as μatm . The CO₂ dissociation constants given by Millero et al. (2006) for 0 to 40 salinity range were utilised. The pH and total alkalinity were measured by potentiometric (Metrohm, Switzerland) Gran titration methods following Standard Operating Procedures (SOP) suggested by DOE (1998).

Statistical analysis

Principal component analysis (PCA) was carried out followed by correlation analysis to check the influence of river discharge associated organic matter and environmental factors on bacterial metabolic rates. Using the correlation matrix, principal components (factors) with eigenvalues > 1 were retained and subjected to a varimax rotation with Kaiser to estimate the proportion of the variance of each attribute explained by each selected factor loadings. Utilizing the loading component theory, we only considered factor loadings (correlation values) greater than 0.5. All the statistical analysis were carried out using the Statistica version 10 software whereas figures were drawn using the Grapher-version 3

and Microsoft Excel.

Result

Hydrographical conditions in study region

Hydrographical variations in study region during three different river discharge patterns are shown in Table 1. Surface water temperature ranged between 27.1 to 31.6 (29.8 ± 1.4 °C) (Figure 3a). Salinity ranged from 0.07 to 34.6 psu (15.4 ± 13.1) having wide variation (Coefficient of variation; CV=84%) and inversely proportional relation to river discharge magnitude (Figure 3b). Relatively higher salinity was observed towards coast than estuary due to tidal influence. Estuary was well mixed during peak discharge period with almost zero salinity (Table 1). Concentrations of TSM varied from 12.4 to 591 (158 ± 203 mg L⁻¹) having higher average values by ~12 times and individual values by ~32 times during peak discharge period to that of no discharge period. A turbidity maximum was observed at station 3 during discharge periods whereas at station 4 during no discharge period (Figure 3c). TSM was inversely proportional to salinity ($r^2=0.7$), suggesting its addition by river discharge. Inorganic nutrients, such as dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP) and dissolved inorganic silicate (DSi), showed variations with reference to river discharge magnitude. DIN varied from 10.4 to 52.9 (22 ± 11.9 µM) whereas DIP ranged from 1.3 to 10.1 (5.9 ± 3.1 µM). DSi varied from 11.2 to 78 (40.8 ± 20.1 µM) having maxima during moderate discharge period. Spatially nutrients (DIN, DIP, DSi) maxima were observed mostly at middle estuary whereas temporally DIN and DIP maxima was observed during peak discharge period and DSi during moderate discharge period. A negative correlation of DIN ($r^2=0.9$) and DSi ($r^2=0.85$) with salinity and strong positive correlation with each other ($r^2=0.9$) (Table 4) suggests their possible addition by river discharge. In the present study, Chl-a concentrations varied from 0.3 to 86.8 (18.5 ± 26.7 mg m⁻³) with higher concentrations in an estuarine region than coastal (Figure 3d). Maximum Chl-a concentrations were observed during moderate discharge period (Table 1). Chl-a was negatively related to DIP ($r^2=0.72$) having maxima during moderate discharge period particularly at the middle estuary.

Spatiotemporal variation of organic matter

As shown in Table 1, POC ranged from 15.2 to 1272 (219 ± 330 µM). Maximum concentrations were observed during peak discharge period (686 ± 391 µM) than moderate discharge period (94.3 ± 39.3 µM) and no discharge period (62.7 ± 25 µM) in estuarine region whereas lower concentrations in the coastal region (Figure 3g). Salinity and POC were inversely related ($r^2=0.57$). PON ranged from 2.2 to 119 (24.5 ± 29.5 µM) having maxima during peak discharge period and similar spatio-temporal trend like POC (Figure 3h). Wide variation was observed for POC (CV =167 %) and PON (CV =137%). Discharge driven organic matter concentrations decreased from the estuary to coast with significant

negative relation with distance (upper estuary to coast) (POC; $r^2=0.93$ $p<0.05$, PON; $r^2=0.67$ $p<0.05$). A significant positive relation between POC and PON ($r^2=0.97$) suggested their common source to estuary. Concentrations of DOC and DON ranged from 127 to 988 (509 ± 264 μM) and 26.6 to 97.3 (51.1 ± 23.1 μM) respectively in estuary whereas in coast it ranged from 72.7 to 176 (117 ± 47.6 μM) (DOC) and 43.8 to 110 (62.4 ± 32.1 μM) (DON). Both DOC and DON concentrations were high during peak discharge period (Figure 3e, 3f), having relatively higher concentrations in estuary than coastal stations. Although the concentrations of both particulate and dissolved organic matter were high during peak discharge period, the ratio of DOC: POC was low indicating relatively higher content of POC than DOC in an estuary. In estuarine region DOC: POC ratio varied between 0.6 to 12.4 (4.9 ± 3.7) having lowest average during peak discharge period (1.4 ± 0.6) and higher during no discharge period (6.5 ± 2.5). In coastal region, relatively higher ratio was observed during moderate discharge (5.1 ± 2.6) than no discharge period (3.4 ± 3.1).

C: N ratio ranged from 2.7 to 12 (7.4 ± 2.5) (Figure 3i). In estuarine region higher C: N ratio was observed during peak discharge period (11 ± 1.1) whereas, during moderate discharge period and no discharge period, ratio was 6.5 ± 0.7 and 5.9 ± 2.6 respectively. At coastal stations ratio was similar during no discharge (6.7 ± 0.3) and moderate discharge periods (6.1 ± 1.3). During moderate discharge period C: N ratio in study region was equal to red field phytoplankton ratio (6.6). Stable isotope ratios of organic carbon ($\delta^{13}\text{C}_{\text{POC}}$) ranged from -32.9 to -22.6 ($-26.7\pm 3.9\%$) and -24.4 to -22.8 ($-23.6\pm 0.8\%$) in estuarine and coastal stations respectively (Figure 3j). Highly depleted $\delta^{13}\text{C}_{\text{POC}}$ ratios were observed during moderate discharge period (-32.9 to -29.7; $-31.5\pm 1.4\%$). In coastal region, $\delta^{13}\text{C}_{\text{POC}}$ ratios were enriched during moderate ($-23.5\pm 1.0\%$) and no discharge periods ($-23.7 \pm 0.9 \%$).

Spatiotemporal variation of bacterial metabolic rates and abundance

The variation in bacterial metabolic rates is depicted in Table 2. Bacterial abundance varied from 0.2 to 3 ($1.3\pm 0.8 \times 10^8$ cells L^{-1}) with wide spatiotemporal variation ($\text{CV}=57\%$) in estuarine region whereas in coastal region it ranged from 0.5-1.6 ($1\pm 0.5 \times 10^8$ cells L^{-1}) (Figure 4a). Higher average values were observed in an estuarine region that decreased towards coastal region having significant negative relation with distance particularly during peak discharge ($R^2=0.82$) and moderate discharge ($R^2=0.2$) periods. Temporally, maximum average abundance was observed in an estuarine region during no discharge period (1.5-3.0; $2.15\pm 0.7 \times 10^8$ cells L^{-1}) followed by peak discharge period (0.9-1.9; $1.3\pm 0.4 \times 10^8$ cells L^{-1}) and least during moderate discharge period (0.2-1.2; $0.7\pm 0.4 \times 10^8$ cells L^{-1}).

BR rates varied 25 fold, ranging between 39.1 to 994 $\mu\text{g C L}^{-1}\text{d}^{-1}$ (269 ± 247 $\mu\text{g C L}^{-1}\text{d}^{-1}$) in estuarine region and 199 to 355 (296 ± 71.3 $\mu\text{g C L}^{-1}\text{d}^{-1}$) in coastal region with large spatiotemporal variation ($\text{CV}=78\%$). Average BR rates decreased in the following trend, peak discharge > moderate discharge >

no discharge. It ranged from 162 to 343 ($264 \pm 79 \mu\text{g CL}^{-1}\text{d}^{-1}$), 78.3 to 994 ($375 \pm 423 \mu\text{g CL}^{-1}\text{d}^{-1}$) and 39.1 to 355 ($222 \pm 113 \mu\text{g C L}^{-1}\text{d}^{-1}$) during no discharge, peak discharge and moderate discharge periods respectively in the entire study region (Figure 4b). Maximum BR rate was observed during peak discharge period at station 2. The BR value decreased with distance towards the coast ($R^2=0.21$) during peak discharge period, while a reverse trend of increase was observed during moderate discharge period towards the coast ($R^2=0.21$). BP rates varied from 0.4 to 33.3 ($8.4 \pm 9.2 \mu\text{g C L}^{-1}\text{d}^{-1}$) having ~7 times higher average rates in estuarine ($10.2 \pm 9.6 \mu\text{g C L}^{-1}\text{d}^{-1}$) than coastal region ($1.4 \pm 1.1 \mu\text{g C L}^{-1}\text{d}^{-1}$) (Figure 4c). In estuarine region average rates were high during peak discharge period, 14.9 to 33.3 ($20.2 \pm 8.7 \mu\text{g C L}^{-1}\text{d}^{-1}$) followed by moderate discharge period 2.5 to 18.8 ($9.1 \pm 7.5 \mu\text{g C L}^{-1}\text{d}^{-1}$) and lowest during no discharge period 1.6 to 3.3 ($3 \pm 1 \mu\text{g C L}^{-1}\text{d}^{-1}$). Significant positive relation of BP with DOC, POC, PON, DSi and DIP and negative relation with salinity (Table 4) indicates that the organic matter and inorganic nutrients carried by river discharge boosted bacterial productivity in the study region.

Higher productivity rates associated with higher C: N ratios having significant positive relation suggests the efficient utilisation of terrigenous organic matter. Maximum BCD was observed during peak discharge (395.2 ± 421.8) in an estuarine region. Quite low BGE's were observed during the present study, and the values ranged from 0.1 to 18 ($4.4 \pm 5.4\%$). Maximum growth efficiency was observed during peak discharge period 1.5 to 18.1 ($10.1 \pm 6.8\%$) followed by moderate discharge period 1.4 to 11.5 ($5.8 \pm 4.2\%$) and low during no discharge period 1 to 1.3 (1.2 ± 0.2) (Figure 4d) in an estuarine region. Majority of BGE values (76%) were $<8\%$ and remaining (24%) were $>8\%$ having wide spatiotemporal variation ($CV=123\%$). Bacterial growth efficiency was significantly related to BP ($r^2=0.76$), indicating the influence of BP on BGE. In coastal region BA, BP and BGE were relatively high during no discharge period than moderate discharge. No data available for the coastal region during peak discharge period due to flood and rough weather conditions.

Surface water pCO₂ levels in Godavari estuary

Surface pCO₂ levels in Godavari estuary and adjacent coastal region displayed significant variations from upper estuary to coastal region. During the study period, pCO₂ level ranged from 362 to 24498 ($5841 \pm 6329 \mu\text{atm}$) having a maximum in an estuarine region ($7414 \pm 6594 \mu\text{atm}$) than coastal region ($1121 \pm 1127 \mu\text{atm}$) (Table 2). In estuarine region, maximum values were observed during moderate discharge ($10670 \pm 10022 \mu\text{atm}$) followed by peak discharge period ($7210 \pm 5089 \mu\text{atm}$) and minimum during no discharge ($4364 \pm 2552 \mu\text{atm}$) period. In coastal region, pCO₂ level was high during no discharge period (1879 ± 1233) to that of moderate discharge period ($364 \pm 3 \mu\text{atm}$). The annual mean of pCO₂ levels decreased linearly from an estuary to coast in the following order: upper estuary

(14162±10609 μatm) > middle estuary (5640±2851 μatm) > lower estuary (4215±3635 μatm) > coastal region (1121±1127 μatm), having maxima in the upper estuary.

Principal Component Analysis implications

The PCA analysis was performed and presented in Figure 5a, b, c, d. Based on the Kaiser criteria (Eigenvalue >1), the first four PC explained 86% of the joint variance (Table 3). The fourth PC did not have any significant loading (<0.5); therefore, only the first three PCs were used for further analysis. PC1 explained 46% of the total variance and had strong negative loadings (>0.8) of, DIP, DOC, POC, BP, TSM, PON, DIN and C: N. As seen in Figure 5 a, the scores of all the stations during the peak discharge were relatively same, and distributed in the second and third quadrant. Therefore, indicating that the increased river discharge lead to the high input of dissolved and particulate organic matter, inorganic nutrients and suspended matter in the estuary. The biotic factors were used as supplementary variables among which BP and BGE showed strong and moderate negative loading respectively, on PC1, suggesting the positive influence of organic matter load and inorganic nutrients on those parameters. PC2 explained 23% total variance with a strong negative loading of Chl-a and positive loading of $\delta^{13}\text{C}_{\text{POC}}$, whilst pCO_2 , DSI (negative), salinity (positive) had a moderate loading (<0.6->0.3). PC2 explained maximum Chl-a in the estuary during moderate discharge period associated with highly depleted $\delta^{13}\text{C}_{\text{POC}}$ and maximum pCO_2 levels. During moderate discharge period, Chl-a and stations 1 to 4 were within same (fourth) quadrant (Figure 5a, b). PC3 (10% of total variance) was moderately loaded with DON (positive) and temperature (negative), indicating higher surface water temperature during no discharge period.

Discussion

Influence of river discharge and associated organic matter on bacterial abundance and metabolic rates

In tropics, monsoon mediated river runoff has a significant impact on estuarine ecology and biogeochemical processes which are interlinked. Elemental and stable isotopic ratios of carbon serve as a very useful tool to identify the source and nature of organic matter and are widely used (Ishiwatari and uzaki 1987; Sarma et al. 2012; Krishna et al. 2015). The high C: N ratio and depleted $\delta^{13}\text{C}_{\text{POC}}$ values during peak discharge period were contributed by increased river discharge associated with a huge load of terrigenous particulate and dissolved organic matter to the estuary. During moderate discharge period, organic matter was dominantly autochthonous as observed from the Chl-a maxima and low C: N ratio (6.5), close to red field ratio. In spite autochthonous organic matter dominance, $\delta^{13}\text{C}_{\text{POC}}$ was highly depleted suggesting its contribution by fresh water planktons. The contribution of

highly depleted $\delta^{13}\text{C}_{\text{POC}}$ isotope ratios (-34 to -40 ‰) by freshwater planktons have been reported earlier (Boschker et al. 2005; Hellings et al. 1999; Hamilton and Lewis 1992). The low saline conditions associated with relatively low TSM could have supported discharge driven fresh water plankton's growth during the moderate discharge period. During the study period (the year 2010) abundant fresh water planktons were observed in study region during moderate discharge period (Bharathi M, 2014 Ph.D. thesis). Our observations suggest significant control of river discharge magnitude and pattern on hydrography as well as quality and quantity of organic matter in the study region.

Having adopted experimental approach to measure bacterial metabolic rates, our results indicate that addition of a huge load of terrigenous organic matter to estuary boosted bacterial metabolic rates. A decrease in average BA was observed from no discharge to moderate discharge period. However, the spatial decline from upper estuary towards the lower estuary during peak discharge period could imply the intolerance of terrigenous bacterial population to increased salinity. Using molecular tools, major bacterial groups identified during the study period (the year 2010) were *Proteobacteria*, *Firmicutes* and *Actinobacteria* (Prasad VR, 2014 Ph.D. thesis). During peak discharge period most of the bacteria belong to the phyla *Proteobacteria* and *Firmicutes* which are known to be heterotrophic terrestrial bacteria. During no discharge period bacterial community was dominated mostly by the members of the genus *Bacillus*. Results suggest a significant impact of river discharge magnitude on the abundance and bacterial community structure.

Unlike Lee et al. (2009), we observed significant positive relation of BP with POC, suggesting a significant influence of allochthonous organic matter on BP rates, which is also evident from the significant positive relationship with DOC, POC, DIP and DSi (Table 4). Significant positive association of BP with C: N ratio ($r^2=0.73$) and a positive relation of BGE with C: N ratio ($r^2=0.49$) suggests the efficient utilisation of terrigenous organic matter by the bacterial community, and hence control of organic substrate on BP and BGE. Observed BP rates were in similar range to those observed for Blanes Bay (Vazquez Dominguez et al. 2007) and Amazon River and tributaries (Benner et al. 1995), but were lower than those observed for tropical Mandovi estuary and adjacent coastal regions (Ram et al. 2003, 2007) and Cochin estuary (Thottathil et al. 2008).

Respiration rates were in similar range as that of the tropical regions but were higher by an order of magnitude to that of the temperate region (Table 5). One extremely high value at station 2 ($994 \mu\text{g C L}^{-1}\text{d}^{-1}$) increased the average BR rate leading to wide variation (CV=113%) during peak discharge period compared to no discharge (CV=33%) and moderate discharge (CV=50%) periods. The maximum (average and individual) respiration rates during peak discharge period were consistent with maximum concentrations of terrigenous organic matter. Observations could imply that the terrigenous

organic matter with higher C: N ratios was utilised at higher energy cost during the peak discharge period resulting into higher respiration rates. Our observations are in corroboration with those of Mc Callister and del Giorgio (2008) who reported 3 to >70 % respiration of terrigenous organic carbon by heterotrophic bacteria and those of Siuda and Chrost (2002) who reported an increase in BR rate with increasing particulate organic matter.

Positive relation of BR and BCD with C: N ratios, indicates control of organic substrate quality on these metabolic rates which is in corroboration with Lee et al. (2009). Moreover, the almost zero salinity of the freshwater filled estuary, especially during peak discharge period, might have supported the faster oxidation of organic matter and hence high respiration rates. Cole et al. (1988) observed that oxidation of organic carbon and CO₂ release is faster in freshwater than in seawater. Maximum BCD during peak discharge period also suggests, in situ produced carbon was not sufficient to meet heterotrophic demands, which is then met from the external source, that is, terrigenous organic matter. It is well-known fact that bacteria prefers and utilise DOC than POC (Eiler et al. 2003), but it was interesting to observe atypical scenario in the present study with respect to bacterial utilisation of organic carbon. Less significant correlation of bacterial metabolic rates with DOC and more significant with POC, suggest the preference and utilisation of abundant form of organic carbon (Table 4; Figure 6 a,b,c). Low DOC: POC ratio (1.4 ± 0.6) during peak discharge period indicates the higher proportion of POC than DOC that was associated with higher BGE, BP and BR rates. This also suggests, irrespective of the dissolved or particulate form of organic carbon, bacteria utilise the abundant one. Other possible reason for terrigenous POC modification could be the terrestrial bacterial population added by the river-runoff must be more efficient in utilising POC. Siuda and Chrost (2002) reported rapid changes in substrate exploitation strategy of bacteria with increasing POM content resulting in the adaptation of bacteria to live in particle-attached stage and effective modification of particulate organic matter. Our observations suggest bacterial community utilises terrigenous organic carbon efficiently to meet carbon demand. Bacterial community structure and assemblage could also play an important role in organic carbon modification which is in corroboration with Tranvik and Hofle (1987) and Logue et al. (2016). Although quantity and quality of organic matter showed significant impact on bacterial metabolic rates, we did not observe any significant impact of temperature on bacterial metabolic rates although some studies reported (Apple et al. 2006; Lee et al. 2009) significant effect of temperature on bacterial respiration rate. Nonetheless, some studies suggest that temperature plays a minor role in controlling BGE (Vazquez Dominguez et al. 2007; Alonso-Saez et al. 2007). Salinity had significant negative impact on BGE and BP whereas a statistically insignificant negative relation with BR and BCD.

Net heterotrophy and pCO₂ levels in estuary

To understand the ecosystem functionality and assessment of the organic carbon transfer to higher trophic level in an estuary, it is necessary to estimate whether it is autotrophic or heterotrophic. BGE has been used as a biological indicator to understand the nature of the estuarine system (Yuan et al. 2010). Usually, estimates of BGE for natural planktonic bacteria range from <0.4% to as high as 80% (Eiler et al. 2003 and references therein). In spite of higher metabolic rates (BP, BR, BCD), observed BGE was quite low, which is in agreement with Amado et al. (2013) who reported higher metabolic rates but low BGE's in tropics to that of temperate ecosystems. BGE is more dependent on organic matter and nutrient resource availability as observed from a significant relation between BGE and DIP ($r^2=0.58$) and BGE and organic matter (POC, PON). Significant positive relation of BGE and BP ($r^2=0.7$) suggests control of BP over BGE. According to Lee et al. (2009), net heterotrophy exists for tropical waters when BGE is <8%. In present study majority (70%) of the BGE values were below 8% suggesting year round net heterotrophy in an estuary. Average BGE in an estuary (5%), suggests ~5% of utilised organic carbon gets in to bacterial biomass and ~95% gets respired as inorganic CO₂. Present observations are similar to Eiler et al. (2003) who reported low BGE's between 0.4 to 10.4% suggesting maximum contribution for CO₂ flux. Yuan et al. (2010) reported >90% bacterial contribution to community respiration and low BGE's in subtropical Hongkong waters. Sarma et al. (2009) reported net heterotrophy in Godavari estuary based on total community production and respiration ratio, where P: R ratio was less than 1. The Present study confirms the significant contribution of heterotrophic bacteria to total respiration rate, therefore, making the study region net heterotrophic.

Compared to other tropical and temperate estuaries, pCO₂ levels were very high in Godavari estuary (Table 6). The addition of loads of organic matter through river discharge and its efficient oxidation by bacteria under low saline conditions could have favoured higher pCO₂ levels in study region as observed from the significant positive relation of pCO₂ with DOC, POC, PON, BP and significant negative relation with salinity (Table 4). Similar observation of higher pCO₂ levels associated with low salinity (<5 psu) was observed in Mandovi and Zuari estuaries (Sarma et al. 2001), even though the levels were quite low (< 2000 μ atm) to that of the present study. Present pCO₂ levels in Godavari estuary were higher than that observed by Bouillon et al. (2003) during dry period (<1000 μ atm) and also higher than that of temperate estuaries considered to be polluted like Rhine and Scheldt (Table 6). It is reported by Abril et al. (2015) that calculated pCO₂ levels were >10 % higher in 60% and >100% higher in 25% of fresh water samples, mostly in organic rich and acidic samples. In the present study, although the high pCO₂ levels associated with high organic matter concentrations, p^H of the water samples was always above 7 with relatively low values during peak discharge period, thereby ruling out the possible overestimation. There might be some overestimations of calculated pCO₂ levels compared to that of measured pCO₂ levels.

Maximum pCO₂ levels in Godavari estuary were ~60 times higher to that of atmospheric pCO₂ levels. Whereas, average value was ~8 times higher to that of atmospheric pCO₂ levels during no discharge period and ~17 times higher during peak discharge and moderate discharge periods, respectively (average atmospheric level ~408 µatm, Scripps observatory data, the year 2010). Therefore, the results of the present study suggest the net heterotrophy in Godavari estuary with significant contribution from heterotrophic bacteria. Yuan et al. (2010) reported linear relation of bacterial respiration and pCO₂ levels (average ~570 µatm) having 50% contribution of bacterial respiration to CO₂ efflux in subtropical Hong Kong water, attributed to the influence of the Pearl river discharge and effluent. Since the respiration rate and pCO₂ levels were high in Godavari estuary to that of Hong Kong waters, bacterial contribution to pCO₂ levels and CO₂ efflux implies to be much higher, which needs further estimation and confirmation.

Conclusion

We conclude that monsoon mediated river discharge magnitude and pattern had significant control on hydrography, nature of organic matter and bacterial metabolic rates in Godavari River estuary and adjacent coastal waters. Organic matter was dominantly autochthonous during moderate discharge whereas allochthonous during peak discharge period. Salinity pattern showed a significant impact on bacterial abundance in the study region, but we did not observe an effect of temperature on the metabolic rates. The heterotrophic bacteria efficiently utilise the terrigenous particulate organic matter when it is abundant. The present study showed that apart from the quality, quantity of organic matter has an impact on bacterial metabolic rates. The observations suggest that organic carbon produced in situ was not sufficient to meet heterotrophic carbon demand which is then sufficed by the external terrestrial organic carbon sources. Further, the majority of the utilised carbon gets respired leading to high pCO₂ levels and heterotrophic conditions in Godavari estuary. The increasing BR rate with terrigenous organic matter input and excess heterotrophy, raise the concern about the occurrence of low dissolved oxygen or hypoxia, which could have an adverse effect on fisheries and biodiversity. The present study attempts to fill the knowledge gap on the bacterial metabolic rates with respect to monsoonal river discharge pattern and contribution to biogeochemical cycling of organic carbon in a tropical monsoonal estuary and at large to global carbon estimates.

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Figure legends

Figure 1. Sampling station locations in the Gautami Godavari estuary: Upper estuary (Station 1); Middle estuary (Station 2- 3); Lower estuary (station 4) and coastal region (5-6).

Figure 2. Godavari river discharge

Figure 3. Hydrographic variations in study region a) Temperature ,b) Salinity, c) TSM , d) Chl-a , e) DOC, f) DON , g) POC, h) PON, i) C:N ratio, j) $\delta^{13}\text{C}_{\text{POC}}$

Figure 4. Spatio-temporal variation of a) Bacterial abundance, b) Bacterial respiration rate, c)

Bacterial productivity rate d) Bacterial growth efficiency in study region.

_●-No discharge period, ▲-Peak discharge period, +-Moderate discharge period.

Figure 5. Principal component analysis showing a) influence of river discharge on bacterial activity and environmental parameters, b) spatiotemporal scale.

Figure 6. Increasing bacterial productivity rate with a) POC, b) PON, c) DOC and d) Bacterial respiration rate with C: N ratio.

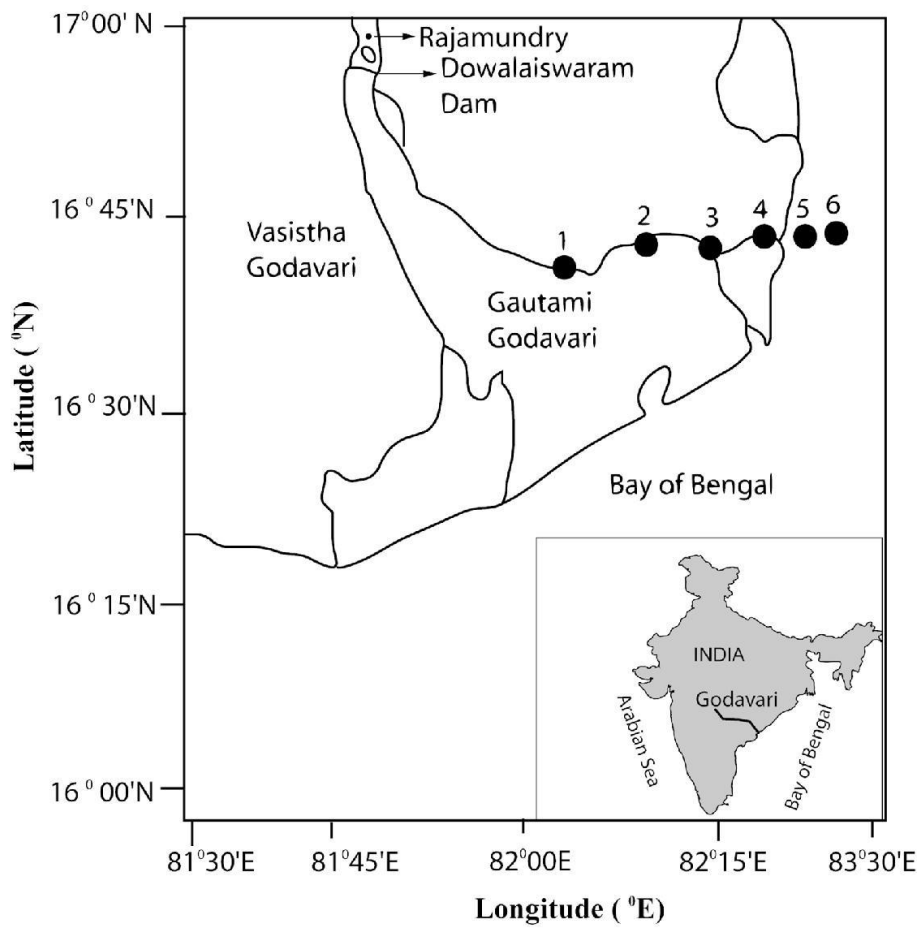


Figure 1

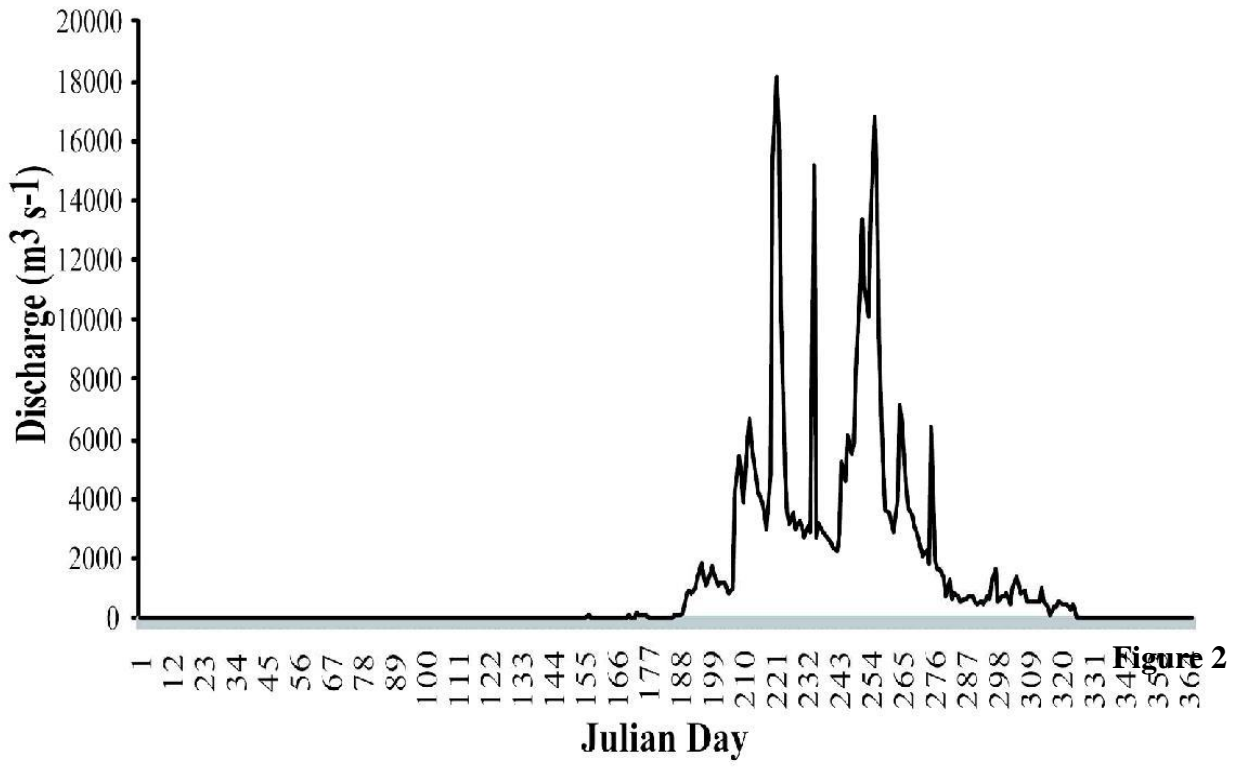


Figure 2

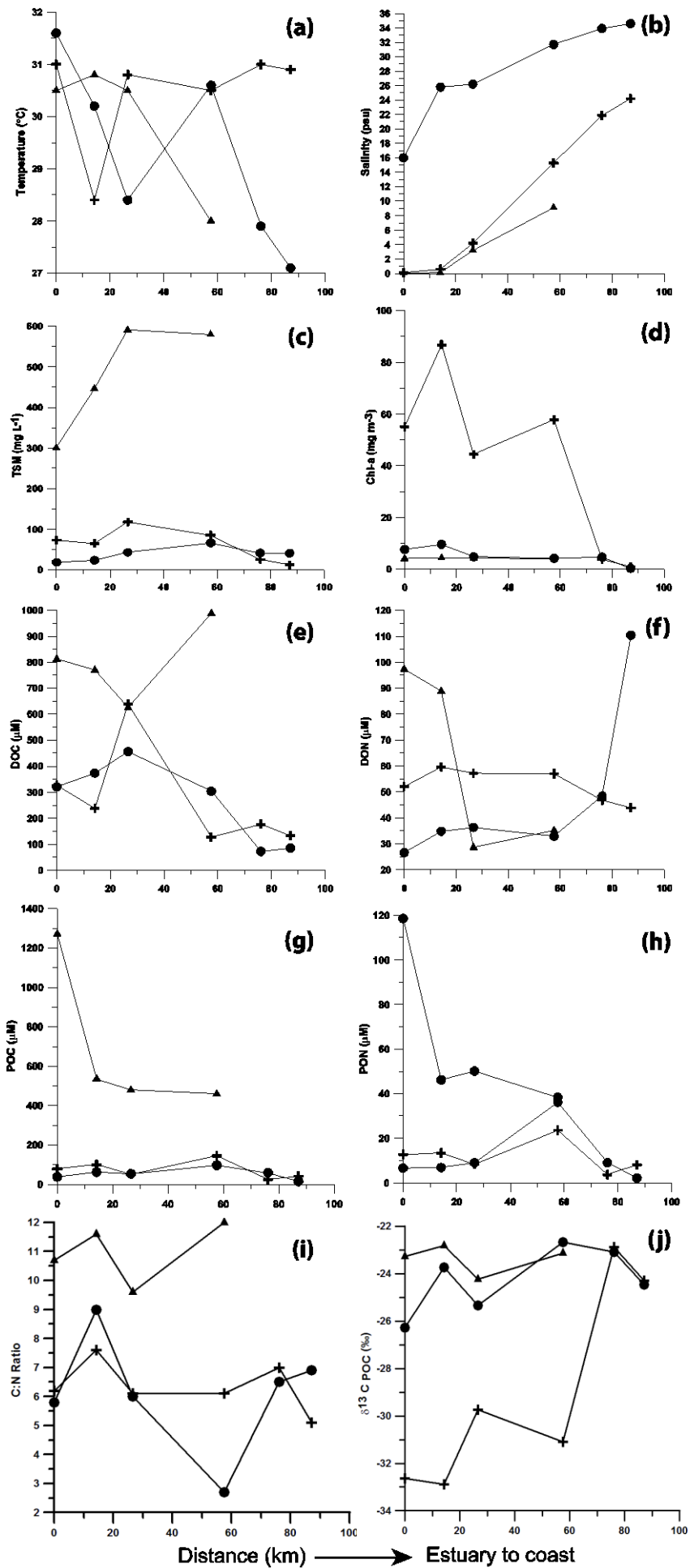


Fig. 3

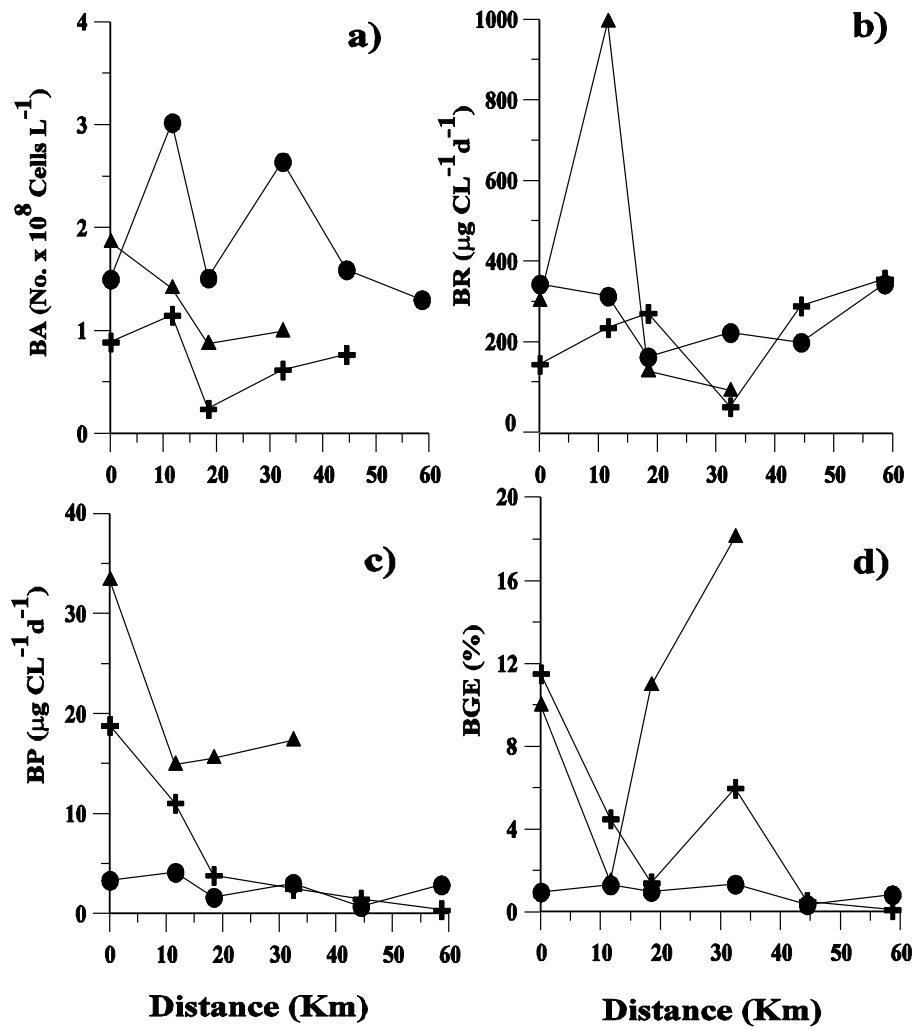


Figure 4

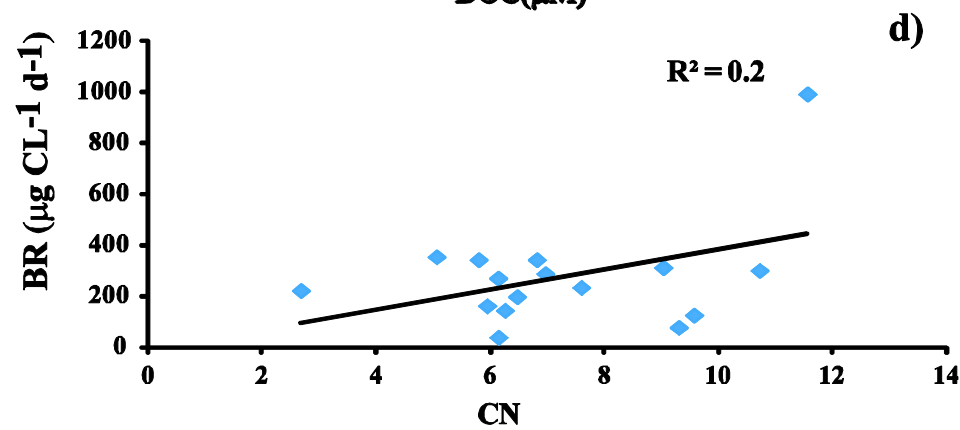
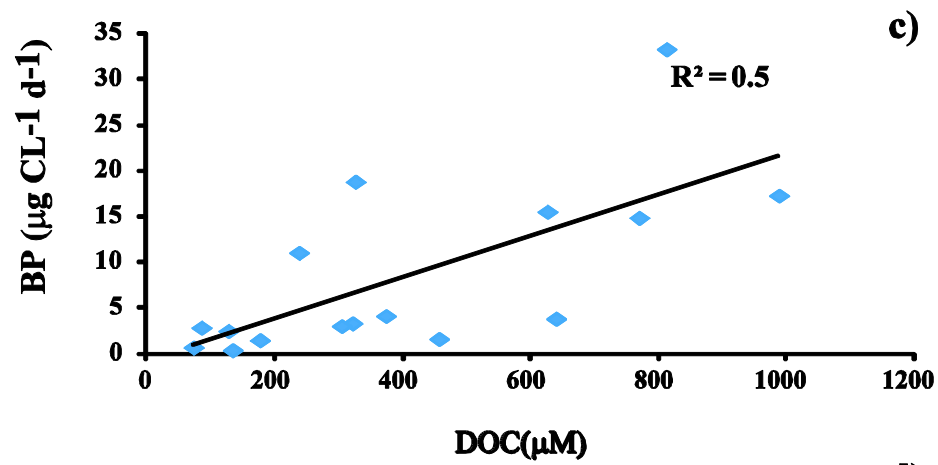
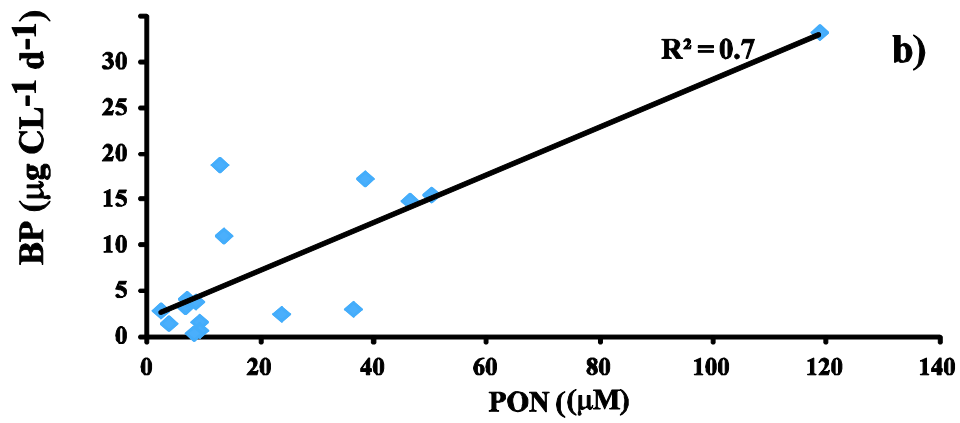
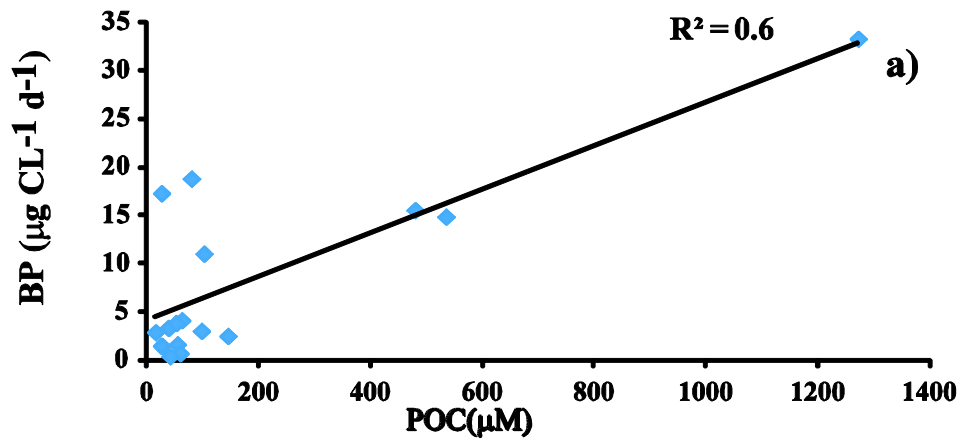


Figure 6

Table 1. Physico-chemical variations in study region

Stn./Disc. period	T (°C)	Sal (PSU)	TSM (mgL ⁻¹)	DIN (µM)	DIP (µM)	DSi (µM)	Chl-a (mgm ⁻³)	DOC (µM)	DON (µM)	POC (µM)	PON (µM)	C:N	δ ¹³ C _{Poc} (‰)
Stn 1 ND	31.6	16.0	18.6	16.5	3.3	36	7.6	321	26.6	38	6.6	5.8	-26.2
Stn 2 ND	30.2	25.8	23.6	13.7	8.1	77.6	9.6	373	34.8	61.9	6.9	9	-23.7
Stn 3 ND	28.4	26.2	43.0	22.8	9.2	54.9	4.8	456	36.3	53.8	9.0	6	-25.3
Stn 4 ND	30.6	31.7	66.4	12.4	4.9	11.4	4.2	304	32.9	97	36.2	2.7	-22.6
Stn 5 ND	27.9	33.9	41.6	10.4	7.1	25.8	4.7	72.7	48.4	58.8	9.1	6.5	-23.0
Stn 6 ND	27.1	34.6	40.8	14.5	5.1	11.2	0.3	85.3	110.4	15.2	2.2	6.9	-24.4
Stn 1 PD	30.5	0.07	301	28.4	9.7	57.4	4.1	812	97.3	1272	118	10.7	-23.2
Stn 2 PD	30.8	0.1	446	27.6	7.8	52.2	4.4	769	88.8	534	46.2	11.6	-22.8
Stn 3 PD	30.5	3.2	591	23.2	4.3	44.4	4.3	626	28.6	479	50.1	9.6	-24.2
Stn 4 PD	28.0	9.1	580	35.3	8.5	31.4	3.9	988	35.2	460	38.3	12	-23.1
Stn 1 MD	31.0	0.1	73.3	52.9	8.9	45.9	55.1	326	52.1	79.2	12.7	6.2	-32.6
Stn 2 MD	28.4	0.6	64.7	38.3	10.1	78	86.8	237	59.5	102	13.4	7.6	-32.9
Stn 3 MD	30.8	4.2	118	12.8	1.3	35.2	44.4	639	57.2	51.6	8.4	6.1	-29.7
Stn 4 MD	30.5	15.3	85.3	15.7	1.7	38.3	57.8	127	57.2	145	23.6	6.1	-31.1
Stn 5 MD	31.0	21.9	25.6	14.8	2	36.2	3.8	176	46.9	25.5	3.7	7	-22.8
Stn 6 MD	30.9	24.2	12.4	12.6	2	17.8	0.9	134	43.8	40.9	8.1	5.1	-24.3

* ND: No discharge period PD: Peak discharge period MD: Moderate discharge period.

* Due to flood and rough weather, sampling was not done for Stn. 5 and 6 during peak discharge period

Table 2. Bacterial metabolic rates and pCO₂ levels variation during study period

Stn./Disch. period	BA (No.x 10⁸cells L⁻¹)	BR (µg CL⁻¹d⁻¹)	BP (µg CL⁻¹d⁻¹)	BGE (%)	BCD (µg CL⁻¹d⁻¹)	pCO₂ (µatm)
Stn 1 ND	1.5	343	3.3	1.0	346	3299
Stn 2 ND	3.0	313	4.1	1.3	317	3398
Stn 3 ND	1.5	163	1.6	1.0	164	2603
Stn 4 ND	2.6	223	3.0	1.3	226	8156
Stn 5 ND	1.6	199	0.7	0.3	199	2751
Stn 6 ND	1.3	343	2.9	0.8	346	1007
Stn 1 PD	1.9	301	33.3	10.0	334	14690
Stn 2 PD	1.4	994	14.9	1.5	1008	5988
Stn 3 PD	0.9	126	15.5	10.9	142	4662
Stn 4 PD	1.0	78.3	17.3	18.1	96	3500
Stn 1 MD	0.9	144	18.8	11.5	163	24498
Stn 2 MD	1.2	235	11.1	4.5	246	10576
Stn 3 MD	0.2	271	3.8	1.4	275	6615
Stn 4 MD	0.6	39.1	2.5	6.0	41.6	991
Stn 5 MD	0.8	289	1.5	0.5	291	362
Stn 6 MD	0.5	355	0.4	0.1	356	366

* ND: No discharge period PD: Peak discharge period MD: Moderate discharge period

Table 3. Factor coordinates of the variables and Eigen values of PCA

Variables	Factor1	Factor 2	Factor 3
pCO ₂	-0.35	-0.68	0.03
Temperature	-0.1	-0.28	-0.74
Salinity	0.72	0.64	0.11
TSM	-0.85	0.15	-0.1
DIN	-0.82	0.25	0
DIP	-0.9	0.21	-0.04
Dsi	-0.66	-0.62	-0.05
Chl-a	0.13	-0.91	0.18
DOC	-0.89	0.05	-0.17
DON	-0.28	-0.03	0.79
POC	-0.89	0.09	0.11
PON	-0.83	0.07	0.05
C:N	-0.8	0.14	0.19
δ ¹³ C _{POC}	-0.2	0.91	-0.11
BA	-0.03	0.39	0
BR	-0.28	0.18	0.06
BP	-0.87	-0.24	0.13
BGE	-0.62	-0.23	-0.01
BCD	-0.32	0.16	0.07

Value number	Eigenvalue Value	% total variance	Cumulative Eigen value	Cumulative %
1	6.43	45.98	6.43	45.98
2	3.2	22.87	9.63	68.85
3	1.3	9.6	11.0	78.5

Table 4. Correlation matrix of physicochemical and biological parameters.

	T	Sal	TSM	DIN	DIP	Dis	Chl-a	DOC	DON	POC	PON:CN ratio	$\delta^{13}C_{POC}$	$\delta^{15}N_{POC}$	BA	BGE	BR	BP	BCD	pCO ₂	
T	1.0																			
Sal	-0.5	1.0																		
TSM	0.3	-0.7*	1.0																	
DIN	-0.42	0.9***	-0.57*	1.00																
DIP	0.22	-0.54	0.87***	-0.39	1.00															
Dis	0.39	-0.85***	0.62*	-0.90***	0.54	1.00														
Chl-a	-0.05	0.01	-0.54	-0.02	-0.72**	-0.10	1.00													
DOC	0.43	-0.62*	0.77**	-0.56*	0.88***	0.72**	-0.65**	1.00												
DON	-0.28	-0.19	0.13	-0.18	0.33	0.14	-0.11	0.18	1.00											
POC	0.29	-0.57*	0.67*	-0.48	0.80**	0.50	-0.47	0.73**	0.44	1.00										
PON	0.33	-0.55	0.68*	-0.46	0.73**	0.46	-0.37	0.69**	0.35	0.97***	1.00									
C:N	0.11	-0.53	0.72**	-0.43	0.83***	0.48	-0.65*	0.64*	0.43	0.68*	0.53	1.00								
$\delta^{13}C_{POC}$	-0.04	0.6*	-0.07	0.72**	0.07	-0.71**	-0.37	-0.18	0.10	0.06	0.08	-0.01	1.00							
$\delta^{15}N_{POC}$	-0.08	0.25	-0.50	0.36	-0.32	-0.17	0.46	-0.22	0.00	-0.44	-0.40	-0.41	-0.11	1.00						
BA	-0.13	0.36	-0.12	0.43	0.18	-0.34	-0.32	0.09	-0.09	0.14	0.21	0.07	0.39	0.22	1.00					
BGE	0.21	-0.68*	0.69**	-0.59*	0.58*	0.47	-0.21	0.48	0.09	0.72**	0.74**	0.49	-0.17	-0.61*	-0.08	1.00				
BR	0.19	-0.17	0.35	-0.11	0.48	0.29	-0.31	0.41	0.47	0.24	0.15	0.51	0.13	0.24	0.09	-0.28	1.00			
BP	0.21	-0.65*	0.70**	-0.61*	0.81**	0.63*	-0.43	0.74**	0.45	0.96***	0.98***	**0.73	-0.11	-0.45	0.14	0.76**	0.24	1.00		
BCD	0.19	-0.20	0.37	-0.13	0.51	0.32	-0.33	0.44	0.49	0.28	0.19	0.53	0.13	0.22	0.09	-0.25	0.99***	0.28	1.00	
pCO ₂	0.11	-0.56*	0.36	-0.61*	0.50	0.66*	-0.13	0.63*	0.27	0.70**	0.74**	0.34	-0.44	-0.11	0.29	0.52	0.11	0.79**	0.14	1.00

Units for temperature (T) -°C; Salinity-PSU; TSM - mg l⁻¹; DIN; DIP; Dis - μM; Chl-a - mg m⁻³; DOC; POC; PON - μM; $\delta^{13}C_{POC}$ - ‰; $\delta^{15}N_{POC}$ - ‰; pCO₂ - μatm; BA - No. × 10⁶ cells l⁻¹; BGE-%; BR, BP, BCD - μg C l⁻¹ d⁻¹.

Table 5. Comparison of bacterial metabolic rates variation from different regions

Study Area	Bacterial Abundance (No.x10 ⁸ cellsL ⁻¹)	Bacterial production (µgCL ⁻¹ d ⁻¹)	Bacterial Respiration (µg C L ⁻¹ d ⁻¹)	Bacterial Growth efficiency (%)	Bacterial Carbon demand (µg CL ⁻¹ d ⁻¹)	Reference
Coastal and estuarine region off Goa-India	40-50	14.4-144	130-610	4.7- 31.1	144-755	Ram et al. 2007
Cochin estuary India	52-110	6.3-199	6.6-430	31.7-48.8	13-630	Thottathil et al. 2008
Blanes Bay and Mediterranean	-	1.7-5.3	33-40	1-30	37-56	Dominguez et al. 2007
Hudson river	0.2-0.6	63-136	48-192	23-70	110-328	Maranger et al. 2005
Amazon river and it's tributaries	9-12.6	10.56-43.9	1.65-39	52-86	12-83	Benner et al. 1995
Subtropical Hong Kong waters	50-900	12-96	48-156	8-30	17-186	Yuan et al. 2010
Coastal Ria de Vigo	13-61	0.2-1.9	0.2-11	29-44	1.2-3.6	Lonborg et al.2011
Godavari estuarine and coastal region-India	0.2 - 3	0.4-33	39-994	0.1-18	42.6-1009	Present study

Table 6. Variations in pCO₂ levels from different regions

Region	pCO₂ (µatm)	Reference
Mandovi-Zuari estuary	< 2000	Sarma et al. 2001
Godavari estuary	<1000	Bouillon et al. 2003
Hoogly estuary	220-1200	Mukhopadhyay et al. 2003
Coachin estuary	200-600	Gupta et al. 2009
Rhine estuary	~25000	Kempe 1982
Scheldt estuary	~15200	Borges and Frankignoulle 2002
Hudson river	~1147	Raymond et al. 1997
Amazon river	100-200	Abril et al. 2009
Hong Kong waters	~570	Yuan et al. 2010
Godavari estuary	362-24498	Present study