

Distribution of size-fractionated phytoplankton biomass from the anthropogenically stressed tropical creek (Thane creek, India)

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Abstract

Size-based partitioning of phytoplankton biomass is a crucial determinant of the type of food web functioning in an aquatic ecosystem. Seasonal and spatial distribution of total as well as size-fractionated phytoplankton biomass (pico, nano and micro-phytoplankton) from the tropical creek (Thane creek, India), was assessed in response to ambient physicochemical parameters. Nanophytoplankton biomass contributed the significant fraction (69%-97%) of total phytoplankton biomass throughout the creek irrespective of the seasons. The ability of nanophytoplankton to proliferate under low solar insolation helped them in sustaining high biomass in nutrient-rich turbid water. Microphytoplankton, which was the second dominant class, contributed 3-19% during pre-monsoon, 11-19% during monsoon and 3-7% during post-monsoon. High suspended sediment load and mesozooplankton grazing pressure have played an essential role in structuring the microphytoplankton biomass. Competitive disadvantage under high nutrient concentration resulted in low picophytoplankton biomass throughout the creek. The dominance of nanophytoplankton biomass deciphered the prevalence of herbivorous based food web in the Thane creek. The Pearson's correlation and the canonical correspondence analysis interpreted nutrient enrichment, suspended sediments and temperature played a significant role in determining the size structure of phytoplankton biomass. The eutrophication status of the study area studied using Trophic state index (TRIX). Availability of surplus nutrient during all the seasons could be the main factor behind the eutrophic to hypereutrophic (6.7-8.8) condition of the Thane creek.

Keywords: size-fractionated chlorophyll; phytoplankton regulation; trophic state Index (TRIX); eutrophication

Introduction

Coastal ecosystems, being at the transitional zone of matter and energy transfer to the sea, perform an essential role in biogeochemical cycles (Qu and Kroeze, 2012). Coastal zones which contribute ~30% of the oceanic primary production are undergoing severe anthropogenic disturbances affecting aquatic biota (Srichandan et al. 2019). Among coastal ecosystems, the estuaries and the creeks are some of the most heavily used and intimate natural ecosystems globally (Barbier et al. 2011). Being transitional systems, they provide essential ecosystem services such as fisheries maintenance, nutrient cycling, water supply, purification and recreation (Barbier et al. 2011). Pollution of these ecosystems, especially those in the proximity of metropolitan cities and its adverse effects on the water column biota have become major concerns in recent years. Among the pelagic biota that is readily affected by indiscriminate addition of pollutants, the phytoplankton can serve as a reliable substitute for marine environmental quality assessment (Amengual-morro et al. 2012).

Even though the phytoplankton assemblages represent various complexities; several attributes of the phytoplankton community can indicate ecological functioning. Delvin et al. (2007) have described the use of different phytoplankton attributes such as biomass, abundance, taxon diversity, seasonal succession and indicator species in the assessment of water quality. Among them, chlorophyll *a* concentration is used as a proxy for the phytoplankton biomass and widely implemented in the water quality assessment. Chlorophyll *a* value is an integrative and a straightforward measure of the phytoplankton response to nutrient enrichment and also an indicator of N and P enrichment in the marine system (Harding et al. 1994).

The use of morphometric descriptors in studying the phytoplankton community response to environmental variations has become the main focus of trait-based classification. Among the various morphometric descriptors, size is considered as a master trait in a recently popular trait-based approach (Weithoff and Beisner, 2019). The phytoplankton size can be studied as body size spectra (Sheldon et al. 1972) or as fractionation of total biomass into the micro, nano and picophytoplankton fraction (Sieburth, 1979). The phytoplankton size structure exhibits strong relationship with various features of phytoplankton physiology (Chisholm, 1992), food web dynamics (Parsons and Lalli, 2002), energy transfer efficiency (San Martin et al. 2006), sinking rate, re-suspension (Sahay et al. 2017) and fish production (Caddy and Griffiths, 1995). Therefore the size fraction measurement could significantly enhance the applicability of data beyond the total chlorophyll *a* alone. Moreover, understanding the relationship between environmental parameters and phytoplankton size distribution is a vital step towards a comprehensive analysis of the spatiotemporal variability in phytoplankton community structure (Iriarte and Purdie, 1994). Identifying the dominant

phytoplankton size class (pico, nano and micro) is also crucial in predicting the type of food chain playing the significant role in energy transfer in a particular ecosystem (Richardson and Jackson, 2007).

Thane creek situated in the Mumbai city, the second-largest coastal city in the world, and is India's premier port. Nearly 8% of the Indian industries situated around Mumbai in three large industrial clusters such as Thane-Belapur, Kalyan-Ambarnath and Patalganga. The Thane-Belapur is Asia's largest industrial zone situated on the east bank of the Thane creek. Whereas, highly urbanised Mumbai and Thane region and also many small scale industries clustered on the western boundary of the creek. The industrial and domestic wastes contribute about $2.94 \times 10^5 \text{ m}^3\text{d}^{-1}$ and $1.45 \times 10^5 \text{ m}^3\text{d}^{-1}$ to $2.60 \times 10^5 \text{ m}^3\text{d}^{-1}$, respectively; exerting enormous pressure on the marine environment (TMC-ES, 2000). These diversified industries (chemical, pharmaceutical, textile, and engineering and major fertiliser complexes) releases high levels of nitrogen and phosphorus through their effluents into the creek through several point sources (Quadros et al. 2009) along with the domestic waste. The creek was also known for luxuriant growth of mangrove, salt marsh vegetation and variety of seafood wealth (Gokhale and Athalye, 1995). Mumbai Port (MbPT) ($18^\circ 54' \text{ N}$; $72^\circ 40' \text{ E}$) and Jawaharlal Nehru Port (JNPT) ($18^\circ 57' \text{ N}$; $72^\circ 57' \text{ E}$), situated in the middle of the Thane creek, are the major gateways for India's imports and exports. These Ports also handles $4.5 \times 10^7 \text{ t}$ of cargo containing crude oil, fertilizers, rock phosphate, sulphur, food grains, metals, chemicals etc. on an annual basis.

Highlighting the importance of phytoplankton size structure several studies have addressed the distribution of phytoplankton size-fractionated biomass from other tropical coastal regions (Sin et al. 2000; Forget et al. 2011; Li et al. 2013; Guenther et al. 2015 and Soria-Piriz et al. 2017). Surprisingly, in the Indian context, the phytoplankton size structure is poorly known (Madhu et al. 2010; Rajaneesh et al. 2018; Srichandan et al. 2019). Even though the creeks, ports and harbour basins are of considerable ecological and economic importance (Anil et al. 2002), the studies related to phytoplankton assemblage from Thane creek- Mumbai Harbour are scarce (Ramaiah and Nair, 1998; Coasta and Anil, 2010) with no information defining the distribution of size-fractionated phytoplankton biomass concerning environmental drivers.

Considering the gap mentioned the phytoplankton biomass dynamics in the Thane creek-Mumbai Harbour was investigated. The main objectives of this study were: (1) to examine temporal and spatial variation in total and size-fractionated phytoplankton chlorophyll (SFC) (2) to investigate mechanisms controlling size structure dynamics in relation to inorganic nutrient enrichment in the system. The hypotheses that we have attempted to test were (i) variation in nutrient modification would have a profound effect on phytoplankton size structure in the anthropogenically stressed

creek; (ii) the nutrient enrichment will favour the dominance of nanophytoplankton fraction. The information gathered with such studies can be useful in understanding the biomass dynamics of the crucial fractions of the phytoplankton size structure and also to predict the type of food chain existing in such ecosystem concerning prevailing environmental conditions.

Materials and Methods

Study site

The Thane creek, which is under the influence of tropical southwest monsoon regime lies at latitudes 19°00' N to 19°15'N and longitudes 72°55' E to 73°00' E. The funnel-shaped Thane creek is a semi-enclosed water body. During the monsoon season, significant horizontal salinity gradients develop along the creek due to freshwater discharge from the Ulhas River (Zingde et al. 1989). The high land runoff along with heavy precipitation (mean rainfall: 2000–2800 mm y⁻¹) during the monsoon period (June–September) leads to efficient annual flushing of the creeks, thereby improving the environmental quality of the creek (Ram et al. 2009).

A seasonal field survey was conducted at nine preselected locations in the Thane creek during 2018 (Fig. 1, Table 1). The study area experienced three seasons: the pre-monsoon (February–May; PrM), the monsoon (June–September; MON), and the post-monsoon (October–January; PoM). The samples from the surface and the near-bottom (~1 m above from bottom) were collected during spring tide in January, April and September 2018 corresponding to PoM, PrM, and MON respectively. The sampling locations were classified under three zones: upper creek (stations 1, 2 and 3), middle creek (stations 4, 5 and 6) and lower creek (stations 7, 8 and 9). The upper creek stations were located at the junction of Ulhas River and Thane creek and the lower creek stations situated outside the mouth of the creek whereas the stations located in the middle of the creek are in the vicinity of JNPT and MbPT area. The rainfall and tidal height data were obtained from the Indian meteorological department (IMD), Pune and Indian tide table survey of India, respectively (Table 2). Duplicate water samples for the analysis of hydrological parameters, nutrients and size-fractionated phytoplankton biomass were collected using 5L capacity Niskin water sampler (Hydro-Bios, Kiel-Holtenau, Germany).

Hydrological parameters

Water temperature was measured using mercury thermometer of accuracy $\pm 0.1^\circ\text{C}$. The pH was measured with a microprocessor-controlled pH meter with an accuracy of ± 0.01 unit. The instrument was calibrated using standard buffered solutions (pH 4.0, 7.0 and 9.2) before the use. The samples for dissolved oxygen (DO) estimation was immediately fixed after collection and measured

by Winkler's method. Salinity was measured by Mohr titration method (Grasshoff, 1983). Evaluation of total suspended sediments (SS) was performed based on the gravimetric method of APHA, (2005).

Nutrient analysis

Seawater for the analysis of dissolved inorganic nutrients was first filtered through pre-combusted 0.45 μm Millipore membrane filter paper. The filtrates were then used for the measurement of nitrate (NO_3^- -N), nitrite (NO_2^- -N), ammonia (NH_4^+ -N), inorganic phosphorus (PO_4^{3-} -P) and dissolved silicate (SiO_3^{2-} -Si) concentration within six h of collection using manual spectrophotometric methods proposed by Strickland and Parsons (1972) and Grasshoff (1983).

Total and size-fractionated phytoplankton biomass (SFC) estimation

For the estimation of the total as well as SFC, 500 ml of seawater was collected in two separate cleaned amber colour plastic bottles and stored in the icebox. In the laboratory, the water samples for total phytoplankton biomass estimation were filtered through Whatman GF/F filter paper. At the same time, size-fractionated chlorophyll *a* estimation were performed by sequentially filtering 500 ml of sample water first through 20 μm nylon mesh followed by 2 μm and 0.2 μm Millipore polycarbonate filters. The cells retained on 20 μm mesh were then concentrated on GF/F filter paper which constitute micro-phytoplankton biomass (MPB) while the fraction of biomass retained by 2 and 0.2 μm filters constitute nano (NPB) and pico-phytoplankton biomass (PPB) respectively. Immediately after filtration pigments were extracted for 24 hrs in 10 ml of 90% acetone in refrigerator. The chlorophyll *a* was measured fluorometrically by a Turner designs fluorometer (Trilogy model 7200) before and after acidification with 10% HCL (Parsons et al., 1984) calibrated with pure chl*a* (Sigma-Aldrich).

Trophic condition

The trophic state index (TRIX) was first described by Vollenweider et al. (1998) and further modified by Giovanardi and Vollenweider, (2004). The methodological tool TRIX is used to define the eutrophication status and the range in an ecosystem (Tugrul et al. 2019). TRIX is a linear combination of four variables related to eutrophication that includes chl*a*, dissolved oxygen (DO) as an absolute percentage deviation from saturation condition, nitrogen and phosphorus. During present study the TRIX was calculated according to Guenther et al. 2014 and the details of the formula is as follows

$$\text{TRIX} = [\text{Log}_{10}(\text{chl } a / \text{DO}\% / \text{DIN} / \text{PO}_4) + a] / b$$

Where, *chl a* is the chlorophyll *a* concentration as $\mu\text{g l}^{-1}$, %DO is an absolute deviation of DO from saturation condition, DIN is the concentration of dissolved inorganic nitrogen ($\mu\text{g l}^{-1}$), and PO_4 is the inorganic phosphorus (P- PO_4 $\mu\text{g l}^{-1}$). The parameters, such as $a=1.5$ and $b=1.2$ used in the equation are the scaled coefficients used to fix the lower and upper limit of the trophic scale between zero and ten. The meaning of each scale value is shown in Table 3.

Data analysis

Seasonal and spatial differences among various environmental variables were analysed by performing one way ANOVA test separately using the SPSS-16.0 software. If the results explain the significant difference ($p < 0.05$) among the variables, a multiple comparison procedure (Tukey-test) was run. Before conducting ANOVA, homogeneity of variance test was performed to check the normality. The variables failing this test were log-transformed. Relatedness of the phytoplankton variables with the physical and chemical parameters was analysed using Pearson's correlation analysis. Canonical correspondence analysis (CCA) and multiple regression analysis were performed in CANOCO 4.5 to identify a set of environmental variables that elucidate the variation in phytoplankton biomass (total and size-fractionated *chl a*). Following the CCA analysis, a plot representative of the model run was obtained in CanoDraw 4.0 (Ter Braak and Similauer, 2002). Monte Carlo permutation test (with manual forward selection, under 999 permutations) was used to select significant environmental variables.

Results

Hydrological parameters

Seasonal distribution of hydrological parameters in Thane creek has been presented in Table 4. Water temperature displayed a distinct spatiotemporal variation. During the three seasons, the temperature ranged between 24.7°C and 32.2°C with the highest temperature during Pre-monsoon (PrM) (av. $30.74^\circ\text{C} \pm 1.16$) which decreased during MON (av. 28.34 ± 0.60) and PoM (av. $26.98^\circ\text{C} \pm 0.95$). During PrM, the temperature progressively reduced towards the mouth from upstream, unlikely during MON and PoM, wherein the definite spatial gradient was absent. As expected, the salinity was highest in PrM, followed by PoM and MON. The spatial variation in salinity was inverse of the temperature profile with the upper creek sustaining comparatively low salinity (av. 32.8 ± 1.1) than the middle (av. 34.4 ± 0.8) and the lower creek (av. 35.2 ± 0.5). The pH ranged from 7.7 to 8.2 with the absence of any specific trend, temporally or spatially. Suspended sediment (SS) in the Thane creek exhibited a wide range of variation ($27.9\text{-}595.2 \text{ mg l}^{-1}$) with the spatiotemporal trend of gradual decrease from the upper to the lower creek during MON and PrM. On the contrary, the

middle creek sustained high SS followed by the upper and the lower region in PoM season. DO concentration in the study region varied from 3.0 mg l⁻¹ to 6.6 mg l⁻¹. The DO concentration was low in MON, while lower values were confined only to the upper creek during PrM and PoM.

The results of ANOVA revealed that the hydrological parameters such as temperature, salinity, pH, and DO exhibited significant spatiotemporal variation while SS displayed only spatial variation ($p < 0.05$) (Table 5).

Nutrients

Noticeable spatiotemporal variation in the concentration of nitrate (NO₃⁻-N) and ammonia (NH₄⁺-N) in the system was discernible. Nitrite (NO₂⁻-N) ranged from 0.29 to 16.5 µmol l⁻¹ with the exceptionally high value during PoM in the upper creek; while nitrate (NO₃⁻-N) ranged from 3.7 to 34.8 µmol l⁻¹ with high concentration during PoM and PrM in the upper and the middle creek. Ammonia (NH₄⁺-N) ranged between 1.5 and 8.2 µmol l⁻¹ with relatively high values in the upper creek compared to the middle and lower creek. The upper creek sustained comparatively higher concentration of phosphate (PO₄³⁻-P) (av. 5.1 µmol l⁻¹ ± 1.6) than in the middle (av. 3.6 µmol l⁻¹ ± 3.2) and the lower creek (av. 2.1 µmol l⁻¹ ± 2.5) with generally high values during PoM throughout the creek. Distribution pattern of nutrients revealed that NO₂⁻-N, NO₃⁻-N and PO₄³⁻-P showed significant spatiotemporal variation ($p < 0.05$) (Table 5).

Total and size-fractionated phytoplankton biomass (SFC)

Seasonal pattern of total *chl a* distribution in the Thane creek demonstrated that PrM sustained the highest biomass, followed by MON and PoM. The total *chl a* in PrM ranged between 3.7 mg m⁻³ and 18.1 mg m⁻³ (av. 8.3 mg m⁻³). In MON, the total *chl a* varied from 2.7 mg m⁻³ to 14.8 mg m⁻³ (av. 7.2 ± 4.1 mg m⁻³) and from 1.8 mg m⁻³ (stn. 3) to 4.5 mg m⁻³ (stn. 7) (av. 2.46 ± 0.9 mg m⁻³) in PoM (Fig.2). There was no significant spatial variation in total *chl a* in the study region (Table 5).

Among the different size-fractionated classes of chlorophyll *a* (SFC), the nanophytoplankton biomass (NPB) displayed significant ($p < 0.05$) spatiotemporal variation and the microphytoplankton biomass (MPB) revealed substantial ($p < 0.05$) seasonal variation (Table 5). The maximum NPB was recorded at the upper creek followed by the lower and the middle creek in PrM. The upper creek continued to maintain high biomass in MON, but low values occurred in the lower creek. During PoM, the biomass difference between the three zones of the creek was not significant ($p > 0.05$). The NPB always dominated the phytoplankton biomass throughout the creek, and their percentage contribution to total *chl a* ranged from 77.1 to 95.5%, 69.1 to 90.8%, and 79.2 to 97.4% during PrM, MON and PoM respectively. The PPB did not display spatiotemporal variation and contributed <7%

to the total phytoplankton biomass throughout the study period. The MPB ranged from 0.1 mg m⁻³ to 1.2 mg m⁻³, and its contribution to total *chl a* was 3-19% during PrM, 11-19% in MON and 3-7% during PoM (Fig. 2 and 3). The relative increase in the contribution of MPB to total *chl a* was evident during PrM and MON (Fig. 2).

Trophic state index (TRIX)

TRIX values calculated in the study area ranged between 6.7 and 8.8 in PrM, 7.1 and 8.3 in MON and 6.9 and 8.3 during PoM (Fig. 4, Table 3). Significant seasonal differences in TRIX value were absent ($p > 0.05$) though marked spatial variation ($p < 0.05$) occurred with the high values in the upper creek followed by the middle and the lower creek (Table 5).

Relationship between *chl a* concentration and environmental variables

The results of Pearson's correlation analysis of total and size fractionated *chl a* with various environmental variables are listed in Table 6. The total *chl a* displayed a significant positive correlation with temperature ($r = 0.54$, $p = 0.00$), TRIX ($r = 0.50$, $p = 0.01$), NH₄⁻-N ($r = 0.43$, $p = 0.03$) and negative correlation with salinity ($r = -0.41$, $p = 0.03$). Among different phytoplankton biomass size classes, the PPB maintained significant positive correlation with TRIX ($r = 0.45$, $p = 0.01$). The NPB exhibited significant positive correlation with temperature ($r = 0.59$, $p = 0.00$), NH₄⁻-N ($r = 0.44$, $p = 0.02$), TRIX ($r = 0.49$, $p = 0.01$) and negative with pH ($r = -0.40$, $p = 0.03$); whereas the MPB was negatively correlated with NO₃⁻-N ($r = -0.49$, $p = 0.01$) and NO₂⁻-N ($r = -0.38$, $p = 0.04$). All phytoplankton size classes were significantly and positively correlated with total *chl a* (Table 6).

Canonical correspondence analysis (seasonal) between total and size-fractionated chlorophyll *a* against hydrological and nutrient (abiotic) parameters indicated 94.4%, 91.6%, 96.2% relationship during PrM, MON and PoM respectively. During PrM, the NPB co-varied together with inorganic nutrient, SS and temperature displaying a weak association. During MON, the NPB present on the negative side and all abiotic parameters positioned on the positive side of the second axis showing a robust negative relationship among them. The relationship between the MPB and inorganic nutrients as well as SS was negative during PrM and MON. In PoM, the NPB and the MPB co-varied together with temperature, SS and inorganic nitrogen (NO₂⁻-N, NO₃⁻-N and NH₄⁻-N) with different affinities (Fig. 5).

Discussion

The Thane creek is subjected to nutrient modifications due to its proximity to diverse industries, urban settlements as well as India's premier ports. The variation in hydrological and nutrient

properties of water leads to the changes in the contribution of size-fractionated *chl a* to the total biomass, thereby affecting the trophic status.

Hydrology

Tidal mixing, river discharge and nutrient inputs are amongst the various factors influencing the spatiotemporal variations in physicochemical parameters (Chowdhury et al. 2017). The results of ANOVA with physicochemical parameters demonstrated the absence of significant differences ($p > 0.005$) between the surface and the bottom waters of Thane creek (Table 5). Hence, the system can be considered as a vertically uniform due to mixing by flood and ebb currents sweeping the creek. Therefore the averaged value of surface and bottom samples was considered for all analysis.

As foreseen in tropical coastal waters, the temperature in the studied region varied in accordance with the air temperature. It showed seasonality due to the variation in solar intensity in different seasons (Anand et al. 2014 and Hardikar et al. 2017). Salinity dilution and distribution in an ecosystem are majorly governed by the variability in freshwater discharge and tidal intensity (Chowdhury et al. 2017). Ulhas River at the northern end drains into the Thane creek by a very narrow channel. Therefore, dilution was confined only to the upper creek during monsoon leading to 2-5 unit freshening compared to its outermost counterpart. During the rest of the period, creek experienced salinity > 33 . SS has been frequently considered as an important parameter affecting the photic depth and thereby controlling phytoplankton community structure in estuarine and coastal waters (Patil and Anil, 2011; Chou et al., 2012). As reported in earlier studies from tropical coastal ecosystems, the re-suspension of bottom sediment due to dredging, turbulence by tidal action and river/terrigenous influx contributed to the high SS load in the Thane creek; thereby increasing turbidity in the water column (Jiufa and Chen, 1998; Satpathy et al. 2010; Hardikar et al. 2017). Dissolved oxygen (DO) concentration in the water column is the result of consumption and replenishment of DO by heterotrophs and autotrophs, respectively. In the water column, DO is replenished through oxygen fluxes across the air-sea interface and also by photosynthetic activity. Simultaneously, it is consumed during oxidation of organic matter and respiration by aquatic flora and fauna (Rixen et al. 2010). It has been observed that DO concentration below 2 mg l^{-1} for a prolonged time may affect aquatic life (Ram et al. 2014). The DO concentration in the Thane Creek was always $> 3 \text{ mg l}^{-1}$, which revealed good oxidising condition in the study area.

Nutrients

The ambient concentration of inorganic nutrients performs a crucial role in phytoplankton ecology in the aquatic ecosystem. Nutrients, particularly inorganic nitrogen and phosphorus, are frequently

referred to as key factors regulating phytoplankton assemblage (Ganguly et al. 2013). Nutrient distribution pattern illustrated that low nutrient (NO_2^- -N, NO_3^- -N and PO_4^{3-} -P) concentration during MON could be due to the combined effect of uptake by phytoplankton and their removal through flushing due to monsoonal runoff (Soria-Piriz et al. 2017). The high concentration during PrM and PoM could be associated with the accumulation of effluents in the system due to reduced flushing. The estuarine sediments are an important source of regenerated nutrients for the water column (Soria-Piriz et al. 2017). The high concentration of NO_2^- -N, NO_3^- -N and PO_4^{3-} -P close to the sediment were associated with diagenetic reaction taking place in the sediment and were supplied to the surface water by tidal mixing (Sin et al. 2000; Ram and Zingde, 2000; Corbett, 2010; Anand et al. 2014). Therefore, it is expected that re-suspension of bed sediment was the common source for these nutrients in the water column. This finding was further supported by the significant positive correlation between the NO_2^- -N, NO_3^- -N, PO_4^{3-} -P and SS (Table 6) and the CCA analysis also indicated their co-variation (Fig.5). Further NO_2^- -N and NO_3^- -N did not display significant negative correlation with salinity, suggesting that diagenesis of organic matter was the major contributor of NO_2^- -N and NO_3^- -N fluxes in the water column. PO_4^{3-} -P ($r = -0.40$; $p = 0.04$) exhibited significant negative correlation with salinity and positive correlation with SS ($r = 0.44$; $p = 0.03$). Therefore, it can be concluded that diagenetic transport from bed sediments and sewage discharge equally contributed to the high values of PO_4^{3-} -P. In a general, terrestrial runoff, demineralisation of organic matter and excretion by zooplankton are among the major sources of NH_4^- -N inputs in the coastal waters. NH_4^- -N recorded comparatively higher values during MON and displayed a significant negative correlation with salinity ($r = -0.48$; $p = 0.01$) thereby revealing the importance of land runoff for the elevated level of NH_4^- -N in the creek. The influence of sewage effluents on the high concentration of NH_4^- -N and PO_4^{3-} -P has also been reported in the Victoria harbour region by Yin and Harrison, (2007).

Spatial and temporal distribution of size-fractionated phytoplankton biomass

The spatiotemporal dynamics in size-fractionated phytoplankton biomass is governed by various factors such as allochthonous nutrient inputs, the strength of grazing and temperature-driven differences in growth rates (Ning et al. 2000). The NPB dominated the whole creek throughout the study period. The nanophytoplankton are known to proliferate efficiently under low solar insolation and also at the lower concentration of ambient nutrient as compared to microphytoplankton (Shiomoto, 1997). In the Thane creek, numbers of factors were collectively responsible for the dominance of the NPB. Among them, the reduced transparency of water column resulting from high

SS load played a significant role irrespective of seasons. A positive correlation of the NPB with temperature and SS also supported the above statement (Table 6; Fig. 5).

Further, in CCA analysis, the NPB displayed inverse relation with nutrients (DIN & P-PO₄³⁻) during MON whereas, during PrM and PoM, they co-varied together with different affinities (Table 6; Fig. 5). The inverse relationship during MON revealed the uptake of these nutrients resulting in high biomass and low nutrient concentration throughout the creek. On the other hand, insignificant correlations during PrM and PoM resulted due to the availability of surplus nutrients in the creek (Jyothibabu et al. 2006). Therefore, it can be concluded that the proficiency of the nanophytoplankton to grow efficiently in the turbid environment made them major players of the phytoplankton community in turbid eutrophic tropical coastal waters. Our findings are in line with the earlier work reported by Madhu et al. 2010 from Cochin backwater and Srichandan et al. 2019 from Mahanadi estuary and Chilika lagoon and also by Guenther et al. 2014 from Brazil.

The MPB was known to increase in the eutrophic ecosystem (Agawin et al. 2000 and Sin et al. 2000). Along with surplus nutrient, the microphytoplankton also requires high light intensity for efficient utilisation of inorganic nutrients for maximum production (Cermeno et al. 2005). The Thane creek with comparatively high SS load (>50 mg l⁻¹) reduced the light insolation throughout the water column (Hardikar et al. 2017). Cloern (1987) also reported that the primary production was mainly limited due to high turbidity in the eutrophic estuary. Therefore, even though the Thane creek showed the eutrophic condition, high SS load throughout the creek hampered the proliferation of the MPB in comparison to the NPB. The finding was further supported by CCA, which revealed a negative relationship between the SS and the MPB. Furthermore, the contribution of the MPB to total biomass was comparatively high in MON and PrM, whereas it reduced to minimal level during PoM. Waning of the MPB during PoM could be due to the export of the MPB through zooplankton grazing. Earlier studies conducted by Gaonkar et al. 2010 reported the high abundance of copepod in the Thane creek during PoM. Further, the chlorophyll:phaeophytin ratio (>1 and <1), which is considered as a proxy for phytoplankton growth or grazing (Welschmeyer and Lorenzen, 1985), was found to be <1 during the same period. Additionally, a high concentration of NO₂ as evidence of grazing pressure (Rajaneesh et al. 2018) was recorded during PoM. Therefore, these findings supported that the reduced water transparency stemmed from high SS load and grazing pressure, might have resulted in low MPB in the study area.

The contribution of the PPB to the total phytoplankton biomass was meagre. Due to their small size, photosynthetic picoplankton are known to dominate the oligotrophic waters (Tada et al. 2003). Therefore, the PPB did not contribute significantly to the total biomass in the Thane creek due to

competitive disadvantage under high nutrient concentration. The preceding discussion concluded that the combined effect of SS, nutrient availability and grazing pressure governed the variability in the contribution of different size classes (PPB, NPB and MPB) to total phytoplankton biomass in the Thane creek.

Phytoplankton size is considered as a master trait controlling the trophic structure of an ecosystem (Litchman et al. 2010). The significant contribution of the NPB to the total biomass had substantial functional implications. In the present study, as explained by Rajaneesh et al. (2018), the dominance of nanophytoplankton indicated the herbivorous based food chain plays a vital role in transferring the energy to the next trophic level.

Trophic state index (TRIX)

Water quality indices comprehend the complex water quality data and facilitate its communication in a meaningful manner. The upper and the lower limit of the TRIX are used for assessing the eutrophication status of an ecosystem and ranges between 0 and 10 (Giovanardi and Vollenweider, 2004). The TRIX scale obtained during the present investigation ranged from 6.7 to 8.8, which classified the Thane creek under eutrophic to hypereutrophic condition (Table 3). Higher TRIX values obtained (8.2) in the upper Thane creek can be linked to high nutrient load associated with sewage discharge as well as diagenetic reactions occurring in the sediment. The gradual dilution of nutrient load towards the mouth of the creek resulted in a concurrent decrease in the TRIX value (7.3). Guenther et al. 2014 also reported similar results with TRIX ranging between 6.6 and 8.3 in the Recife harbor (NE Brazil) classifying the system as hypereutrophic.

Regulation of phytoplankton biomass

Top-down and bottom-up control are the two different mechanisms that regulate the phytoplankton population in an ecosystem (Kerimoglu et al. 2012). During the present study, the stepwise multiple regression analysis showed that the % variance increased with each addition when nutrients were added to the model as independent variables. Hence it can be concluded that nutrient availability may perhaps elucidate the bottom-up regulation of the phytoplankton biomass in the Thane creek. Similarly, Chl:phaeo ratio (>1 and <1) can also be considered as a proxy for zooplankton grazing (Welschmeyer and Lorenzen, 1985). Release of phaeopigments, through autolysis under the low light condition for a prolonged period and also as egested faecal material by grazers, is a factor ruling the bottom-up and top-down regulation of phytoplankton. During PrM and MON, Chl:phaeo ratio was >1 throughout the study region while the ratio was <1 in PoM (Fig.6). The regulation of phytoplankton during PrM and MON can be considered as bottom-up, and it is tricky to conclude

whether the whole phytoplankton growth was regulated through top-down/bottom-up control in PoM. The low Chl:Pheo ratio (<1) observed during PoM might have resulted from the autolysis of the phytoplankton cells due to the limited light condition or due to the increase grazing pressure (Daley and Brown, 1973; Shuman and Lorenzen, 1975). However, in the case of MPB, there is a possibility of zooplankton grazing. Still, in the absence of grazing studies, it is difficult to assess whether the increase in pheophytin concentration [thereby depressing the chl:pheo ratio (>1)] was due to cell death or through herbivory. Therefore, the study concluded that the regulation of phytoplankton biomass in the Thane creek is a seasonal process.

Conclusion

The study reinforces the hypothesis of NPB dominated the total phytoplankton biomass throughout the study region. Based on the observations of the present study, it can be concluded that the eutrophic tropical coastal ecosystems with high SS load favour the proliferation of NPB, which is also substantiated in the literature. Since the mesozooplankton feeding is known to be size-selective and prefers the larger food particles, grazing pressure and reduce solar insolation limited the growth of microphytoplankton. SS load and nutrient inputs are the main factors controlling the dominance of different size classes (Pico, Nano and Micro). Providing the contrasting role of different size classes in terms of response to environmental perturbations, this kind of studies will be complementary to those analyses focussing on the response of phytoplankton biomass as a whole. The results of the trophic state index (TRIX) can act as references for future research and also for environmental management. Even though herbivorous food chain is more effective in energy transfer due to less trophic level, multivorous food chain is known to be more stable due to increased functional diversity and contributes towards the healthy structure of the ecosystem. Therefore, the present study emphasises the need for monitoring studies concerning the dominance of single size class (nano) and the eutrophication control and management.

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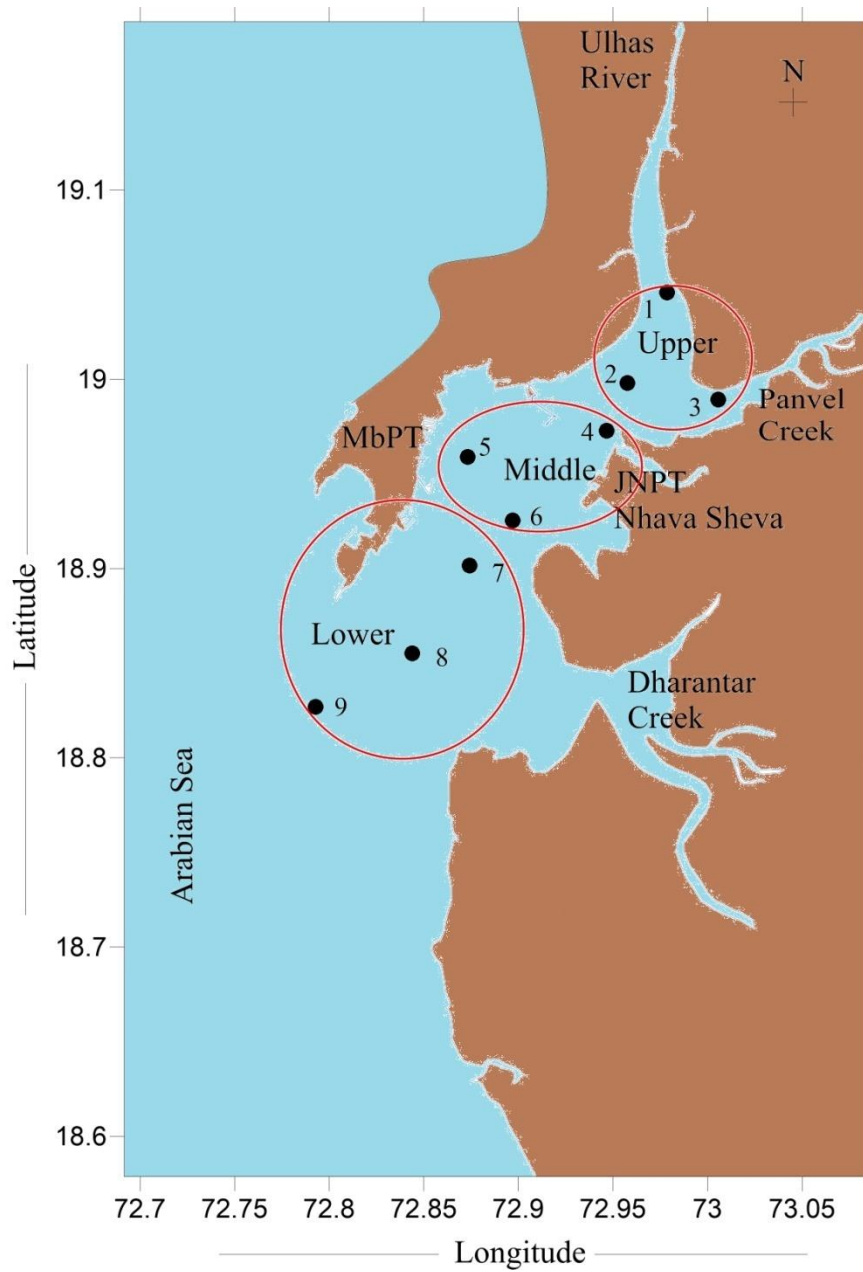


Figure 1. Map showing sampling locations at the Thane creek

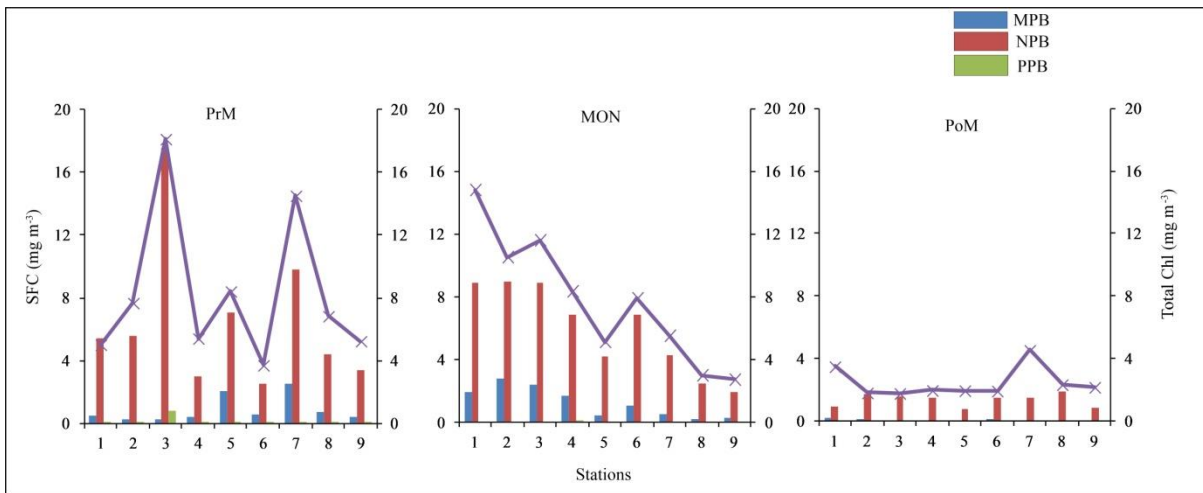


Figure 2. Spatial and temporal distribution of total and size fractionated phytoplankton biomass in the study region (PPB- picophytoplankton biomass, NPB- nanophytoplankton biomass, MPB- Microphytoplankton biomass)

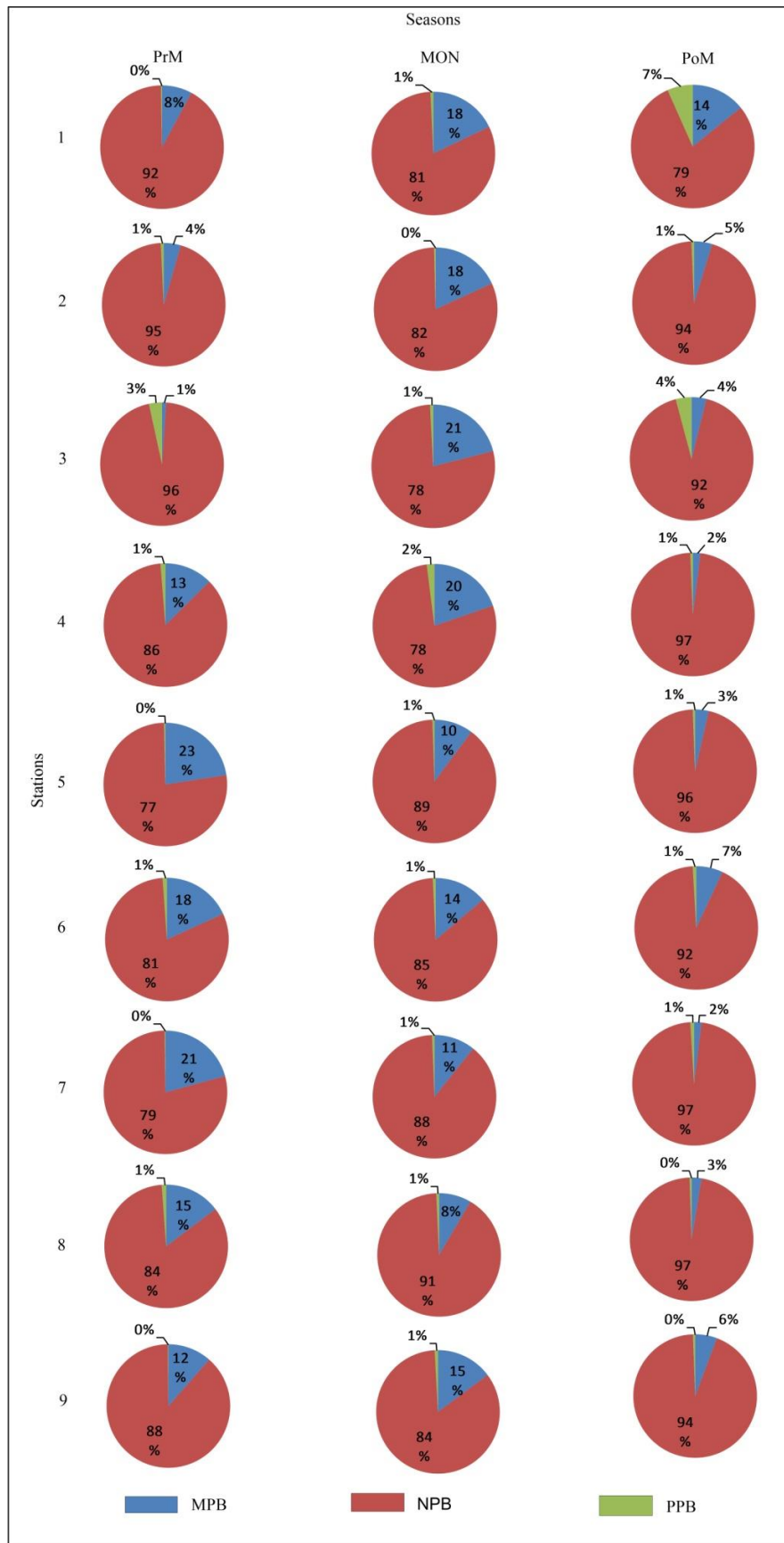


Figure 3. Percentage contribution of MPB- Microphytoplankton biomass, NPB- nanophytoplankton biomass, PPB- picophytoplankton biomass to total chl *a* in the study region

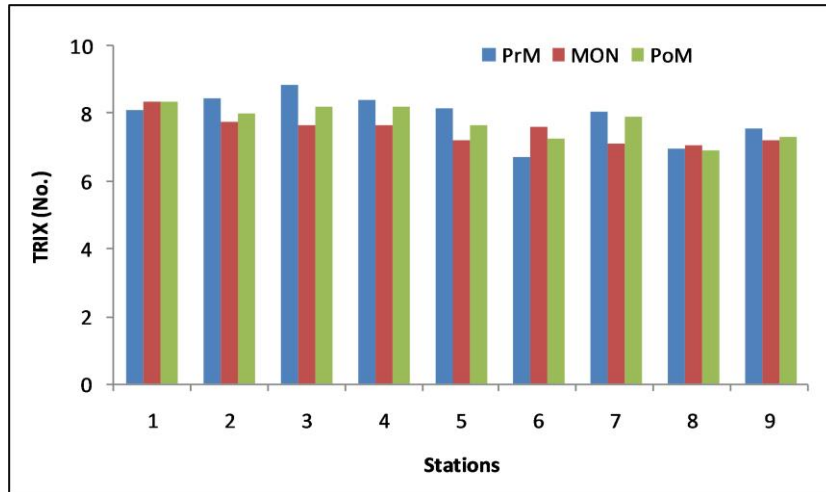


Figure 4. Spatial and temporal distribution of TRIX values in the Thane creek

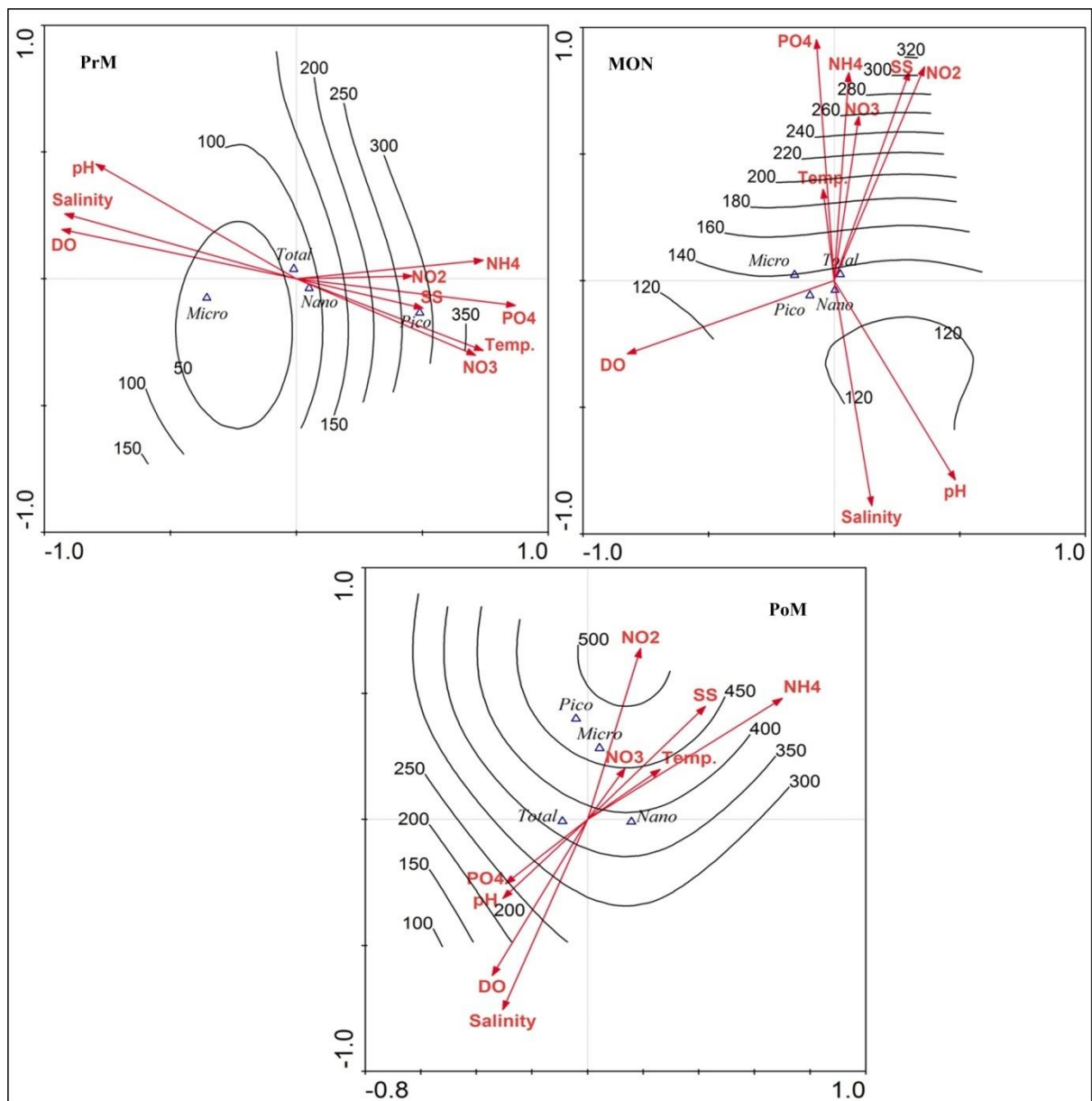


Figure 5. Result of canonical correspondence analysis showing the relationship between environmental parameters and total as well as size fractionated chlorophyll (contour line shows the distribution of SS load)

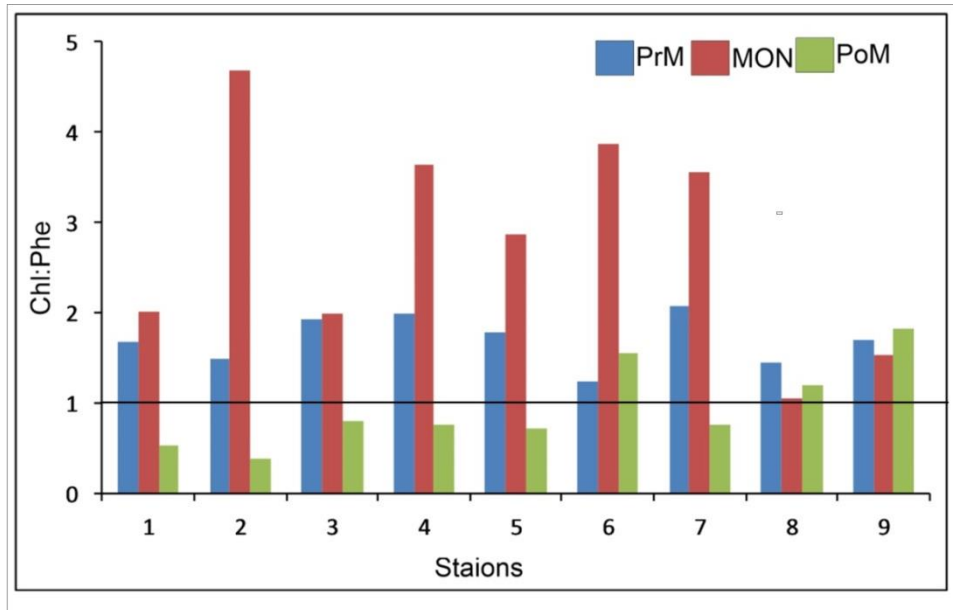


Figure 6. Spatial and temporal distribution of chlorophyll a:phaeophytin ratio (chl:phe) in the Thane creek

Table 1 List and details of sampling locations in Thane Creek

Station No.	Latitude (N)	Longitude (E)	Approximate depth (m)	Distance from mouth (km)
1	19° 2'45.00"N	72°58'43.00"E	6	25.68
2	18°59'53.00"N	72°57'50.00"E	8	20.59
3	18°59'21.00"N	73° 0'20.00"E	11	23.05
4	18°58'40.51"N	72°56'43.22"E	11.5	17.60
5	18°55'22.00"N	72°53'58.00"E	9.5	9.84
6	18°54'3.16"N	72°52'23.11"E	12	6.20
7	18°51'50.00"N	72°53'49.00"E	9	6.44
8	18°52'48.00"N	72°48'59.00"E	11	3.26
9	18°51'26.62"N	72°50'10.10"E	15	0

Table 2 Seasonal rainfall (monthly averaged) and tidal height data (HT- high tide, LT- low tide) for the sampling dates

Season	Average Rainfall (mm)	Sampling Date (d-m-y)	Tidal Height (m)	
			HT	LT
PoM	19.21	17-01-2018	3.76	1.82
		18-01-2018	3.87	1.69
		19-01-2018	3.93	1.58
		20-01-2018	3.92	1.49
PrM	0.38	13-04-2018	3.06	1.41
		14-04-2018	3.3	1.21
		15-04-2018	3.96	1.99
		16-04-2018	3.82	0.89
MON	177.58	17-04-2018	4.07	0.81
		25-09-2018	4.22	0.89
		26-09-2018	4.28	0.73
		27-09-2018	4.27	0.93
		28-09-2018	4.18	0.91

Table 3 Methodological tool, indicators and ranges used for Eutrophication assessment in Thane Creek

Method	Indicators	Eutrophication status	Eutrophication range
TRIX ^{abc}	D% O ₂ , DIN= (NO ₃ + NO ₂ + NH ₄), PO ₄ and Chl- <i>a</i>	High/Oligotrophic	<4
		Good/Mesotrophic	4-5
		Moderate/Eutrophic	5-6
		Bad/Hypereutrophic	6-10

^a Fiori et al. 2016

^b Pavluk and vaate, 2013

^c Giovanardi and Vollenweider, 2004

Table 4 Ranges, averages and standard deviation of physical and chemical parameters

Parameters	Premonsoon			Monsoon			Postmonsoon		
	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD
Salinity	33.10	35.60	34.77 \pm 0.94	30.02	35.99	33.63 \pm 1.77	32.76	35.16	34.00 \pm 0.80
Temperature ($^{\circ}$ C)	28.88	32.25	30.75 \pm 1.23	27.25	28.75	28.31 \pm 0.62	24.75	28.25	27 \pm 1.00
DO (mg l^{-1})	3.09	6.57	5.25 \pm 1.41	3.15	5.17	3.93 \pm 0.77	3.01	6.02	4.84 \pm 0.90
pH	7.74	8.07	7.94 \pm 0.11	7.86	8.20	8.05 \pm 0.12	7.85	8.20	8.01 \pm 0.13
SS (mg l^{-1})	27.95	595.17	163.37 \pm 203.04	65.56	355.18	152.06 \pm 92.65	60.67	552.76	276.77 \pm 171.7
Nitrite ($\mu\text{m l}^{-1}$)	0.20	6.82	3.13 \pm 2.46	0.18	3.48	0.78 \pm 1.06	1.66	20.14	8.84 \pm 6.83
Nitrate ($\mu\text{m l}^{-1}$)	1.64	24.78	12.26 \pm 8.09	1.58	7.75	4.51 \pm 1.87	5.09	44.25	27.73 \pm 11078
Ammonia ($\mu\text{m l}^{-1}$)	2.79	15.50	6.42 \pm 4.43	1.24	14.30	5.42 \pm 3.78	0.53	10.57	3.89 \pm 3.55
Phosphate ($\mu\text{m l}^{-1}$)	5.72	40.40	21.81 \pm 11.41	4.29	25.54	10.71 \pm 6.15	14.82	60.88	40.47 \pm 16.41
Silicate ($\mu\text{m l}^{-1}$)	3.89	30.05	14.78 \pm 10.16	7.08	26.36	17.77 \pm 5.73	-	-	-

Table 5 Summary of one-way ANOVA analysis

Source	Dependent variables	F	Significance
Location	Temperature	7.059	.005
	Salinity	25.404	.000
	DO	14.467	.000
	SS	4.801	.021
	pH	19.019	.000
	Nitrite	20.641	.000
	Nitrate	4.761	.022
	Ammonia	0.682	.518
	Phosphate	5.149	.017
	Pico	1.542	.241
	Nano	3.527	.048
	Micro	1.148	.339
	Total	3.081	.071
	TRIX	6.763	.006
	Seasons	Temperature	58.335
Salinity		6.073	.010
DO		11.505	.001
SS		2.352	.124
pH		4.033	.036
Nitrite		23.339	.000
Nitrate		29.447	.000
Ammonia		1.001	.387
Phosphate		8.315	.003
Pico		0.896	.426
Nano		6.147	.002
Micro		11.165	.001
Total		9.286	.002
TRIX		0.732	.495

Table 6 Results of Pearson's correlation analysis between physical, chemical and biological parameters

	Temp	Salinity	DO	SS	pH	NO2	NO3	NH4	PO4	Pico	Nano	Micro	Total
Temp	1												
Salinity	0.067	1											
DO	-0.103	.524**	1										
SS	-0.058	-.446*	-0.314	1									
pH	-.505**	.531**	0.242	-0.257	1								
NO ₂	-0.121	-0.309	-0.247	0.329	-.455*	1							
NO ₃	-0.198	-0.189	-0.073	.641**	-0.310	.590**	1						
NH ₄	0.357	-.480*	-0.291	0.251	-.525**	0.097	0.026	1					
DIN	-0.103	-0.347	-0.198	.637**	-.494**	.773**	.934**	0.280					
PO ₄	0.029	-.395*	-0.242	.444*	-.537**	.544**	.758**	0.046	1				
Pico	0.369	-0.281	-0.286	0.167	-0.377	-0.028	0.094	0.378	0.180	1			
Nano	.588**	-0.369	-0.215	0.022	-.395*	-0.304	-0.278	.435*	-0.094	.742**	1		
Micro	0.232	-0.286	0.161	-0.195	-0.135	-.382*	-.477*	0.147	-0.375	0.016	.604**	1	
Total	.536**	-.408*	-0.120	-0.010	-0.349	-0.317	-0.317	.429*	-0.092	.619**	.953**	.690**	1
TRIX	.464*	-.555**	-0.323	.412*	-.766**	.403*	.459*	.508**	.590**	.452*	.486*	0.175	.504**

** . Correlation is significant at the 0.01 level

* . Correlation is significant at the 0.05 level