

A brief description on spatial variability in nutrient-phytoplankton relationship in a shallow tidal creek-Thane Creek, India

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Abstract

Anthropogenic influence on nutrient-phytoplankton relationship was studied in Thane Creek. The upper creek was highly loaded with N-forms through anthropogenic inputs. The elevated level of nutrients enhanced the chlorophyll *a* and phytoplankton density. A huge disparity was observed in the distribution of nitrate in the upper creek which can be linked to the 10 times higher density by *Skeletonema costatum*. CCA analysis carried out on species-environment parameters implied that phytoplankton species which were found in ascendancy (*Thalassiosira* sp., *Skeletonema* sp., >*Leptocylindrus* sp., >*Plagioselmis* sp., >*Chaetoceros* sp., >*Cylindrotheca* sp., >*Teleaulax* sp., >*Alexandrium* sp., >*Nitzschia* sp., >*Gyrodinium* sp., >*Surirella* sp. >*Guinardia* sp., and *Pyramimonas* sp.) were more correlated to environmental variables like nitrate, ammonia, phosphate and salinity. The correlation between nitrate, ammonia and the phytoplankton showed that they were the essential nutrients responsible for their high density and distribution along the creek.

Keywords: Anthropogenic input, Phytoplankton, Chlorophyll *a*, Nutrients, Tidal Creek.

Introduction

Over-enrichment of shallow coastal waters (creeks/estuaries) is a global problem that is brought through physical and chemical conditions with space and time (Heiss et al. 2016). The causes of over-enrichment are closely connected to the nutrient inputs (mainly nitrogen and phosphorus compounds) from point (usually industrial discharge) and nonpoint (agriculture and other anthropogenic activities) sources in the drainage basin (Wang, Liu, and Zheng, 2013). Over the decade the anthropogenic input of nutrients (N&P) into these systems has increased dramatically which is responsible for the degradation of water quality (Ulibarrena and Conzonno, 2015). The transport and distribution of anthropogenically generated nutrients in these systems depend mainly on residence time (Lopez et al. 2006). N inputs into the coastal waters are now 2 to 20 times higher than pre-industrialized times; additionally, N experiences a complicated oxidation-reduction cycle. However, N production and consumption mainly involve the physical-chemical environment including mixing, salinity gradient and biological activities (Wu et al. 2016).

The anthropogenic nutrient loading to the shallow coastal waters (creeks/estuaries) from agricultural and wastewater effluent not only changes concentration but also the nutrient ratios (Seitzinger et al. 2010). High phytoplankton biomass and densities in most aquatic systems are based on inferences from the stoichiometry of nitrogen (N) and phosphorus (P) (Huszar et al. 2006) and the relative availability of these elements in nature (Lewis and Wurtsbaugh, 2008). Macronutrient, especially N-forms (nitrite, nitrate and ammonia) is necessary element for the growth of phytoplankton along with phosphorus. However, over-enrichment of these nutrients (N&P) can enhance the fast growth of phytoplankton which is considered a sensitive biological indicator of eutrophication, anthropogenic pressures and nitrogen loading (Brito et al. 2012). Therefore, phytoplankton is recognized as a key factor in assessing the water quality status.

Creeks being small tidal inlets are often the first point of entry of point and non-point source, runoff from upland regions (Van Dolah et al. 2003; Mallin and Lewitus, 2004). Thane Creek on the west coast of India, one of the major cities located around Mumbai is opening into the Arabian Sea through the Mumbai Harbour. Speedy industrialization and human aggregation in Mumbai city have resulted in the production and disposal of enormous quantities of wastewater into the adjacent creek adversely affecting the water quality and resulting in stress on the biota (Zingde et al. 1989; Ramaiah and Ramaiah et al. 1995). Tiwari and Nair, 1998; Ramaiah and Ramaiah, 1998 studied phytoplankton biomass, density distribution and community structure of this creek. The work of Quadros et al. 2001 on the water quality status of Thane creek established that the dissolved oxygen, suspended solids, salinity and phytoplankton are key parameters that govern the quality status of the

creek. The ecological status of ports and harbours in Thane, Mumbai were also elaborately studied by Sawant et al. 2007; Gaonkar et al. 2010a and 2010b. Hardikar et al. 2021 studied the distribution of size-fractionated phytoplankton biomass in Thane Creek and also defined the quality status of the creek. The study reported that nanophytoplankton biomass contributed a significant portion of the total phytoplankton biomass. Additionally, the study also addressed the ecological status of the creek and defined the region as eutrophic to hypereutrophic (TRIX=6.7-8.8). Although studies on phytoplankton distribution is been carried out in Thane Creek from 1995 onwards, they are found to be intermittent. Therefore, the present study emphasized the spatial distribution of phytoplankton and their relationship with the environmental variables and also about the zonal (upper, middle and lower creek) classification of phytoplankton assemblage against anthropogenically generated nutrients.

Methods and methodology

Description of the study region

The Mumbai city is separated from the mainland by the Thane Creek system which comprises Thane Creek and Mumbai Harbour. Mumbai city is the second largest coastal city in the world; nearly 8% of the industries are situated around Mumbai in three large industrial clusters as Thane-Belapur, Kalyan-Ambernath and Patalganga. Thane-Belapur is Asia's largest industrial zone situated at the east bank of the Thane creek (TMC-ES, 2000). The funnel-shaped Thane Creek is a semi-enclosed water body that lies at latitudes 19°00' N to 19°15'N and longitudes 72°55' E to 73°00' E (Zingde et al. 1989). The length of the Thane Creek from the mouth to the head is about 27 km with a width of 15 km at the mouth (off Colaba) narrowing to a few hundred meters at the head. Thane Creek receives a huge quantum of wastes through wastewater treatment facility (WWTF) of Municipal Corporation of Greater Mumbai, Thane Municipal Corporation, Thane-Belapur Industrial Association, Maharashtra Industrial Development Corporation, City Industrial Development Corporation and several industries (Vijay et al. 2015). Two commercial ports, Mumbai (MbPT) and Jawaharlal Nehru (JNPT) Ports are located towards the mouth of Mumbai Bay or Harbour. The creek is broad but shallow, forcing frequent dredging to maintain the navigational depths in the channel and at the berths. Domestic wastewater and a variety of industrial effluents result in the anthropogenic fluxes of pollutants along the eastern and western banks of Thane Creek impacting the water quality and removal of contaminants by natural processes that happen simultaneously.

Sampling strategy

The sampling stations were classified as upper (station 1, 2 &3), middle (4&5) and lower creek (station 6&7) (Figure 1). Sampling was carried out during December 2018 (winter). Samples for

environmental variables, chlorophyll *a* (biomass) and phytoplankton taxonomy were collected from surface and near-bottom water using a 5 L capacity Niskin water sampler (Germany).

Estimation of environmental variables

A hand-held mercury thermometer of accuracy $\pm 0.1^{\circ}\text{C}$ was used to measure water temperature. Salinity was estimated by Mohr titration and dissolved oxygen (DO) by Winkler's method as recommended by Grasshoff, 1983. Dissolved inorganic nutrients (nitrite, nitrate, ammonia and phosphate) were collected in clean polyethylene bottles and transported to the laboratory in an icebox. After filtering the samples through Millipore filter paper (0.45 μm) nutrient concentration was measured spectrophotometrically within 6 h of the collection as described by Grasshoff, 1983.

Estimation of Chlorophyll *a*, phytoplankton density and assemblage

For the estimation of chlorophyll *a*, 500 ml of water sample were filtered through Whatman GF/F filter paper (47 mm diameter; pore size 0.7 μm) and extracted in 90% acetone overnight at 4°C . The chlorophyll *a* was measured using a fluorometer (Turner Trilogy, Model 7200) following the procedure of Parson et al. 1984. For phytoplankton taxonomic identification and density enumeration, 500 ml of seawater sample was fixed with Lugol's iodine. Later the samples were concentrated to 10 ml after sedimentation in dark. Using the standard taxonomic keys of Subrahmanyam, 1946; Tomas, 1997, the phytoplankton was identified up to genus level using 1 ml aliquot from each concentrate on Sedgwick Rafter (United Kingdom) chamber. The sample was further analyzed under a compound light microscope (400 X magnifications) and density was expressed in cells l^{-1} .

Statistical analysis

Excel data analysis was used to carry out a t-Test to find the difference in the environmental variables and chlorophyll *a* between surface and near-bottom water (Paired Two Samples for means). To examine inter and the intra-group relationship between phytoplankton assemblage and environmental variables, Pearson's correlation matrix (*r*) were computed using SPSS-PASW Statistics 18. Shannon-Weiner diversity index ($H' \log_2$) was performed using the PRIMER v 6.1.4 software package (PRIMER-E Ltd.). To understand the main environmental variables affecting the distribution of key phytoplankton species in the study region Canonical Correspondence Analysis (CCA) was intended. CCA was performed using the CANOCO 4.5 statistical software. Followed by CCA analysis a representative bi-plot was obtained through CanoDraw software.

Results

Spatial variation in environmental parameters

Sampling was carried out in Thane Creek during December 2018, the first month of winter. For the ease of understanding the creek scenario, it was categorized into three zones viz., upper creek (stations 1,2,&3) the over-enriched shallow region of the creek that receives the anthropogenically generated nutrients; middle creek (stations 4&5) encompasses the Port area (JNPT and MbPT), that has maximum navigation of cargo vessels plying in the channel; lower creek (stations 6&7), encircle the harbour which is the wider region of the creek and connects with the adjacent coastal water (Figure 1).

The temperature varied from 21.5-29°C, with a moderately low temperature only at station 5 (21.5-22°C) otherwise, the temperature ranged between 25.2 and 29°C (Figure 2a). A progressive increase in salinity was observed from the upper (32.2-33.8 ppt) to the lower creek (35.6-35.8 ppt) during the study period (Figure 2b). Dissolved oxygen (DO) concentration did not show any trend and the range was between 4.1 and 5.9 mg l⁻¹ (Figure 2c). Nutrient distribution in the study region showed a wide variation with the peak concentration of nitrite, nitrate, ammonia and phosphate in the upper creek. Along the creek, the nitrite concentration showed a decreasing trend from upper to lower creek that ranged between 0.2 - 23.2µM, with the maximum concentration at station 3 (Figure 3a). Nitrate also showed a similar trend as that of nitrite with a high concentration in the water column of the upper creek (4.4-23.6µM) and gradually decreased towards the lower creek (4.2-7.4µM). At station 1 huge disparity in the distribution of nitrate was observed from the surface (4.4µM) to near-bottom water (20µM) (Figure 3b). The concentration of ammonia exhibited a sudden spike at the surface water of station 3 (20.5 µM) otherwise, ammonia in the water column ranged from 1.1 to 9.5µM (Figure 3c). The dissolved inorganic form of nitrogen-DIN (nitrite, nitrate and ammonia) in the water column showed a decreasing trend from upper to lower creek (10.2-60.6µM) (Figure 3d). Nitrate was the major contributor to the DIN concentration. A sudden fall in phosphate concentration was observed from the upper creek (4.4-6µM) to the middle creek (0.6-1.8µM) and continued with a concentration range of 2.2 to 2.4µM in the lower creek (Figure 3e). N:P ratio in the study region showed that the upper and lower creek are N-limited, while the middle creek showed P- limitation (Figure 3f).

Chlorophyll a (or biomass), phytoplankton density, community structure and species distribution

Chlorophyll *a* and phytoplankton density were studied in Thane Creek and it was found that the upper creek (stations 1 to 3) exhibited an elevated level of the above-mentioned phytoplankton components. Chlorophyll *a* was comparatively high in the upper creek (8.0-16.8 mg m⁻³) than in the

middle (0.8-1.3 mg m⁻³) and lower creek (0.5-0.6 mg m⁻³) (Figure 4a). Phytoplankton density followed the same pattern as that of chlorophyll *a* (Figure 4b). The discrepancy in the total phytoplankton density was observed at station 1 with a three-fold high density at the surface compared to the near-bottom water and the total density ranged between 4.2 and 762.2 x 10⁴ cells l⁻¹ in the water column. The phytoplankton community encountered during the study period were diatom (28), dinoflagellate (8), cryptophyte (2), chlorophyte (1) and euglenophyte (1). The phytoplankton genera with density ≥10,000 cells l⁻¹ were considered key players in controlling the nutrient cycling of the creek (Table 1). *Thalassiosira* sp., *Skeletonema* sp., *Leptocylindrus* sp., *Plagioselmis* sp., *Chaetoceros* sp., *Cylindrotheca* sp., *Teleaulax* sp., *Alexandrium* sp., *Nitzschia* sp., *Gyrodinium* sp., *Surirella* sp., *Guinardia* sp., and *Pyramimonas* sp. were the phytoplankton species in the upper creek. Among them, *Thalassiosira* sp. was the dominant species and was found profusely in the near-bottom water (Table 1). The repeated presence of diatoms like *Skeletonema* sp., *Thalassiosira* sp., *Cylindrotheca* sp., *Nitzschia* sp. were seen in the middle and lower creek along with dinoflagellate-*Alexandrium* sp. From the study, it was understood that *Guinardia* sp., *Surirella* sp., *Scrippsiella* sp., *Torodinium* sp., *Plagioselmis* sp., *Teleaulax* sp., and *Pyramimonas* sp. were encountered only in the upper creek. Density wise, *Guinardia* sp., *Surirella* sp., *Gymnodinium* sp., *Scrippsiella* sp., and *Torodinium* sp. were found in low density compared to *Plagioselmis* sp., *Teleaulax* sp., and *Pyramimonas* sp. which exhibited high density (Table 1). The major phytoplankton are depicted in microphotographs (Plate 1 a-q).

Statistical analysis

Based upon the t-Test carried out there was no significant (p>0.05) difference in the environmental variables and chlorophyll *a* between surface and near-bottom water with few exceptions. Therefore, for carrying out statistical analysis the surface and near-bottom water values were averaged for each parameter (Table 2)

Pearson's correlation was carried out to explain the relationship between environmental variables (temperature, salinity, dissolved oxygen and nutrients) and chlorophyll *a* (Table 3). Salinity showed a significant negative correlation with nitrite (r= -0.98; p<0.01), phosphate (r=-0.88; p<0.01) and DIN (r= -0.93; p<0.01), while nitrite was positively correlated to phosphate (r=0.84; p<0.05) and DIN (r=0.89; p<0.01). Significant positive correlation was observed between phosphate and DIN (r=0.76; p<0.05). Chlorophyll *a* showed significant negative correlation with salinity (r=-0.91; p<0.01) and positively correlated with nitrite (r=0.90; p<0.01) and phosphate (r=0.82; p<0.05).

The diversity index for Thane Creek was evaluated using Shannon-Weiner diversity index (H' at \log_2) as depicted in Figure 4c. The diversity index varied from 1.1 to 2.9 with very low diversity of station 1 (1.1).

The phytoplankton assemblage study revealed that the discrepancy in the abundance of major genera and their distribution was mainly dependent upon the variance in the nutrient stoichiometry. CCA analysis was carried out to explain the above fact and 93.4% of the total variation in the species-environmental relationship was established (Figure 5). Axis-I displayed 78.4% of total variability and was influenced by environmental variables such as temperature, DO (dissolved oxygen) and NH_4 (ammonia). On the other hand, Axis- II was correlated with salinity, NO_3 (nitrate) and NO_2 (nitrite) which explained 15 % of the total variation. The importance of carrying out CCA was to identify the nutrient preference of each genus. Phytoplankton genera like *Cylindrotheca* sp., *Nitzschia* sp., *Alexandrium* sp., *Pyramimonas* sp., *Plagioselmis* sp., *Teleaulax* sp. and *Pleurosigma* sp. displayed a strong positive correlation with NH_4 . On the other hand, *Thalassiosira* sp., *Leptocylindrus* sp., *Chaetoceros* sp., *Torodinium* sp. and *Gyrodinium* sp., covaried together with PO_4 , while, *Guinardia* sp. and *Gymnodinium* sp. displayed positive loading with salinity and NO_3 respectively. On the negative side of the first axis, *Skeletonema* sp. and *Scrippsiella* sp. showed an affinity towards temperature and DO. The negative correlation of *Skeletonema* sp. and *Scrippsiella* sp. with NO_3 and PO_4 indicates that these nutrients are found to be essential for their proliferation.

Discussion

Spatial variation in environmental parameters

During December, the first month of winter, the temperature usually varies from 18-32.4°C (av.27°C) (NIO, 2018), therefore, the huge difference (7°C) in the temperature (Figure 2a) can be related to the time of measurement (Alkan et al. 2013). Additionally, as the temperature did not show any significant correlation with any of the observed environmental parameters and chlorophyll *a* (Table 3), the disparity in the temperature can be considered inconsequential. The salinity difference between lower and upper creek was found to be 2.0-3.3 ppt (Figure 2b) which can be related to the tidal asymmetry in the creek. For any coastal waters, creeks and estuaries tidal asymmetry is the dominant factor for the movement of particles (Castaing and Allen, 1981). As per the research carried out by Thomas et al. 2019, Thane Creek exhibited low tidal amplitude in the upper creek which increased in the mid and further decreased towards the lower creek. Therefore, the salinity gradient from the lower (35.6-35.8 ppt) to the upper creek (32.3-33.8 ppt) could be due to the tidal asymmetry in the study region. Moreover, the discrepancy in salinity in the upper creek can also be

due to the effect of freshwater runoff into the creek from Ulhas Estuary (NIO, 2019). Dissolved oxygen (DO) is an important parameter in assessing water quality because it influences the organisms living within a body of water. DO in the water column was in the range of 4.1 to 5.9 mg l⁻¹ and did not show any significant correlation with the environmental parameters and chlorophyll *a*.

The study region showed a wide variation in nutrient distribution with a peak concentration of nitrite, nitrate, ammonia and phosphate in the upper creek. Peak values of nitrite (20.1-23.2µM) and nitrate (20.6-23.6µM) concentration in the upper creek indicated the influence of anthropogenic load into the region (Figure 3b). Normally nitrite gets oxidized to nitrate very fast in an oxygen-sufficient water body, however, in Thane Creek build-up of nitrite was observed in the upper creek. The DO concentration in Thane Creek during the study period never reached below 3 mg l⁻¹ although the upper creek received organic load from different sources, simultaneously nitrite build-up also occurred. The probable reason could be due to inhibition of nitrite oxidation which is a microbial process (Nitrobacter) that blocked the immediate oxidation of nitrite to nitrate (Ram and Zingde 2000; Martin, 1970; NIO, 1992 &2019).

The huge disparity in the nitrate concentration at station 1 from the surface (4.4µM) to near-bottom water (20µM) (Figure 3b) can be related to the high density of *Skeletonema costatum* at surface water (564 x 10⁴ cells l⁻¹) which was 10 times higher than near-bottom water and lead to an enormous drawdown of nitrate (Table 1). The possible reason for the high density of *Skeletonema costatum* at surface water could be the temperature. The CCA result also confirmed the above findings as *Skeletonema costatum* showed positive loading with temperature (Figure 5). The studies made by Lomas and Glibert, 2000; Li et al. 2010 mentioned that high levels of nitrate can lead to *Skeletonema costatum* bloom. The threshold of *Skeletonema costatum* to store nitrate internally was much higher than other diatoms (Dortch et al. 1984; Lomas and Glibert, 2000), which facilitated the apparent drawdown of nitrate at station 1. Although the organic matter was high in the upper creek, ammonia concentration was found to be low (except at station 3) compared to nitrite and nitrate which could be due to effective assimilation by phytoplankton (Dugdale et al. 2007). CCA results also indicated the same, as phytoplankton (*Cylindrotheca* sp., *Nitzschia* sp., *Alexandrium* sp., *Pyramimonas* sp., *Plagioselmis* sp., *Teleaulax* sp. and *Pleurosigma* sp.) showed a positive loading with ammonia (Figure 5). Further, high DIN concentration in the upper creek signified the anthropogenic loading of nutrients into the creek and the main contributor was nitrate (55.8%), while the remaining fraction of DIN was equally contributed by nitrite (23.6%) and ammonia (20.5%). Phosphate distribution depicted that a high concentration in the upper creek was due to the intrusion of sewage and/or industrial effluents. According to the study made by Hardikar et al. 2021 in the

same creek, the increase in phosphate can be due to diagenetic transport from bed sediments and from sewage discharge too. N:P ratio that defines the N and P limited condition of an environment demonstrated that the upper and lower creek as an N-limited province while the middle creek behaved as a P-limited condition (Figure 3f). The N-limitation could be due to the high molar ratio of phosphate (>1) to nitrogen, although N-forms (nitrite, nitrate and ammonia) were found to be high in the upper creek (stations 1,2&3). In contrast, the middle creek (stations 4&5), except at surface water of station 4 showed P-limitation mainly because the molar ratio of phosphorus was <1 . The study made by Ryther and Dunstan, 1971 explained that there is no indication of any “normal” or “optimum” nitrogen to phosphorus ratio in algae. The N:P ratio is between 5:1 and 15:1 which are most commonly encountered and averaged ratio of 10:1 is a reasonable working value.

Nitrite ($r=-0.98$; $p<0.01$), phosphate ($r=-0.88$; $p<0.01$) and DIN ($r=-0.93$; $p<0.01$) showed a significant negative correlation with salinity indicating the source of these nutrients are not through land runoff instead through point and non-point sources (Vijay et al. 2015). The study made by Wu et al. 2016 reported that the inverse relationship of DIN and salinity can be due to the effluent inputs into the system which are heavily loaded with N-forms. It was observed that phosphate ($r=0.84$; $p<0.05$) and DIN ($r=0.89$; $p<0.01$) was positively correlated to nitrite, while DIN was positively correlated ($r=0.76$; $p<0.05$) to phosphate (Table 3). The positive correlation of nitrite to phosphate ($r=0.84$; $p<0.05$) illustrated that both N-forms and phosphate are from the same source such as pollutants from the discharges of open drains/nallas as well as effluents from domestic and industrial wastewater treatment facilities (WWTF) (Dongke Yu, 2012; Vijay et al. 2015).

Chlorophyll a, phytoplankton density, community structure and species distribution

Anthropogenically derived nutrient inputs from sewage/domestic discharges and industrial effluents lead to elevated concentration of chlorophyll *a* and phytoplankton density. Hardikar et al. 2021 studied the total and size-fractionated (pico, nano and micro) chlorophyll *a* in Thane Creek and found that nanophytoplankton biomass always dominated throughout the study region without any significant spatial variation in total biomass. However, in the present study prominent spatial variability in chlorophyll *a* (biomass) was seen, with an elevated concentration in the upper creek. Although the season was the same, the disparity in the total phytoplankton biomass between the earlier (1.8-4.4 mg m^{-3} ; January 2018) and the present study (0.5-16.8 mg m^{-3} ; December 2018) was noteworthy. Additionally, the negative correlation of chlorophyll *a* with salinity ($r=-0.91$; $p<0.01$) anticipated that anthropogenic input in the upper creek enhanced chlorophyll *a* where relatively low saline condition prevailed. The study made by Doering et al. 2006 reported that chlorophyll *a* responded to nutrient loading through the anthropogenic activity that is brought by the river

discharge carrying the major source of N & P which ultimately hiked the biomass (Chlorophyll *a*). However, in the present study, the nutrient loading is more through anthropogenic interventions (originating from domestic wastewater and a variety of industries). Altogether, Thane Creek receives around 1260 MLD of sewage (NIO, 2018) of which the major discharge outlets are through subsurface outfall of the Thane-Belapur belt, Asia's largest industrial zone which is in the eastern part of the creek. In the present study the upper creek which showed a nutrient-enriched niche is also in the eastern zone, therefore the elevated chlorophyll *a* concentration can be related to the anthropogenically mediated nutrients. The positive correlation of chlorophyll *a* with nitrite ($r=0.90$; $p<0.01$) indicated that high nitrite in upper creek enhanced chlorophyll *a*. The study made by Polat and Terbiyik, 2013 reported that nitrate+nitrite resulted in high chlorophyll *a*, the present study is also in agreement and pointed towards the hike in chlorophyll *a* in the upper creek was due to high concentration of nitrite+nitrate. Over-enriched upper creek behaved as a separate niche with few phytoplankton (*Thalassiosira* sp., and *Skeletonema costatum*) in outburst conditions promoting the region with high density (Table 1). Phytoplankton annual cycle is characterised by a winter/early spring diatom bloom, *Thalassiosira* spp. is reported as a major component of spring diatom bloom in a shallow euphotic zone with high nutrients (Waite et al. 2005; Popovich et al. 2008). Besides the above mention species, the density of *Nitzschia* sp., *Leptocylindrus* sp., *Chaetoceros* sp., *Alexandrium* sp., *Gymnodinium* sp., *Plagioselmis* sp., *Teleaulax* sp., and *Pyramimonas* sp. also collectively contributed to the high phytoplankton density in the upper creek (Table 1). Closely observing the distribution of *Plagioselmis* sp., *Teleaulax* sp., and *Pyramimonas* sp. density, they were encountered only in the upper creek during the study period. The high nitrogen condition and relatively low salinity that prevailed in the upper creek favoured the growth of *Plagioselmis* sp. and *Teleaulax* sp. (Cryptophytes) and *Pyramimonas* sp. (Chlorophytes). The study made by Chowdhury et al. 2017 in Kundalika Estuary also denoted that Cryptophytes (*Cryptomonas* sp.) preferred high nitrogen demand for their maximum proliferation. Gonzalez Rio et al. 2007 reported that phytoplankton groups especially the chlorophytes that preferred intermediate salinity could not compensate for the losses caused by salinity shock. Therefore, it can be assumed that the relatively low saline condition in the upper creek enhanced the growth of *Pyramimonas* sp. As a whole, it can be depicted that low tidal activity in the upper creek in addition to the nutrient-replete conditions might have enhanced the growth of phytoplankton. In contrast, there was a drastic decrease in the phytoplankton density in the middle and lower creek because of high tidal activity that limited phytoplankton growth in the nutrient-deplete regions (Engelhardt et al. 2004 and Snow et al. 2000). The major deciding factors for the phytoplankton density in the middle and lower creek were dilution by coastal water due to the tidal activity.

Shannon-Wiener diversity index can be used as a proxy for ecosystem modification under eutrophication or pollution stress. Shannon Wiener index as a pollution index suggests the following scale: 0–1 for high pollution (poor), 1–2 for moderate pollution (bad), 2–3 for marginal pollution (moderate), and 3–4 for incipient pollution (good) (Balloch et al. 1976). The surface water showed a relatively high diversity index compared to near-bottom water. However, due to the single species dominance of *Skeletonema costatum* at station 1 (upper creek), the diversity index came down to 1.1 and 1.8 which categorized station 1 to moderately polluted/bad condition (Figure 4c). The upper creek of Thane receives anthropogenically generated nutrient load derived from agricultural, domestic and industrial effluents, which becomes the main source of N-forms that promoted few species to dominate.

CCA species-environment biplot described the phytoplankton species which were found in association with different nutrients are discussed further (Figure 5). It is observed that *Thalassiosira* sp., *Leptocylindrus* sp., *Chaetoceros* sp., *Gyrodinium* sp. *Torodinium* sp. and *Gymnodinium* sp. were associated with PO₄ (phosphate) and NO₃ (nitrate). Whereas, *Nitzschia* sp., *Pleurosigma* sp., *Cylindrotheca* sp., *Pyramimonas* sp., *Teleaulax* sp., *Plagioselmis* sp. and *Alexandrium* sp. was linked to NH₄ (ammonia). Ryther and Dunstan, 1971 stated nitrate, nitrite and ammonia as the key nutrients for phytoplankton growth and biomass in coastal waters. The study as a whole specified that phytoplankton community composition and abundance were principally governed by the change in the concentration of nutrients.

The present study stood aside from the early published work of Hardikar et al. 2021 in the same location by unifying the phytoplankton assemblage with nutrient stoichiometry. Even though diatom demonstrated supremacy by being distributed throughout the creek they showed high density in the upper creek. Furthermore, cryptophytes (*Plagioselmis* sp., *Teleaulax* sp.) and chlorophytes (*Pyramimonas* sp.) showed a restricted distribution to the region where relatively low saline and over-enriched condition existed. The effluent inundated water with a high nutrient load acted as an N-source that helped in the proliferation of the phytoplankton in the upper creek, therefore, it can be defined that the upper creek behaved as a source of N-forms.

Being a tidal creek, the upper creek was less influenced by the tidal water due to tidal asymmetry. The study made by Aubrey and Speer, 1985, tidal asymmetry can change the length of flood and ebb. Additionally, the study made in Thane Creek by Naidu and Sarma 2002 reported that eddies and residual current promote the mixing capacity in the southern region of the creek, therefore, high sedimentation was found in the central and upper creek. Overall the study signifies that tide played a trivial role in nutrient-phytoplankton relationship especially in the upper creek.

Conclusion

The brief study on the spatial variability in the nutrient-phytoplankton relationship revealed that N-forms controlled the growth and proliferation of phytoplankton. In the study region, there existed a salinity gradient due to tidal asymmetry making the upper creek relatively low saline compared to the lower creek which is closer to the adjacent coastal water. Furthermore, the study could identify the build-up of nitrite in the upper creek although adequate dissolved oxygen was available, which can be defined as a peculiar feature of this creek. Because of the over-enrichment of the creek due to anthropogenic intervention (originating from domestic wastewater and a variety of industries), the upper creek behaved as a separate niche. Elevated concentration of chlorophyll *a* and phytoplankton density was seen in the upper creek due to nutrient enrichment of the region which lead to the outburst of diatoms like *Thalassiosira* sp. and *Skeletonema costatum*. species in the upper creek. Species like *Plagioselmis* sp., *Teleaulax* sp. and *Pyramimonas* sp. were encountered only in the upper creek where relatively low salinity and high N-forms prevailed. Through the present study, it was understood that the over-enriched upper creek can be considered as a source of N-forms. Therefore, the study as a whole specifies that chlorophyll *a*, phytoplankton community and abundance were principally governed by the change in nutrient stoichiometry. A detailed study is required in this creek to capture the transport and dispersion of the anthropogenically generated nutrient along the creek. This may help in understanding the further over-enrichment of the creek which may lead to a phytoplankton outburst (bloom) and single species dominance. Henceforth, a meticulous study on the nutrient loading and their drawdown by phytoplankton need to be addressed immediately for the sustainability of the creek.

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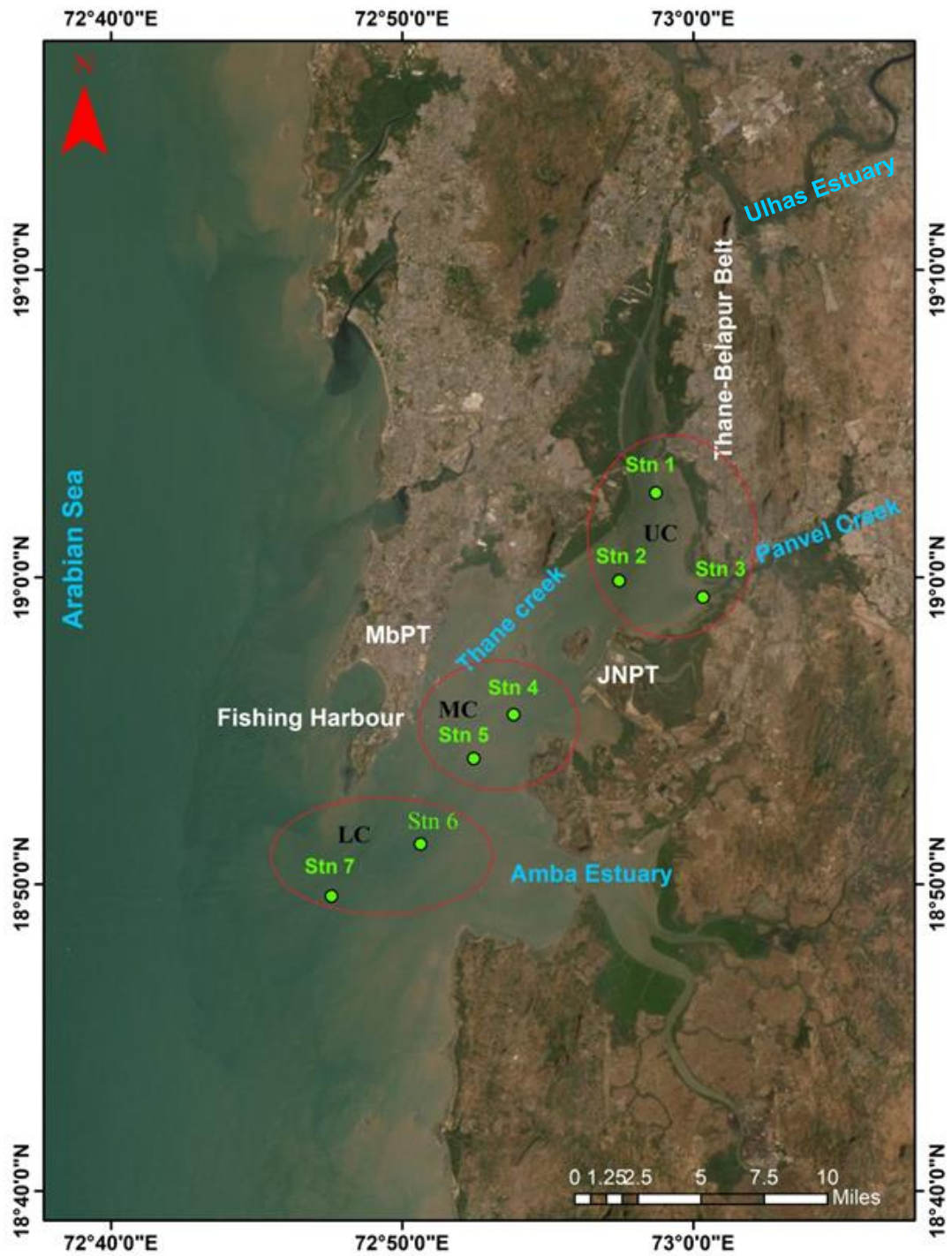


Figure 1. Sampling locations in Thane Creek

(UC=upper creek; MC=middle creek; LC=lower creek; JNPT=Jawaharlal Nehru Port Trust;
MbPT=Mumbai Port Trust)

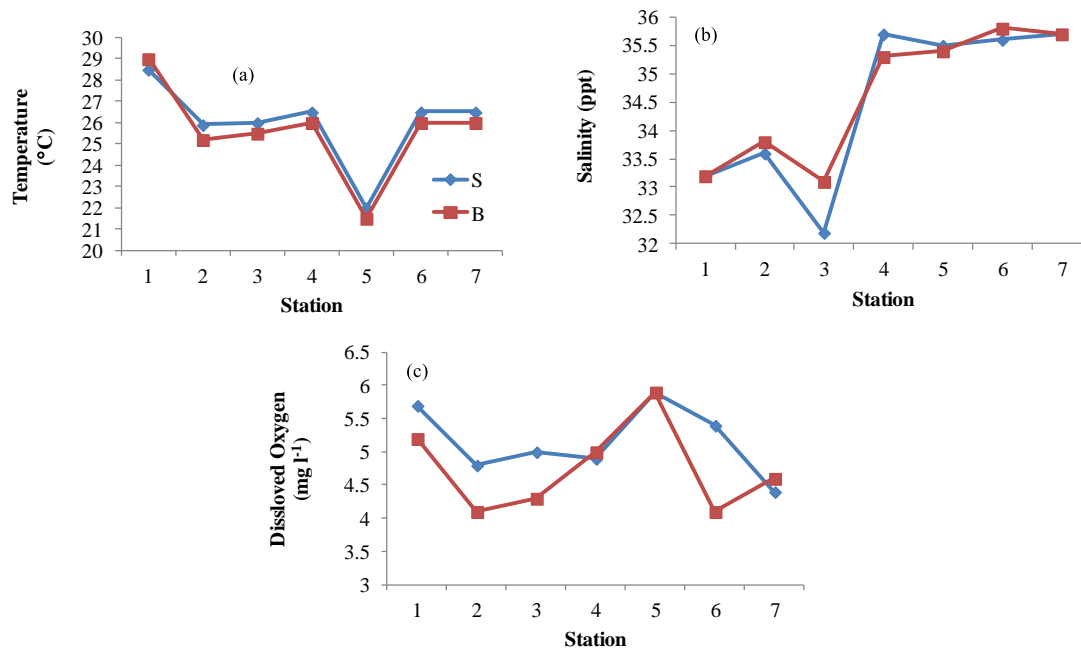


Figure 2. Distribution of (a) Temperature (b) Salinity and (c) Dissolved oxygen in the study region (S=surface; B=Near-bottom)

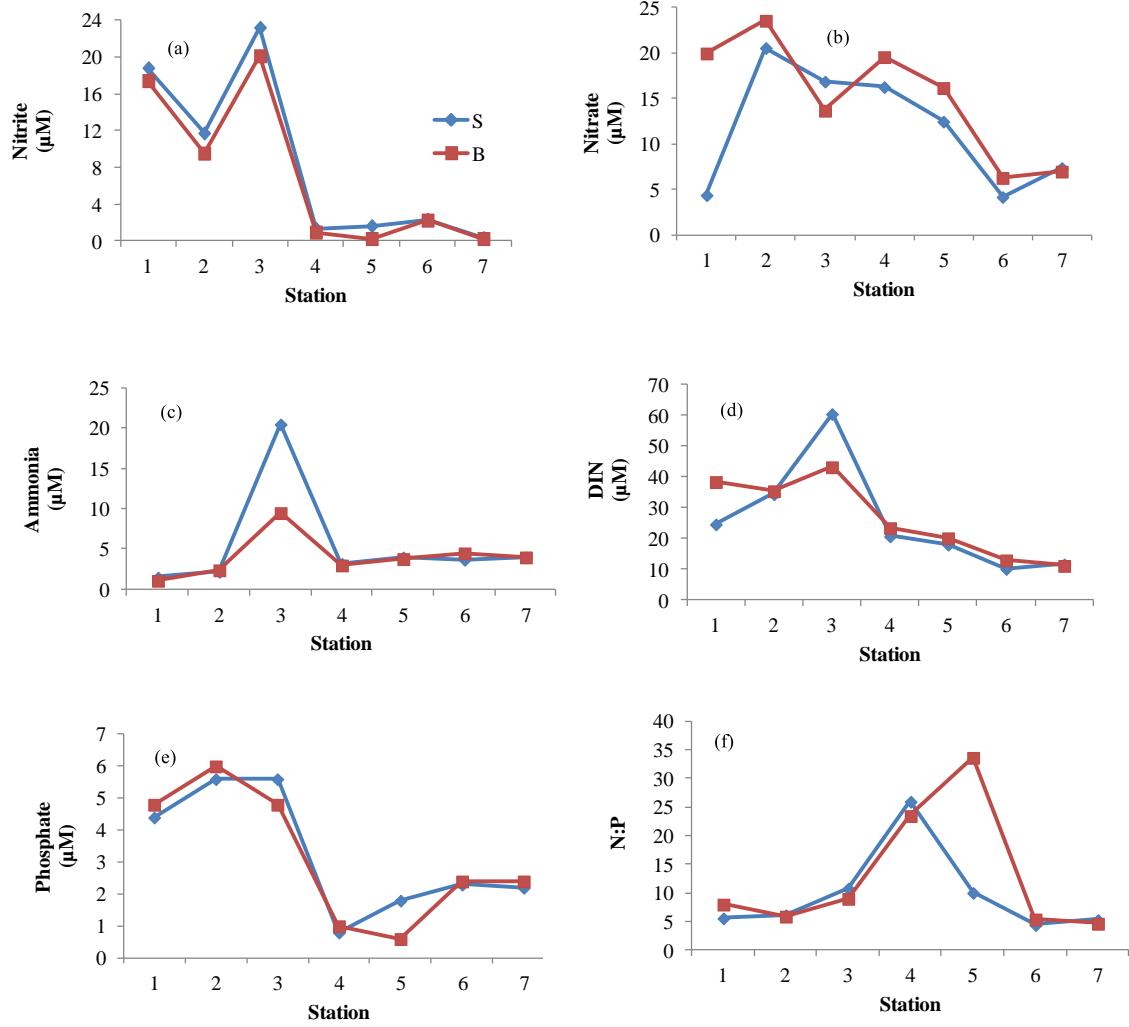


Figure 3. Distribution of (a) Nitrite (b) Nitrate (c) Ammonia (d) DIN (e) Phosphate (f) N:P ratio in the study region (S=surface; B=Near-bottom)

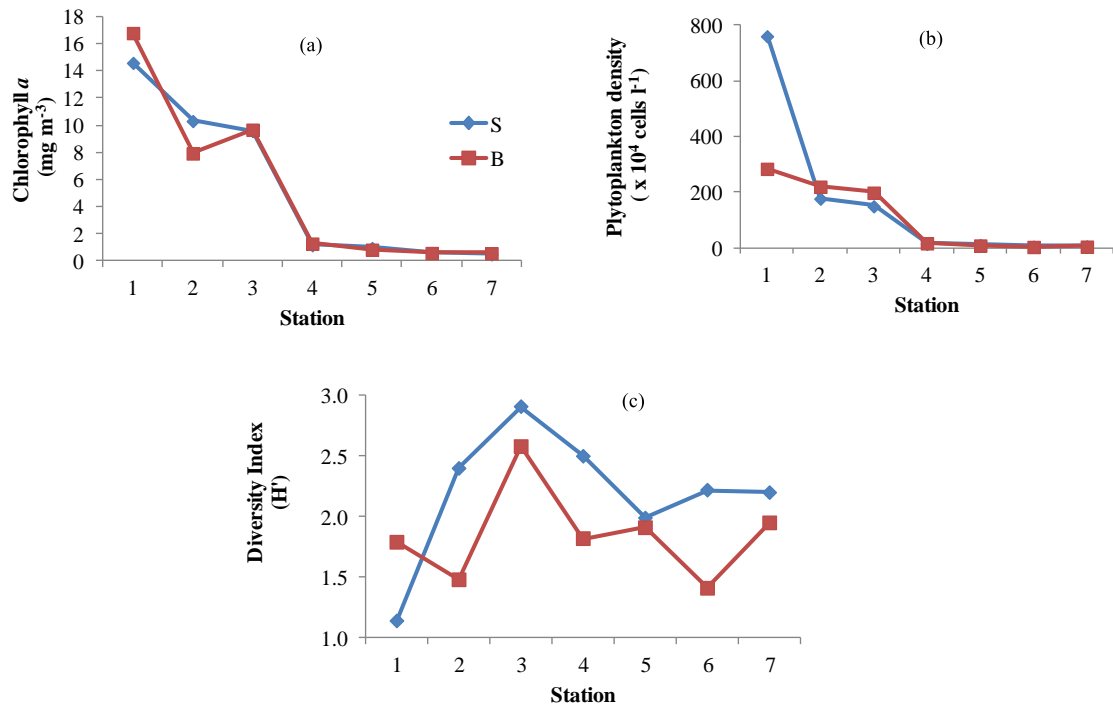


Figure 4. Distribution of (a) Chlorophyll *a* (b) Phytoplankton density (c) Phytoplankton diversity index in the study region (S=Surface; B=Near-bottom)

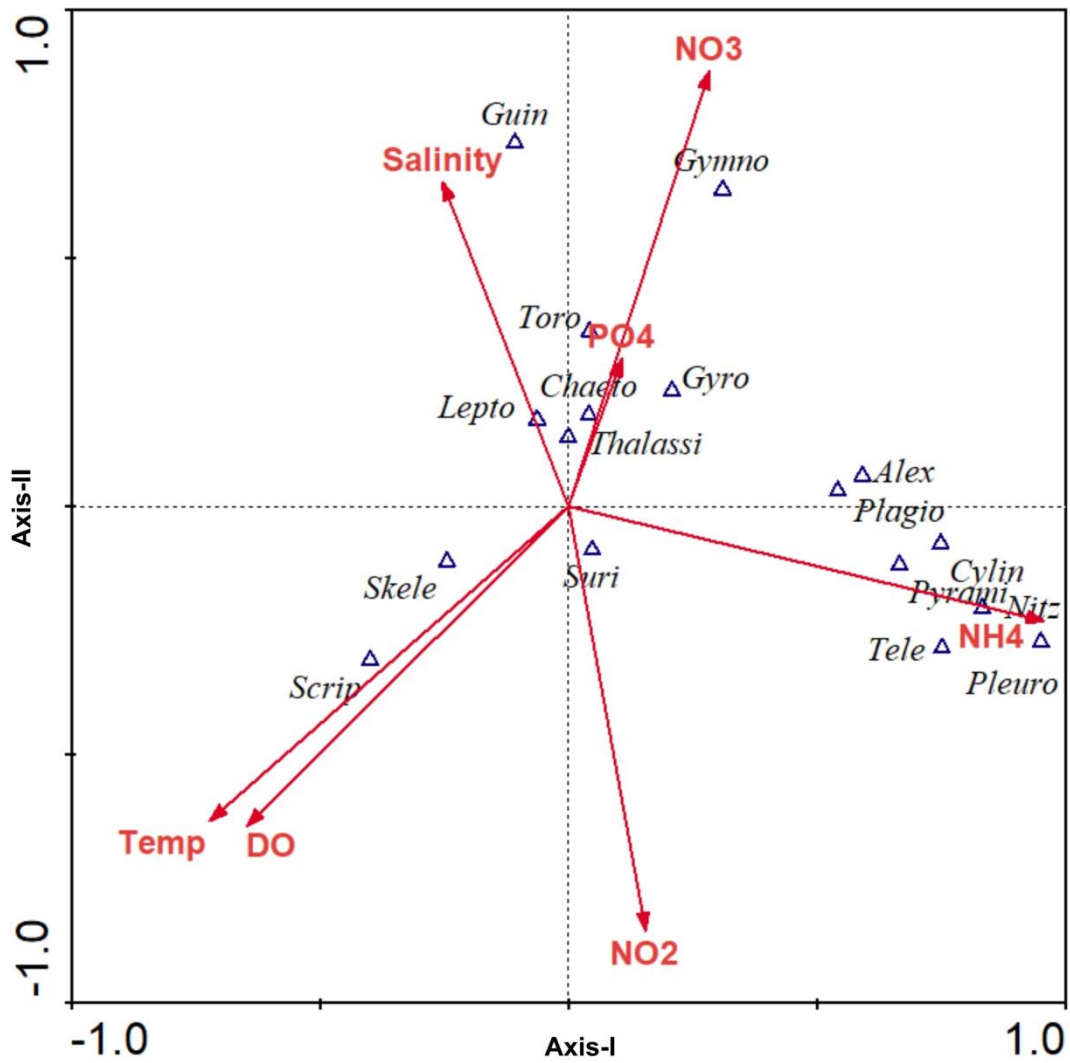


Figure 5. CCA graph of species-environmental data in the study region

(Temp=Temperature, DO=Dissolved Oxygen, NO₂=nitrite, NO₃=nitrate, NH₄=ammonia, PO₄=Phosphate, Pleuro=*Pleurosigma* sp., Cylin=*Cylindrotheca* sp., Nitz=*Nitzschia* sp., Gyro=*Gyrodinium* sp., Gymmo=*Gymnodinium* sp., Alex=*Alexandrium* sp., Thalassi=*Thalassiosira* sp., Chaeto=*Chaetoceros* sp., Lepto=*Leptocylindrus* sp., Pyrami=*Pyramimonas* sp., Plagio=*Plagioselmis* sp., Toro=*Torodinium* sp., Guin=*Guinardia* sp., Scrip=*Scrippsiella* sp., Skele=*Skeletonma* sp., Suri=*Surirella* sp., Tele=*Teleaulax* sp.)

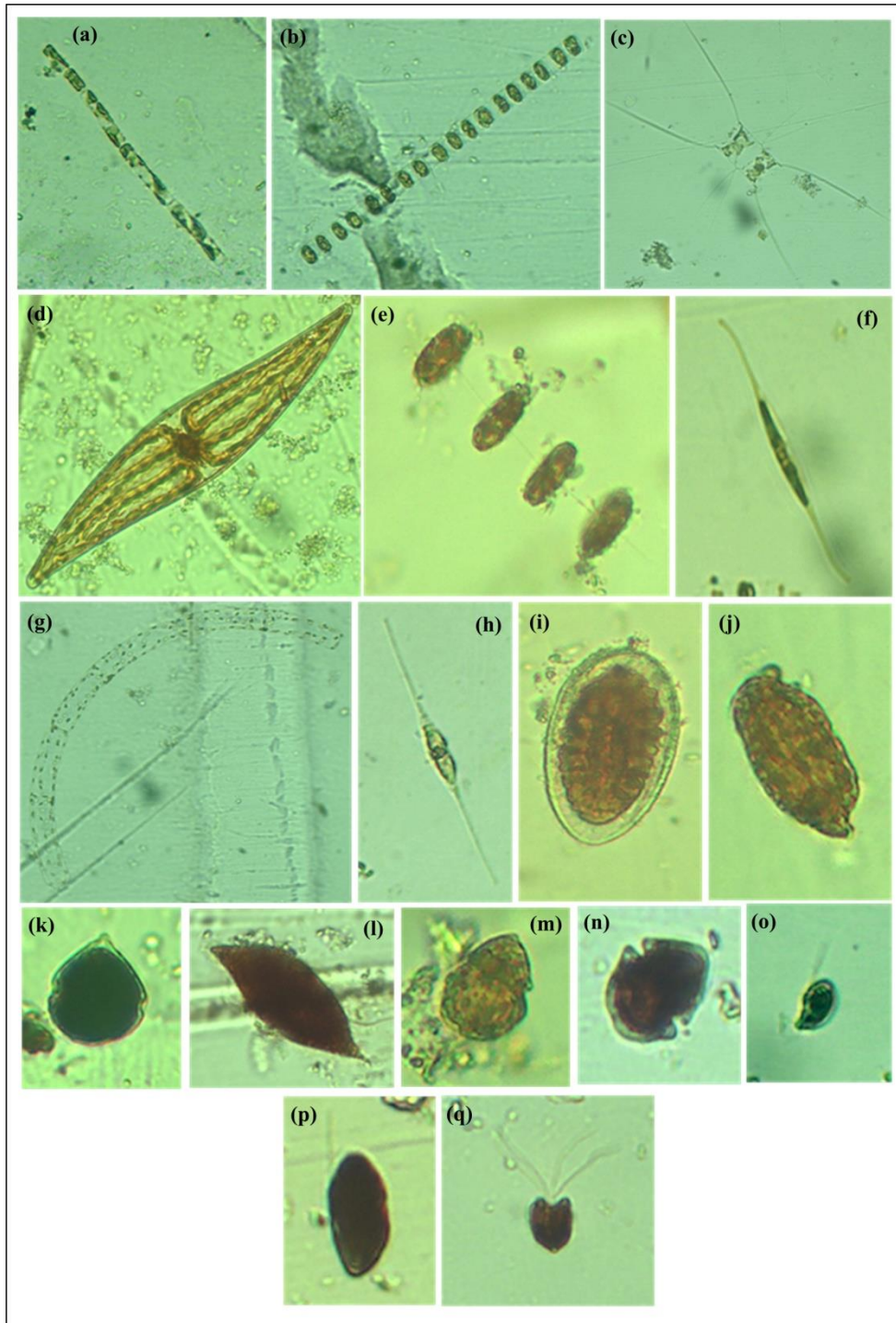


Plate 1: Photographs of major phytoplankton genera in Thane Creek

- (a) *Leptocylindrus* sp. (b) *Skeletonema costatum* (c) *Chaetoceros* sp. (d) *Pleurosigma* sp. (e)
Thalassiosira sp. (f) *Nitzschia* sp. (g) *Guinardia* sp. (h) *Cylindrotheca* sp. (i) *Surirella* sp. (j)
Torodinium sp. (k) *Scrippsiella* sp. (l) *Gyrodinium* sp. (m) *Gymnodinium* sp. (n) *Alexandrium* sp. (o)
Plagioselmis sp. (p) *Teleaulax* sp. (q) *Pyramimonas* sp.

Table 1. Major phytoplankton species ($\times 10^4$ cells l^{-1}) encountered during the study. Near-bottom values are given in parenthesis

Phytoplankton Genera	1	2	3	4	5	6	7
Depth (m)	(7m)	(4.5m)	(5m)	(9m)	(6m)	(15m)	(15m)
DIATOM							
<i>Chaetoceros</i> sp.	4.8 (4.8)	5.8 (1.4)	2.4 (1.6)	0 (0)	0 (0)	0 (0)	0 (0.8)
<i>Cylindrotheca</i> sp.	4.4 (4)	3 (3.6)	2.2 (40)	2.4 (3.4)	1.2 (0.8)	0.6 (0.2)	0.8 (0.2)
<i>Guinardia</i> sp.	0.8 (0.2)	1.8 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Leptocylindrus</i> sp.	7.8 (7)	4.8 (5.4)	3 (0.6)	0 (0.16)	0.4 (0)	0 (0)	0 (0)
<i>Nitzschia</i> sp.	1 (3.6)	0.6 (1.8)	0.8 (28)	0.8 (1)	0.2 (0.2)	0.4 (0)	0.2 (0.2)
<i>Pleurosigma</i> sp.	1 (0.6)	0.12 (0.8)	1.82 (19.6)	0 (0.6)	0 (0.02)	0.4 (0)	0.2 (0.22)
<i>Skeletonema</i> sp.	*564 (59.2)	51.6 (62)	19.8 (26.6)	1.8 (0.8)	1.2 (2.8)	1.6 (2.2)	0 (1.2)
<i>Surirella</i> sp.	2 (1.2)	0.2 (0.8)	0.2 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Thalassiosira</i> sp.	163.2 (183.2)	75.6 (140)	41.2 (70)	4.8 (9.2)	6.4 (3.2)	1.6 (1)	2.6 (2.6)
DINOFLAGELLATES							
<i>Alexandrium</i> sp.	2.4 (3.6)	9 (0.6)	19.2 (3.2)	2 (0.2)	0.6 (1)	0.2 (0.2)	0.2 (0)
<i>Gymnodinium</i> sp.	0.2 (0)	1 (0)	0 (0.4)	0.2 (0)	0.2 (0)	0 (0)	0.2 (0)
<i>Gyrodinium</i> sp.	2.6 (2.2)	4 (0.4)	2.4 (1)	1 (0.02)	0 (0)	0 (0)	0.6 (0)
<i>Scrippsiella</i> sp.	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Torodinium</i> sp.	0.4 (0.4)	1.2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CRYPTOPHYTES							
<i>Plagioselmis</i> sp.	2.4 (6.4)	14 (0.8)	26.4 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Teleaulax</i> sp.	2.4 (3.8)	1 (0.8)	20.8 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)
CHLOROPHYTES							
<i>Pyramimonas</i> sp.	0.4 (2)	2.4 (0.2)	9.2 (0)	0 (0)	0 (0)	0 (0)	0 (0)

(-)=absent; * *Skeletonema costatum* outburst count

Table 2. t-test for the environmental variables

Parameters	t stat	t critical	P
Temperature (°C)	2.56	1.94	0.02
Salinity (ppt)	-0.75	1.94	0.24
Dissolved Oxygen (mg l ⁻¹)	2.01	1.94	0.05
Nitrite (µM)	2.89	1.94	0.01
Nitrate (µM)	-1.54	1.94	0.08
Ammonia (µM)	0.97	1.94	0.18
DIN (µM)	-0.18	1.94	0.42
Phosphate (µM)	0.42	1.94	0.34
N:P	-0.89	1.94	0.20
Chlorophyll <i>a</i> (mg m ⁻³)	0.005	1.94	0.49

Table 3. Summary of Pearson's correlation

	Temp	Salinity	DO	NO ₂	NO ₃	NH ₄	PO ₄	DIN	N:P	Chl
Temperature	1									
Salinity	-0.34	1								
DO	-0.40	0.14	1							
NO ₂	0.42	-0.98**	-0.13	1						
NO ₃	-0.19	-0.44	-0.06	0.29	1					
NH ₄	-0.14	-0.46	-0.27	0.50	0.02	1				
PO ₄	0.39	-0.88**	-0.43	0.84*	0.36	0.29	1			
DIN	0.14	-0.93**	-0.19	0.89**	0.59	0.63	0.76*	1		
N:P ratio	-0.51	0.30	0.54	-0.34	0.40	-0.03	-0.63	-0.06	1	
Chl	0.54	-0.91**	-0.003	0.90**	0.34	0.10	0.82*	0.73	-0.37	1

*p<0.05; **p<0.01 (DO=dissolved oxygen; NO₂=nitrite; NO₃=nitrate; NH₄=ammonia; PO₄=phosphate; DIN=Dissolved inorganic nitrogen; Chl=Chlorophyll *a*)