

Bloom report of *Noctiluca scintillans*, *Gymnodinium wulfii* and *Guinardia delicatula* from Shastri Estuary, West Coast of India

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Abstract

The present study reports the concurrent blooms of *Noctiluca scintillans*, *Gymnodinium wulfii* and *Guinardia delicatula* from Shastri Estuary. The bloom of *Gymnodinium wulfii* is reported for the first time from the west coast of India. Additionally, the blooms of the three phytoplankton are also reported for the first time from Shastri Estuary. The environmental relationships of these blooms was studied using CCA and it was observed that *Noctiluca scintillans* and *Gymnodinium wulfii* displayed a positive correlation with temperature, salinity, phosphate and nitrate while *Guinardia delicatula* showed affinity towards silicate and ammonia. Considering that the density of chaetognaths and jellyfish, the major predators of copepods was not sufficient enough for the reduction of copepod density, the reduced copepod abundance is attributed to the toxic thin layer of dinoflagellate blooms.

Keywords: *Noctiluca scintillans*, Phytoplankton bloom, diatom, dinoflagellate, copepods.

Introduction

Phytoplankton biomass, primary production and community composition in the estuarine transitional zone are influenced by anthropogenic interventions and climate variability which intersect to drive complex patterns of change over time (Cloern and Jassby, 2008). An important pattern is the occurrence of seasonal or episodic bursts of biomass as blooms. The phytoplankton bloom is often regulated by different nutrient species, mainly nitrogen, phosphorus and silicate (Yin et al. 2000; Twomey and Thompson 2001). Hydrodynamic conditions like residence time and water column stratification/stability are also important factors for the development of phytoplankton blooms in an estuary. The highly variable physical environment and nutrient regime promote different strategies at different times and bloom species are often selected among those present at suitable inoculums before the bloom (Smayda and Reynolds 2001).

Tropical estuaries are usually rich in nutrients, delivered through river discharges making them mesotrophic to eutrophic (Huang et al. 2012; Mahoney and Bishop 2017). Nutrient enrichment eventually influences phytoplankton growth rates and bloom development (Glibert 2016; Cloern et al. 2014). Blooms are also considered a major ecological event that provides a considerable portion of annual primary production and energy transfer sustaining the whole marine food web (Carstensen et al. 2015). Bloom can cause a variety of deleterious effects on the marine ecosystem including the production of toxins, with subsequent accumulation and transport by feeding interactions through pelagic and benthic marine food webs.

There also exists an inter-linkage between phytoplankton and zooplankton community structures where a change in phytoplankton community composition can influence the food web structure and energy flow in the pelagic ecosystem (Kiorboe 1993). Copepods the dominant mesozooplanktonic grazers in the marine realm have specific feeding and reproductive performance which depend on specific food traits, such as prey size (Hansen et al. 1994; Saiz et al. 2014 and Moller 2007), prey concentration (Saiz et al. 1999), prey motility (Kiorboe and Saiz 1995) and food quality (Broglio et al. 2003; Kleppel and Burkart 1995).

Incidence of *Noctiluca scintillans/miliaris* ((Macarty) Kofoid and Swey 1921) bloom from the Northeastern Arabian Sea (NEAS) has been reported by researchers since 1900 and some of the latest reports are Gomes et al. 2008; Padmakumar et al. 2012; Madhu et al. 2012; Gomes et al. 2014; Sulochanan et al. 2014; Lotliker et al. 2018 and Sarma et al. 2019. *N. scintillans* is a heterotrophic dinoflagellate encountered in the Arabian Sea that displays mixotrophic behaviour and can fix carbon through photosynthesis with the help of the autotrophic endosymbiont, *Pedinomonas noctiluca* present in them (Goes and Gomes 2016). *N. scintillans* do not have a strict prey selection and can graze up on bacteria, phytoplankton, protozoans, copepods, copepod eggs, and fish eggs (Nawata and Sibaoka 1983; Kirchner et al. 1996). Other than *N. scintillans*, dinoflagellate bloom of *Ceratium polykrikoides*, *Karenia brevis*, *Karenia mikimotoi*, *Protoperidinium* sp. were reported along the southwest coast of India by O’Herals 2001; Iyer et al. 2008; Sanilkumar et al. 2009 and Madhu et al. 2011. Similarly, the bloom of diatoms, which is the major phytoplankton group has been reported from the west coast of India (*Coscinodiscus* spp., *Proboscia alata*, *Rhizosolenia* spp., *Fragilaria oceanic*, *Thalassiosira* spp., *Chaetoceros* spp., *Asterionella japonica*, *Skeketonema costatum*, *Psuedo-nitzschia* spp., and *Haslea gretharum*) by Mitbavkar and Anil 2002; Padmakumar 2007; Patil and Anil 2008; D’Costa and Anil 2010; Padmakumar et al. 2012 and Thomas et al. 2022.

Shastri Estuary is a confluence of the Shastri River and the Arabian Sea. It is a shallow tropical estuary that does not have any effluent discharge from point sources (NIO 2018). Shastri River is one of the few undammed, unpolluted and near pristine rivers in the Northern Western Ghats. The present study mainly intended to report the concurrent blooms of *Noctiluca scintillans*, *Gymnodinium wulfii* and *Guinardia delicatula* and their relationship with the environmental variables.

Materials and methods

Study area description

The Shastri Estuary is fed by the Shastri River which originates in the Western Ghats after following a meandering course of about 64 km and debouches into the Arabian Sea at Jaigad, Maharashtra. Small tributaries namely Gad, Kapshi, and Bav join the Shastri Estuary. Shastri Estuary has two bays viz. Dhamankhol Bay (depth of 7 to 8 m) and Jaigad Harbour (depth of 12 to 13 m). The adjoining peninsula at the mouth of Shastri Estuary has Jaigad Port, which is also an active fish landing centre and has bauxite export. Shastri Estuary experiences limited tidal impact and tidal ingress is expected to be weak from the middle to upper segments. Shastri Estuary has a mixed semi-diurnal tide with two unequal high and low water occurring each tidal day.

Sampling strategy

A monitoring study was carried out during 1st to 4th of December 2020 in Shastri Estuary and water samples were collected from mouth to mid estuary of Shastri. The sampling stations C1, C2 and C3 were classified as coastal stations which were in the estuary mouth while station U4 was located in the mid-estuary (Figure 1). Samples for analysis of abiotic and biotic parameters were collected from the surface and near-bottom using a 5 L capacity Niskin water sampler (Germany).

Satellite data processing

MODIS-Aqua Level-3 HDF data were downloaded for the non-bloom (24th November 2020) and bloom period (1st December 2020) through NASA Internet server Ocean Color Web. For the analysis of the chlorophyll *a* data along the west coast of India, 9km resolution data (eight days averaged) were used for the study (Figures 2a,b&c). Hourly wind data were also downloaded from ERA-5 for December 2020 and used in this study (Figure 2d).

Estimation of physico-chemical parameters

Water sample for temperature was measured using a hand-held mercury thermometer of accuracy $\pm 0.1^\circ\text{C}$. Salinity was estimated by Mohr titration methodology and dissolved oxygen (DO) by Winkler's method as recommended by Grasshoff et al. 1983. Dissolved inorganic nutrients (nitrite- NO_2 , nitrate- NO_3 , ammonia- NH_4 , phosphate- PO_4 , and silicate- SiO_4) 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) concentration of nutrients in the water sample was measured spectrophotometrically within 6 h of the collection as described by Grasshoff et al.1983.

Estimation of Chlorophyll *a*, phytoplankton density and diversity

For chlorophyll *a* estimation, 500 ml of water sample was filtered through Whatman GF/F filter paper (47 mm diameter; pore size 0.7 μm) and extracted in 90% acetone kept overnight at 4°C. The chlorophyll *a* was estimated fluorometrically (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. After sedimentation in dark the samples were concentrated at 10 ml. An aliquot of 1 ml from each concentrate was taken into Sedgwick Rafter (United Kingdom) chamber and analyzed under a compound light microscope (MOTIC BA 410, 400 X magnifications). The phytoplankton was identified up to the lowest possible taxonomic level using the standard taxonomic keys of Subrahmanyam 1946 and Tomas 1997 and the density was expressed in cells L^{-1} .

Criteria for bloom count

The least cell concentrations that can be attributed to the blooms of the various taxonomic classes were 1.3×10^4 cells/L for Bacillariophyceae-Diatoms (Sanilkumar et al. 2009), 1.0×10^4 and 9.0×10^4 cells/L for *Noctiluca* (Padmakumar et al. 2016) and other Dinophyceae, respectively (Iyer et al. 2008).

Collection and estimation of mesozooplankton

Mesozooplankton samples were collected with Heron Tranter (HT) net having a mouth area 0.25m^2 and mesh size $200\mu\text{m}$ towed horizontally just below the surface for 5 minutes with a boat speed of approximately 1.5 knots. The net is fitted with calibrated flow meter (General Oceanics Model 2030, USA) diagonally, to quantify the volume of water filtered through the net. Collected samples were preserved in a 4% formaldehyde solution.

In the laboratory, biomass estimation was determined by the displacement volume method. Collected mesozooplankton were filtered through a piece of clean, dried bolting silk and filtered zooplankton was transferred with a spatula to a measuring cylinder with a known volume of 4% formaldehyde solution. The displacement volume is obtained by recording the volume of fixative in the measuring cylinder displaced by the mesozooplankton and the total biomass was expressed as $\text{ml}/100\text{m}^3$ (Harris et al. 2000)

After estimation of biomass, the mesozooplankton was primarily sorted into the major taxonomic groups according to the standard identification manuals (Newell and Newell 1973; Todd and Laverack 1991) under the stereomicroscope (LEICA, Model-S6-D). Subsamples were done by 10 ml Stempel pipette and the density of mesozooplankton was expressed as (no. of individuals $/\text{m}^3$).

Statistical analysis

Pearson's correlation was carried out to study the linearity of phytoplankton species with environmental variables. Canonical correspondence analysis (CCA) was intended to understand the main environmental variables affecting the distribution of major phytoplankton species in the study region. CCA was performed using the CANOCO 4.5 statistical software. Followed by CCA analysis a representative plot was obtained through CanoDraw software.

Results

The present study was a routine monitoring program and the study period was conducted between the 1st and 4th of December 2020. During the sampling period, green patches were observed on the surface water of stations C1-C3 (Plate 1a&b). Later satellite imagery from the NASA internet server, Ocean Color Web was downloaded for 9 km resolution data (eight days averaged) for 24th November 2020 (non-bloom period), 1st December 2020 (bloom period) and 5th December 2020 (post sampling) Figure 2 a, b&c. Further wind data was derived from ERA-5 to investigate the direction of the bloom. The results indicated that the wind was northerly with a maximum wind speed of 10 m/s. However, in the study region, the wind speed was 4 m/s. Therefore, it can be concluded that the bloom was moving from north to south (Figure 2d).

The range of environment variables like temperature, salinity, dissolved oxygen (DO), and concentration of nutrients (N, P & Si) in the study region are indicated in Table 1. Surface water temperature ranged between 25.5 and 29°C while at the near-bottom it ranged from 25 to 28.5°C at sampled locations. During the study period, salinity showed spatial variation with relatively low salinity in the surface water (33.8 PSU) at station U4 which is in the mid estuary. Meanwhile, stations C1, C2 and C3 which are in the mouth of Shastri Estuary had relatively high salinity of 34.5 PSU (Table 1). Dissolved Oxygen (DO) ranged between 4.64 and 6.16 mg l^{-1} in the surface water and was moderately high at stations C1, C2 and C3 than at station U4 (4.64 mg l^{-1}). The near-bottom water DO ranged from 3.43 to 4.87 mg l^{-1} and was relatively low in concentration compared to

surface water (Table 1). Nutrients like nitrite, nitrate, ammonia, phosphate and silicate varied to various extent in the study region (Table1). Noticeably low nitrite concentration was observed in the surface water of stations C1 (0.06 μM) and C2 (0.19 μM) compared to stations C3 (0.56 μM) and U4 (0.40 μM). Nitrate was found to be lower at station C2 (0.10 μM) compared to other stations during the same period. Ammonia varied widely in the surface water of various stations (0.26 to 1.13 μM) while in near-bottom water the difference between the stations was meagre (0.32 to 0.84 μM). Phosphate concentration in the surface water was more or less evenly distributed except the minor drop at station U4. However, the overall average of phosphate was 0.67 μM at the surface and 0.85 μM in near-bottom water. Silicate was comparatively high at station U4 in surface water (28.28 μM) and near-bottom water (26.77 μM) than at stations C1, C2 and C3 which ranged between 5.29 and 9.74 μM (Table 1).

Chlorophyll *a* in the study region was maximum at C1 (6.35 mg m^{-3}) and minimum at C2 (2.32 mg m^{-3}) in the surface water. The near-bottom water chlorophyll *a* ranged between 0.38 and 1.64 mg m^{-3} which was comparatively lower than the surface water (Table 1; Figure 3). Phytoplankton population density at surface water ranged between 143 and 491 $\times 10^3$ cells L^{-1} and at near-bottom water the density ranged from 48 to 141 $\times 10^3$ cells L^{-1} (Table 1). High chlorophyll *a* coincided with high phytoplankton population density especially in the surface waters of stations C1, C3 and U4 where phytoplankton species like *N. scintillans*, *G. wulfii* and *G. delicatula* were encountered in high abundances (Table 2). *N. scintillans* and *G. wulfii* were observed in the mouth of Shastri Estuary at stations C1 and C3 respectively in high density. *N. scintillans* was found with a maximum density at surface water of station C1 (24 $\times 10^3$ cells L^{-1}) and followed a decreasing trend from stations C2 (12 $\times 10^3$ cells L^{-1}), C3 (10 $\times 10^3$ cells L^{-1}) to U4 (0.8 $\times 10^3$ cells L^{-1}). Whereas, the bloom of *G. wulfii* was encountered at station C3 with a density of 106 $\times 10^3$ cells L^{-1} during the same period. *G. wulfii* cells were also found at stations C1, C2 and U4 but at comparatively lower densities (Table 2). Other than *N. scintillans* and *G. wulfii*, dinoflagellates like *Oxytoxum* sp. and *Gyrodinium* sp. were also found in relatively high density. During the same time, the diatom *G. delicatula* was observed in the surface water of station U4 with a maximum density of 184 $\times 10^3$ cells L^{-1} and was completely absent at stations C2 and C3 (Table 2). The other dominant diatoms species were *Cylindrotheca closterium*, *Leptocylindrus danicus*, *Chaetoceros* sp. and *Skeletonema costatum*. (Table 2). *Plagioselmis* sp. which belongs to class Cryptophytes was also found plentiful at stations C1, C3 and U4.

The high density of *N. scintillans* in the study region was indicative of the occurrence of this particular species as bloom (based on bloom criteria selection as described in the methodology) in the study region. The bloom trails and visuals of this species are depicted in Plate 1 a, b&c while the microscopic images are shown in Plate 1d&e. As the other two species, *G. wulfii* which has never been reported from Indian waters and *G. delicatula* which is not reported from the west coast of India, therefore their present cell count (density) is considered as bloom density.

Copepods the immediate grazers of phytoplankton are the major group in any mesozooplankton sample. During the study period, copepod density was found to be lower at stations C3 (10 ind./ m^3), C1 (18 ind./ m^3) and C2 (27 ind./ m^3) compared to station U4 (470 ind./ m^3). Other than copepods, appendicularians were the second dominant mesozooplankton community during the study period (Figure 4).

Pearson's correlation analysis was performed to find the linearity of the bloom species with environmental parameters (Table 3). It was found that *N. scintillans* was positively correlated with water temperature ($r=0.85$; $p<0.05$) and DO ($r=0.83$; $p<0.05$) and negatively correlated with ammonia ($r= -0.84$; $p<0.05$) and silicate ($r= -0.81$; $p<0.05$). On the other hand, *G. wulfii* exhibited a positive correlation with nitrate ($r=0.96$; $p<0.05$). *G. delicatula* showed a positive correlation with ammonia ($r=0.99$; $p<0.05$) and silicate ($r=0.98$; $p<0.05$) while a negative correlation was displayed

with water temperature ($r = -0.99$; $p < 0.05$), DO ($r = -0.96$; $p < 0.05$), salinity ($r = -0.99$; $p < 0.05$) and phosphate ($r = -0.97$; $p < 0.05$).

Further, CCA analysis (Figure 5a&b) was carried out which is a multivariate method to elucidate the relationship between biological assemblages of species and their environment. The CCA result explained 90.9% and 84.8% of the total variation in surface and bottom waters respectively (Figure 5). In surface water axis-I displayed 69.6% of total variability and the environmental variables such as temperature, salinity, DO (dissolved oxygen) and phosphate showed negative loading while ammonia and silicate showed positive loading. The second axis explained 21.3% of total variation where nitrate and nitrite correlated positively to this axis. A similar trend was observed in bottom water except for nitrite which displayed a negative correlation to the first axis.

Phytoplankton species such as *N.scintillans*, *Gyrodinium* sp., *Oxytoxum* sp., *Bacteriastrum* sp., *Cylindrotheca closterium*, *Pseudo-nitzschia* sp., *Hemidiscus* sp., *Alexandrium* sp., *Rhizosolenia setigera*., *Plagioselmis* sp., *G. wulfii* and *Scrippsiella trochoidea* displayed a positive correlation with temperature, salinity, DO and phosphate and a negative correlation with ammonia and silicate. On the other hand, an opposite correlation was displayed by *G. delicatula*, *Thalassiosira* sp., *Chaetoceros* sp., *Rhizosolenia* sp., *Leptocylindrus danicus*, *Ceratium furca* and *Protoperidinium* sp., with ammonia and silicate. On the second axis *Thalassiosira* sp., *Rhizosolenia robusta*., *Navicula delicatula*., *Pinnularia* sp., *Gymnodinium brevis*. and *Dinophysis caudate* displayed a positive correlation with nitrate and nitrite while *Nitzschia* sp. and *Pleurosigma* sp. were correlated with nitrite only. Species like *Torodinium* sp., *Navicula distans*, *Navicula* sp., *Protoperidinium stenii*, *Gonyaulax spinifera* and *Warnowia* sp. showed a negative correlation with nitrate and nitrite (Figure 5a). The near-bottom water also exhibited a similar kind of correlation as that of surface water with minor deviations (Figure 5b). In surface water, *Skeletonema costatum* located near the centre of the graph showed a minor influence of environmental parameters while in bottom water it displayed a strong positive correlation with nitrate.

Discussion

Temperature showed a typical tropical condition with an average temperature of 27.8°C, while salinity displayed a spatial variation with relatively low salinity in the mid estuary (33.8 PSU) compared to stations at the mouth (C1-C3). The low salinity in the mid estuary was attributed to the weak tidal effect (NIO 2018). Nutrients (N&P) were not high as found in other estuaries which receive anthropogenically generated nutrients as Shastri Estuary does not have any effluent release from point sources (NIO 2018).

During the study period, *N. scintillans* bloom was encountered in the mouth of Shastri Estuary. The occurrence of *N. scintillans* bloom in the mouth of Shastri Estuary can be attributed to the transport of cells from the northern Arabian Sea. Lotliker et al. 2018 reported that *N. scintillans* bloom is observed between 17° and 21°N and 66° to 70°E annually from the northern Arabian Sea during the winter monsoon (November to February). This indicated that the bloom outbreak had occurred in the northern Arabian Sea which was pushed to south by the northerly wind. This assumption was well supported by the present results as the wind in the study region was blowing northerly (Figure 2d).

Additionally, the satellite imagery of chlorophyll *a* also indicated the progress of high biomass from north to south along the coastline (Figure 2a, b, c&d).

N. scintillans bloom showed a decreasing trend along the estuary with maximum density at C1 (24×10^3 cells L⁻¹) followed by C2 (12×10^3 cells L⁻¹), C3 (10×10^3 cells L⁻¹) and minimum in the mid-estuary at station U4 (0.8×10^3 cells L⁻¹). The present density of *N. scintillans* at three locations (C1-C3) in Shastri Estuary was in accordance with the previously reported bloom density (17,111 cells L⁻¹) in the Andaman Sea by Dharani et al. (2004) and Dwivedi et al. (2015) in the NEAS (3×10^3 cells L⁻¹). Furthermore, the correlation between environmental variables and *N. scintillans* bloom

indicated a positive correlation with temperature ($r=0.85$; $p<0.05$) and DO ($r=0.83$; $p<0.05$) whereas a negative correlation with ammonia ($r=-0.84$; $p<0.05$) and silicate ($r=-0.81$; $p<0.05$) (Table 3). Moreover, the CCA results also clearly indicated a positive loading of *N. scintillans* with temperature, salinity, DO and phosphate while a negative loading with ammonia and silicate. Sulochanan et al. (2014) reported that water temperature in the range of 28.2-33°C promoted the proliferation of *Noctiluca* in the West Coast of India. Therefore, it can be concluded that environmental variables like temperature, salinity, DO and phosphate favoured the growth of *N. scintillans* in the study region.

In addition to this, the complete absence of *N. scintillans* in the near-bottom water of all sampled locations was a noticeable feature. The complete absence of this species can be attributed to the lack of sufficient prey availability in the near-bottom water and also due to the larger cell size ($>20\mu\text{m}$). The study made by Baliarsingh et al. (2018) in the NEAS explained that the discrepancy in the abundance of *N. scintillans* in bottom water depends on the quantum of prey availability. Also, the larger cell size with positive buoyancy would have restricted green *Noctiluca* sp. to the upper few meters of the water column.

G. wulfii bloom (106×10^3 cells L^{-1}) was also encountered in the mouth of Shastri Estuary in the surface water of the C3 location. This particular bloom is the first report from Indian waters. This species has over 500 occurrence records in Global Biodiversity Information Facility (GBIF) (Thessen et al. 2012). The information on the geographical distribution of *G. wulfii* is scanty and the first report of this particular species is from the Barents Sea (Schiller 1933). This species has also been reported from the Bering and Chuckchi Seas and eastern Russian waters (Konovalova 1998). Ratkova and Wassmann (2005) and Lewis et al. (2018) also reported this species from the Chupa Estuary of the White Sea and the Barents Sea and the Caspian Sea respectively. The introduction of this exotic species into the coastal waters of Shastri Estuary might be through ballast water exchange. Jaigad Port, which is located at the mouth of Shastri Estuary, is an international port operating for the export-import of raw materials such as bauxite/iron ore, coal and fly ash, etc (NIO 2018). The correlation and CCA study pointed out that *G. wulfii* showed a significant positive correlation with nitrate ($r=0.96$; $p<0.05$) Table 3, while the CCA result indicated a positive affinity towards temperature, salinity, DO and phosphate and negative relation with ammonia and silicate (Figure 5a). Therefore, it can be concluded that the proliferation of this exotic species was favoured by the environmental variables described above.

G. delicatula bloom is also reported for the first time from the west coast of India. However, this particular species in bloom condition has been reported from the east coast of India (Murty et al. 2017). *G. delicatula* bloom (184×10^3 cells L^{-1}) at station U4 can be attributed to high silicate concentration ($28.28 \mu\text{M}$) in the surface water which promoted the proliferation of this particular species to single species dominance. The correlation results substantiated the above finding as *G. delicatula* showed a positive correlation with silicate ($r=0.98$; $p<0.05$) and ammonia ($r=0.99$; $p<0.05$) and a negative correlation with temperature ($r=0.99$; $p<0.05$), salinity ($r=0.99$; $p<0.05$), DO ($r=0.96$; $p<0.05$) and phosphate ($r=0.97$; $p<0.05$) (Table 3). The CCA analysis also pointed towards the positive correlation of *G. delicatula* to silicate and ammonia (Figure 5a). Dissolved inorganic silica is a major nutrient for bloom-forming diatom and nitrate:silicate ratio influences the composition of phytoplankton (Officer and Ryther 1980; Tsunogai and Watanabe 1983). Although nitrate and silicate are considered the causative agent for these blooms, an increase in silicate concentration is always accompanied by diatom blooms (Officer and Ryther 1980). Mohanty et al. (2007) reported that silicate utilization by diatoms for the siliceous frustules formation is one of the most key mechanisms that regulate the growth and proliferation of phytoplankton. The author also reported that an increase in silicate concentration (13.36 to $47.9 \mu\text{mol L}^{-1}$) was associated with the diatom bloom in the Rushikuliya River, Bay of Bengal. Therefore, it can be concluded that the bloom of *G. delicatula* in Shastri Estuary was due to the the increase in silicate and ammonia concentration.

Copepods are key components of the 'classical' food web, where they mainly function as herbivores, forming an important trophic link between phytoplankton as primary producers and higher trophic levels (Cushing 1989; Vargas et al. 2010). They are the most numerically dominant group contributing more than 80% to the total mesozooplankton communities (Schminke 2007). However, in the present study, the density of copepod was found to be low at stations C2 (10 ind./m³), C1 (18 ind./m³) and C3 (27 ind./m³) compared to station U4 (470 ind./m³) (Figure 4). At these stations, the bloom of *N. scintillans* and *G. wulfii* was also encountered. Therefore, the reduction in the density of the copepod population at these stations can be assumed as an avoidance of the thin toxic layer produced by these dinoflagellate blooms. True et al. (2018) reported that copepods have a strong incentive to sense and avoid nearby, spatially discrete patches of toxic algae to improve fitness by avoiding exposure and/or ingestion and associated negative impacts. The study also suggested that harmful algae not only produce deleterious physiological effects in copepod grazers, but chemical-induced behavioural responses can also alter grazer distribution and top-down control via avoidance reactions. Another assumption is the predators, pelagic copepods have many predators, mainly chaetognaths, jellyfish, fish and even whale (Kiorboe 2011). However, during the present sampling period, the population density of chaetognaths was between 1 and 2 ind./m³, whose density was not sufficient enough to graze the copepods to the low numbers as recorded in this study. On the other hand, it was observed that the population density of appendicularians was found to be the second highest (on average) in the mesozooplankton community (Figure 4). Appendicularians are filter feeders with amazing filtration apparatus. The filters are made of strands of mucus that allow only the smallest food particles <0.0004 in (<1µm) into the tube leading to the mouth (<http://www.encyclopedia.com>). Therefore, it can be concluded that the copepod density was affected by toxic thin layer of dinoflagellate bloom.

Conclusion

During the present study, three simultaneous blooms of *N. scintillans*, *G. wulfii* and *G. delicatula* were encountered in the mouth and mid-estuarine region of Shastri Estuary respectively. *N. scintillans* bloom is a seasonal scenario in the northeastern Arabian Sea and has been studied extensively along this coast. However, from Shastri Estuary, *N. scintillans* bloom is reported for the first time. Similarly *G. delicatula* the diatom bloom is also reported for the first time from the west coast of India. This particular species has been observed in very high density from the east coast of India. The noteworthy feature of this study was the bloom report of *G. wulfii* from Shastri Estuary and also from Indian waters. This species is reportedly found only in temperate waters but never in bloom conditions. This is the first report of this particular species from Indian water in bloom condition. Further, the relationship of these species with environmental variables was studied and it was found that temperature, salinity, DO and phosphate were found to favour the proliferation of *N. scintillans* and *G. wulfii*. Conversely, *G. wulfii* showed affinity to silicate and ammonia. The study also focused on the relationship of the bloom species with copepods and a reduction was observed in their density at the station where *N. scintillans* and *G. wulfii* were in bloom condition. Considerable reduction in the contribution of copepods at the bloom outbreak areas can hamper the energy transfer efficiency leading to the waning of the plankton food chain. Therefore, it is advised to have a long-term observation of the coastal water of Shastri Estuary to decipher the bloom outbreak.

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Competing interests

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Credit Author Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors contribution

1. Haridevi C.K. (Conceptualization, sample collection and analysis, data synthesis, drafting, review and editing of the manuscript)
2. Revati Harikar (Involved in the sample collection, statistical analysis and proofing)
3. Jubin Thomas (Satellite data retrieving from NASA server and its interpretation)
4. Umesh Kumar Pradhan (Project leader, execution of the sampling, in charge of chemistry data).
5. Aparnamol T.A (Involved in fieldwork and assisted in the laboratory)
6. Rakesh P.S (In charge of mesozooplankton data)
7. Vivek M.A (Mesozooplankton sample analysis)
8. Balaram Sahu (Assisted in fieldwork, water quality parameter analysis)
9. Muhammed Rafeeq (Initial support to generate the satellite data)

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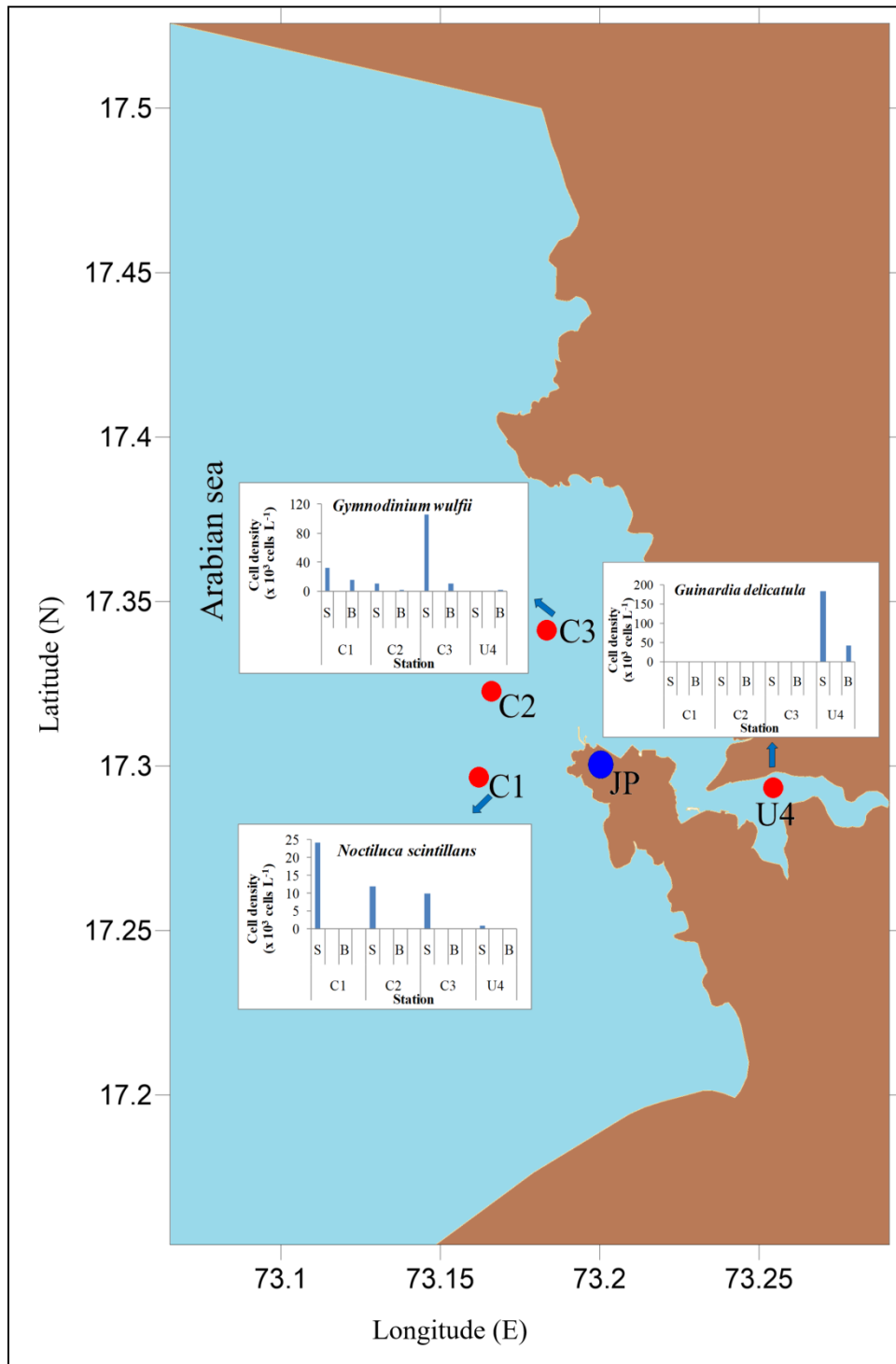


Figure 1. Map of the sampling location in Shastri Estuary and the bloom species density
 JP= Jaigad Port (blue dot)

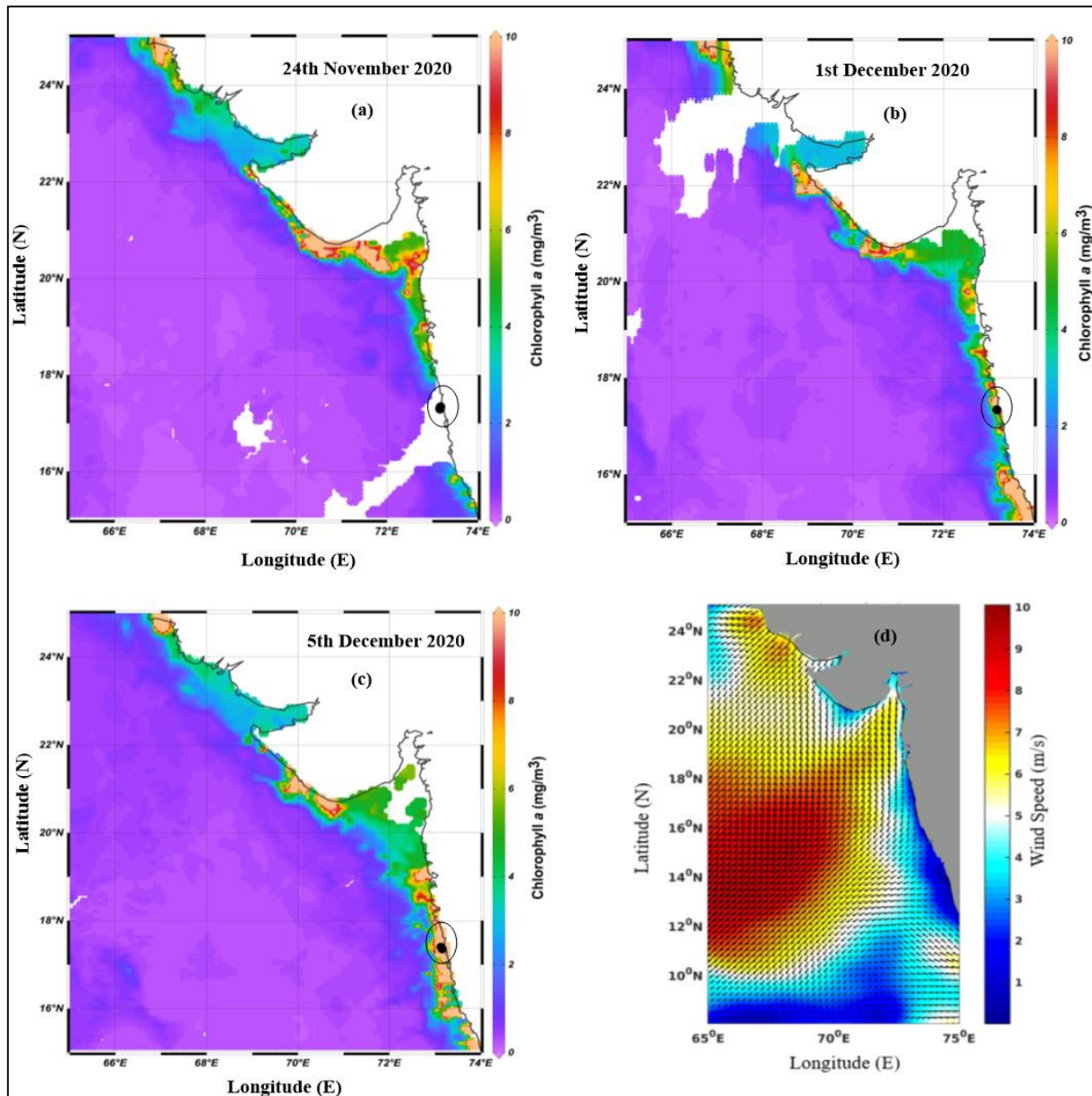


Figure 2. Satellite image of chlorophyll *a* distribution along the west coast during (a) 24th November 2020 (non-bloom period) (b) 1st December 2020 (sampling period) 5th December 2020-post sampling period (d) ERA5 derived wind speed and direction along the west coast during December 2020. The sampling location is given in black dot.

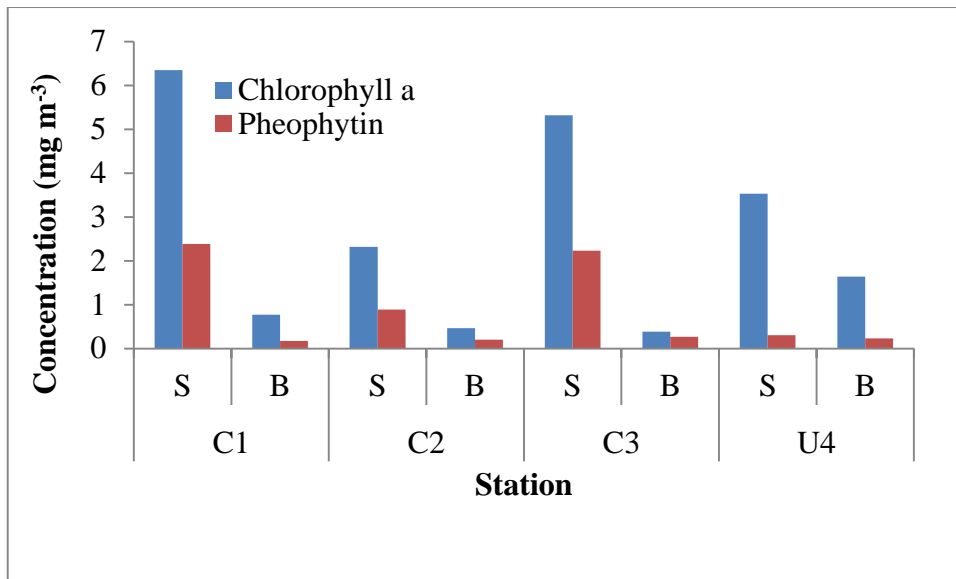


Figure 3. Distribution of chlorophyll *a* and Pheophytin at the surface (S) and near-bottom (B) waters at sampled stations in Shastri Estuary.

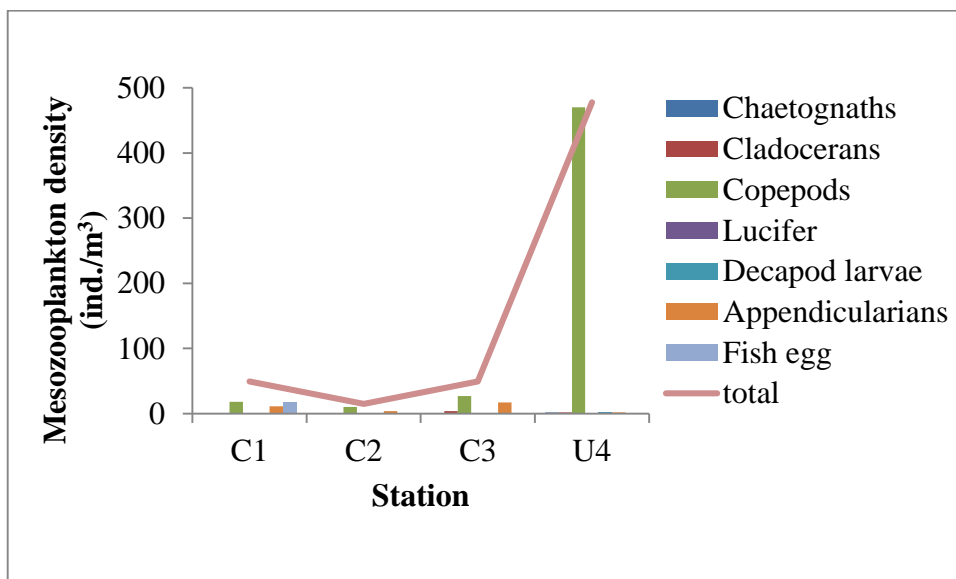


Figure 4. Distribution of mesozooplankton community in Shastri Estuary

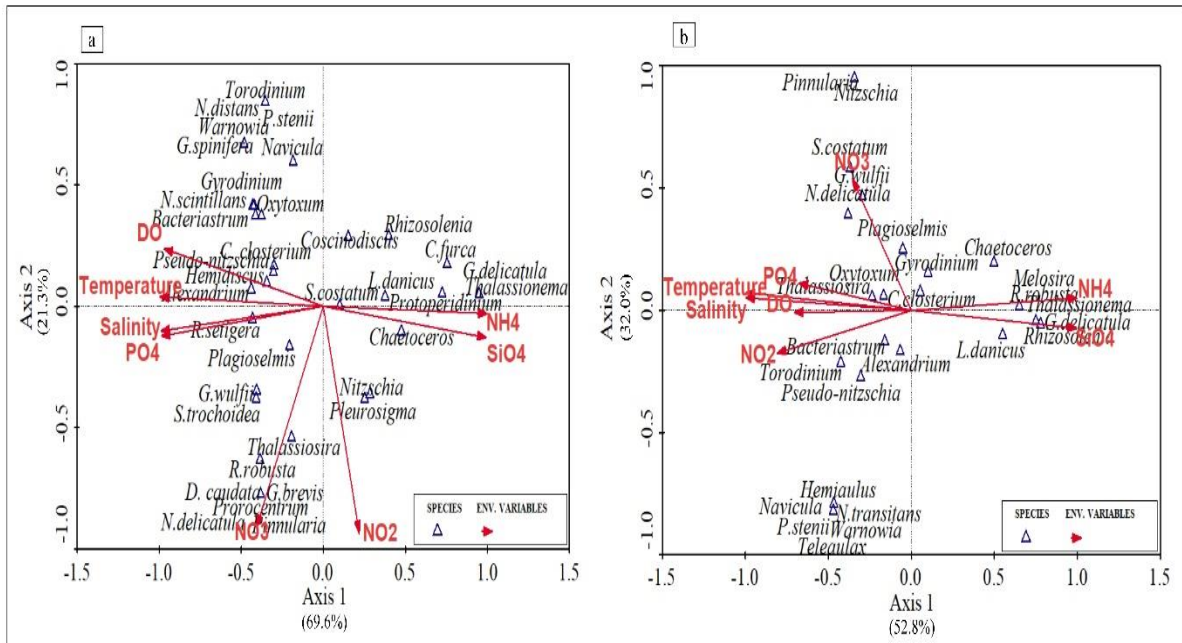


Figure 5. CCA graph of species-environmental relationship in the study region at (a) surface and (b) near-bottom waters

Table.1 Spatial variation of physico-chemical and plankton components at surface and near-bottom (parenthesis) waters of Shastri Estuary

Station code/Parameters	C1	C2	C3	U4
Water temperature (°C)	29.0±0.0 (28.5±0.0)	28.5±0.0 (28.5±0.0)	28.5±0.0 (28.5±0.0)	25.5±0.2 (25.0±0.3)
Salinity (PSU)	34.5±0.0 (34.6±0.0)	34.5±0.0 (34.6±0.14)	34.5±0.0 (34.5±0.14)	33.8±0.56 (34.1±0.73)
Dissolved oxygen (mg L ⁻¹)	6.10±0.0 (4.87±0.04)	6.16±0.06 (4.23±0.04)	5.70±0.0 (3.43±0.04)	4.64±0.06 (3.48±0.03)
Nitrite(μM)	0.06±0.06 (0.75±0.07)	0.19±0.01 (1.37±0.04)	0.56±0.06 (1.45±0.07)	0.40±0.0 (0.40±0.14)
Nitrate(μM)	0.36±0.06 (0.41±0.01)	0.10±0.0 (0.49±0.01)	1.85±0.07 (1.71±0.01)	0.35±0.21 (0.32±0.54)
Ammonia(μM)	0.26±0.06 (0.35±0.07)	0.36±0.06 (0.32±0.03)	0.37±0.04 (0.44±0.06)	1.13±0.18 (0.84±0.02)
Phosphate(μM)	0.85±0.07 (0.56±0.06)	0.72±0.03 (0.98±0.03)	0.85±0.07 (1.51±0.01)	0.26±0.06 (0.34±0.08)
Silicate(μM)	5.65±0.23 (7.46±0.12)	5.29±0.18 (8.70±0.09)	9.74±0.11 (9.66±0.17)	28.28±0.22 (26.77±0.25)
Chlorophyll <i>a</i> (mg m ⁻³)	6.35±1.39 (0.77±0.08)	2.32±0.10 (0.46±0.06)	5.32±0.01 (0.38±0.04)	3.53±1.07 (1.64±0.28)
Phytoplankton population density (x 10 ³ cells L ⁻¹)	341±0.83 (118±0.22)	143±0.52 (103±0.10)	491±0.48 (48±0.20)	435±0.77 (141±0.15)
Copepod density (individual/m ³)	18.0±2	10.0±1	27.0±3	470.0±8

Table 2. Phytoplankton species recorded at the surface (s) and near-bottom (b) water of sampled stations in Shastri Estuary (cell count =no.x10³ cells L⁻¹)

Station	C1		C2		C3		U4	
	S	B	S	B	S	B	S	B
Phytoplankton Species	S	B	S	B	S	B	S	B
<i>Alexandrium</i> sp.	8	2	0.3	2	6	0	0	1.7
<i>Bacteriastrium</i> sp.	0.2	4	2	3.2	1	0	0	2
<i>Ceratium furca</i>	0	0	0.2	0	0	0	1.2	0.1
<i>Chaetoceros</i> sp.	6	0	4	0	30	4	74	12
<i>Coscinodiscus</i> sp.	2	0	0.2	0.2	0.4	0	2	0
<i>Cylindrotheca closterium</i>	60	10	22	4	48	2	14	10
<i>Dinophysis caudata</i>	0	0	0	0	6	0	0	0
<i>Gonyaulax spinifera</i>	4	0	0	0	0	0	0	0
<i>Guinardia</i> sp.	0.1	0	0	0	0	0	0.2	0
<i>Guinardia delicatula</i>	0.1	0	0	0	0	0	184	43
<i>Gymnodinium wulfii</i>	32	16	10	2	106	10	0	2
<i>Gymnodinium brevis</i>	0	0	0	0	4	0	0	0
<i>Gyrodinium</i> sp.	14	10.6	12	0.8	8	0	0.2	8.2
<i>Hemiaulus</i> sp.	0	0	0	2	0	0	0	0
<i>Hemidiscus</i> sp.	0.2	0.3	2	0	2	0	0.2	0.1
<i>Leptocylindrus danicus</i>	4	1.8	16	4	21	0.7	56	28
<i>Melosira</i> sp.	0	0	0	0	0	0	0	4
<i>Navicula</i> sp.	6	0	2	6	0	0.1	2	0
<i>Navicula delicatula</i>	0	12	0	0	2	0	0	0
<i>Navicula transitans</i>	0	0	0	4	0	0	0	0
<i>Navicula distans</i>	1	0	0	0	0	0	0	0
<i>Nitzschia</i> sp.	0	0	0	0	4	2	4	0
<i>Noctiluca scintillans</i>	24	0	12	0	10	0	0.8	0
<i>Odontella mobiliensis</i>	0	0	0	0	0	0	0	0
<i>Oxytoxum</i> sp.	22	6	6	2	8	0	2	2
<i>Pinnularia</i> sp.	0	0	0	0	2	2	0	0
<i>Plagioselmis</i> sp.	34	4	4	2	48	4	18	4
<i>Pleurosigma</i> sp.	0	0.1	0	0	2.2	0	2	0
<i>Pleurosigma elongatum</i>	0	0	0	0.4	0	0	0	0
<i>Procentrum</i> sp.	0	0	0	0	6	0	0	0
<i>Protoperdinium</i> sp.	0	0	0.2	0	0.2	0	2	0.1
<i>Protoperdinium stenii</i>	4	0	0	2	0	0	0	0
<i>Pseudo-nitzschia</i> sp.	76	30	45	56	92	6.8	20	10.8
<i>Rhizosolenia</i> sp.	2	0.1	0	0	0	0	3.2	4
<i>Rhizosolenia imbricata</i>	0	0.1	0	0.1	0	0	0	0
<i>Rhizosolenia robusta</i>	0	0	0.1	0.1	1.1	0.4	0	4
<i>Rhizosolenia setigera</i>	2	0	0	0	2	0	0	0
<i>Scrippsiella</i> sp.	0	0	0	0	8	0	0	0
<i>Scrippsiella trochoidea</i>	3	0	0	0.2	0	0	0	0
<i>Skeletonema costatum</i>	32	16	0	1	30	12	39	0
<i>Teleaulax</i> sp.	0	0	0	2	0	0	0	0
<i>Thalassionema</i> sp.	0	0	0	0	0	0	2	3
<i>Thalassiosira</i> sp.	2	3	2	5	43	4	8	2
<i>Thalassiothrix</i> sp.	0	0	0	0	0.1	0	0	0
<i>Torodinium</i> sp.	0.4	2	3	2	0	0	0.2	0
<i>Warnowia</i> sp.	2	0	0	2	0	0	0	0
Total density (no. x10 ³ cells L ⁻¹)	341	118	143	103	491	48	435	141

*Bloom species and bloom count are given in bold

Table 3 Pearson's correlation coefficient between bloom species and environmental variables at surface and near-bottom (parenthesis) waters of Shastri Estuary

Parameter	WT	DO	Sal	PO ₄	NO ₂	NO ₃	NH ₄	SiO ₄	NS	GW
WT	1.00 (1.00)									
DO	0.96 (0.51)	1.00 (1.00)								
Sal	0.97 (1.00)	0.94 (0.57)	1.00 (1.00)							
PO ₄	0.98 (0.66)	0.89 (-0.32)	0.96 (0.60)	1.00 (1.00)						
NO ₂	-0.38 (0.78)	-0.53 (-0.08)	-0.22 (0.74)	-0.22 (0.93)	1.00 (1.00)					
NO ₃	0.21 (0.42)	-0.03 (-0.51)	0.30 (-0.36)	0.39 (0.91)	0.78 (0.69)	1.00 (1.00)				
NH ₄	-1.00 (-0.98)	-0.97 (-0.64)	-0.97 (-0.99)	-0.98 (-0.52)	0.38 (-0.71)	-0.20 (-0.23)	1.00 (1.00)			
SiO ₄	-0.98 (-1.00)	-0.99 (-0.59)	-0.97 (-1.00)	-0.93 (-0.58)	0.45 (-0.72)	-0.08 (-0.35)	0.99 (0.99)	1.00 (1.00)		
NS	0.85* (-)	0.83* (-)	0.69 (-)	0.79 (-)	-0.71 (-)	-0.11 (-)	-0.84* (-)	-0.81* (-)	1.00 (-)	
GW	0.47 (0.54)	0.25 (0.54)	0.54 (0.55)	0.64 (0.12)	0.58 (0.02)	0.96* (0.25)	-0.47 (-0.47)	-0.35 (-0.57)	0.16 (-)	1.00 (1.00)
GD	-0.99* (-1.00*)	-0.96* (-0.51)	-0.99* (-0.99*)	-0.97* (-0.65)	0.28 (-0.78)	-0.26 (-0.42)	0.99* (0.97*)	0.98* (0.99*)	-0.76 (-)	-0.52 (-0.54)

*Significance at p<0.05

WT=water temperature (°C); DO=dissolved oxygen (mg L⁻¹); Sal=salinity (PSU); PO₄=phosphate (μM L⁻¹); NO₂=nitrite (μM L⁻¹); NO₃=nitrate (μM L⁻¹); NH₄=ammonia (μM L⁻¹); SiO₄=silicate (μM L⁻¹); NS=*Noctiluca scintillans* (no. x10³ cells L⁻¹); GW=*Gymnodinium wulfii* (no. x10³ cells L⁻¹); and GD= *Guinardia delicatula* (no. x10³ cells L⁻¹); (-)=NS absent in near-bottom water.

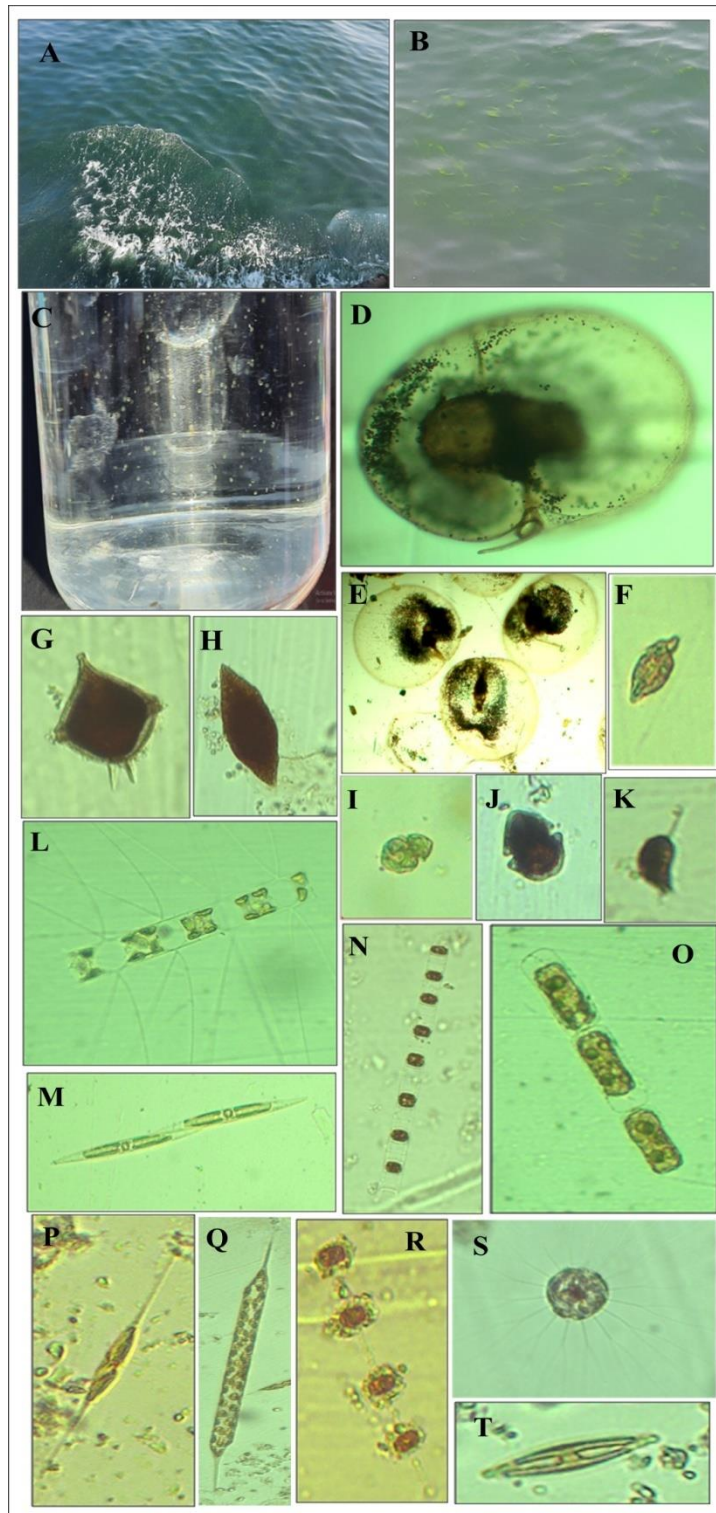


Plate 1. A&B- bloom trail, C- *Noctiluca scintillans* cells in filtering flask, D&E- *Noctiluca scintillans* under microscope, F- *Oxytoxum* sp., G- *Protoperidinium* sp., H- *Gyrodinium* sp., I- *Gymnodinium wulfii*, J- *Alexandrium* sp., K- *Plagioselmis* sp., L- *Chaetoceros* sp., M- *Pseudo-nitzschia* sp., N- *Skeletonema* sp., O- *Guinardia delicatula*, P- *Cylindrotheca* sp., Q- *Rhizosolenia* sp., R- *Thalassiosira* sp., S- *Bacteriastrum* sp., T- *Navicula* sp.