

Effect of environmental perturbations on the occurrence of phytoplankton blooms during south west monsoon in a tropical bay

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Abstract

Arabian Sea adjoining the west-coast of India is a unique tropical habitat influenced by south-west monsoon. This phenomenon brings in characteristic seasonal changes in the abiotic and biotic factors. The phytoplankton communities, which are heterogenous in form and function, are influenced by such environmental changes. In order to elucidate the influence of environmental perturbations on phytoplankton bloom during monsoon observations at 24h intervals were carried out during the 2008 monsoon-season. This paper provides insights to phytoplankton bloom formation based on the observations of live phytoplankton cells using FlowCAM first of its kind information from the region. Six phytoplankton bloom situation were encountered and each of them differed in characteristics. The signals of the blooms corresponded positively with chlorophyll fluorescence and it could be ascertained that the blooms could last for a maximum period of 6-days. The occurrence of blooms also coincided with increase in transparency (>50 cm) or reduced turbidity. At the onset of the monsoon the first bloom noticed was dominated by nano+pico-phytoplankton whereas in subsequent ones micro-phytoplankton dominated the blooms. We observed that cyclicity and characteristic in the intra-seasonal phytoplankton blooms is governed by the intensity and length of perturbations and eventually play an important role in trophic dynamics.

Key words: tropical bay, phytoplankton, diatoms, dinoflagellates, blooms, monsoon

1. Introduction

In recent years concerned with the global increase in harmful algal blooms/red tide phenomenon, studies related to dynamics of phytoplankton population have gained importance. Phytoplankton abundance and species composition in an estuarine ecosystem are closely linked to various physical (advection, light, temperature salinity, etc), chemical (pH, nutrients) and biological (grazing) factors as well as interactions among them. Hence the continual documentation of phytoplankton population dynamics along with relevant environmental variables can offer important information on functioning of the ecosystem. Phytoplankton community, a source of organic carbon and energy for higher trophic levels, ultimately determines the success of fisheries. The understanding of phytoplankton dynamics is, therefore, central to the understanding of how estuarine ecosystems work and how they respond to environmental stresses imposed by natural and anthropogenic activities (Cloern, 1979).

Until recently, in the algal bloom monitoring programs, phytoplankton analysis is traditionally based upon cell counts using microscopy. However, microscope counting requires fixation, and available techniques for optimal preservation and enumeration of phytoplankton are taxon-specific. This consideration implies that, for accurate enumeration of the whole phytoplankton, counting of the live samples will be the ideal solution.

Dona Paula bay, located along the west-coast of India, Goa, is a dynamic aquatic ecosystem exhibiting a strong seasonal gradient, both in environmental variables and plankton assemblages, because of the tight physico-chemical and biological coupling. This area is strongly influenced by the southwest monsoon during June to September and the changes associated with its onset have marked effects on the phytoplankton community, food-web and production (Bhattathiri et al., 1976; Devassy and Goes, 1988). Earlier observations based on low resolution sampling (monthly observations) indicated that the oscillations in freshwater discharge

during monsoon have a bearing on the nature of phytoplankton blooms (Patil and Anil, 2008). In order to strengthen the observation on phytoplankton community and relating the changes to oscillations in monsoon related events we collected phytoplankton samples along with related environmental parameters at every 24 hour intervals from a fixed station, Dona Paula, Goa (Fig.1). This paper presents information relevant to the period starting from May 28, 2008 to September 30, 2008 covering the events during one south-west monsoon. The observations on phytoplankton were carried out through FlowCAM on live samples, first of its kind from the region and presents the intra-monsoon season phytoplankton dynamics.

2. Materials and Methods

2.1. Study Site

Surface water samples were collected from 28th May 2008 to 30th September 2008 in the Dona Paula Bay at a fixed location (Longitude: 73.00⁰59'E; Latitude: 15.00⁰25'N). This sampling covers the events during one south west monsoon (onset of the monsoon to the end of the monsoon).

2.2. Meteorological parameters

Data regarding meteorological parameters like solar radiation (MW cm^{-2}), wind speed (m sec^{-1}), wind direction (degrees), air temperature ($^{\circ}\text{C}$), relative humidity (%) and atmospheric pressure (mbar) were obtained from AWS, NIO, Goa. The rainfall (mm) and sunshine hours [the time of brightness (solar radiation $> 1 \text{ mw cm}^{-2}$) available in a day length] data were obtained from the Indian Meteorological Department, Panaji, Goa. For interpretation, daily average values have been used.

2.3. Water Sampling

Water samples were collected from surface using bucket. Temperature of the collected water sample was measured immediately at the study site. The depth of the light penetration was measured using secchi disc at the sampling station. Salinity samples were analyzed by Autosol. Dissolved oxygen and nutrients (NH_4 , $\text{NO}_3 - \text{N}$, $\text{NO}_2 - \text{N}$, $\text{PO}_4 - \text{P}$, and SiO_3) were analyzed by following standard procedures. Nutrients were mostly analyzed once in three days. The oxygen-saturation was calculated using temperature, salinity and dissolved oxygen values (Benson and Crause, 1984). Turbidity of the samples was measured in the laboratory using Turner designs, Trilogy equipped with a turbidity module.

*2.4. Chlorophyll *a*, Size fractionation of chlorophyll fluorescence and quantum yield*

A known volume of sea water was filtered through GF/F Whatman filter paper, and the filter papers were immediately used for chlorophyll *a* analysis by 90% acetone extraction method. The fluorescence readings of the extracted chlorophyll were taken in a Trilogy (Turner designs), equipped with a chlorophyll module. The same module was further used for the determination of size fractionated chlorophyll fluorescence and DCMU based quantum yield. Phytoplankton size fractionation (nanoplankton + picophytoplankton and microphytoplankton) was measured through *in vivo* chlorophyll fluorescence measurements of the sea water samples (total fluorescence, tF) as well as the samples filtered through 20μ mesh (Nano and picoplankton; $nan+picoF$) using Trilogy. The difference in the fluorescence readings would give the fluorescence of microphytoplankton ($mF=tF-nan+picoF$). *In-vivo* chlorophyll fluorescence with (F_{DCMU}) and without (IVF) the addition of DCMU was measured daily to measure quantum efficiency. DCMU (<1% in ethanol) was added to a final concentration of $\sim 20\mu\text{M}$ to the dark-adapted samples. Fluorescence values were adjusted for background fluorescence (determined

from GF/F filtered sea water) and used to calculate the quantum efficiency ($F_v/F_m = [(F_{DCMU} - IVF) / F_{DCMU}]$).

2.5. FlowCAM analysis

Phytoplankton composition was analyzed live using FlowCAM following a standard procedure. The samples were analyzed in auto-image mode, in which the flow sample stream is sampled regularly by the imaging system. Therefore, every particle, including planktons, was counted and imaged. For each sample, a maximum of either 106 particles or 2-5 ml samples were analyzed in duplicates. A 10x objective and 100 micron flow cell was used in the sample analysis and the instrument was factory calibrated. Before loading the sample the sea water sample was prefiltered and raw fluorescence values were measured using Triology equipped with chlorophyll a module. If the fluorescence values of the sea water sample and the 100 micron prefilter sample was same then the samples were analyzed using 10 x objective and 100 micron flowcell and if the fluorescence values of the the total sample was greater than 100 micron filtered sample then 4x and 300 micron flow cell was used to analyzed the sample for greater than 100 micron particles. Invalid images, bubbles, repeated images were removed from the database. The identification of phytoplankton species were carried out based on the identification keys (Hasle and Syvertsen, 1997; Round et al., 1990; Desikachary, 1987; Subrahmanyam, 1946; 1959).

2.6. Spearman's rank correlation test

Spearman's rank correlation test was performed to evaluate the relationships between the dominant groups (total phytoplankton, diatoms and dinoflagellates) and different size fractions with various observed environmental parameters that may be responsible for regulating their

population. This test was performed using the software Statistica release 5.0 by Statsoft, after transformation of only biological data i.e. fourth root ($\sqrt[4]{}$).

3. Results

3.1. Hydrographic conditions

Surface seawater temperature ranged from a maximum of 32 °C to a minimum of 25.8 °C (Fig. 2A). The sea water temperature was >30 °C before the onset of the monsoon and <30 °C during rest of the monsoon period. Low temperature coincided with low saline waters (<30). Salinity ranged from 2 - 35.4 (Fig. 2A). During the early phase of monsoon salinity level decreased gradually and there after oscillations in salinity was observed. Low salinity in the surface layer was due to freshwater influx from the Zuari River as well as precipitation during the monsoon period.

The DO concentrations showed large variations during the study period ranging from a high of 7.26 ml L⁻¹ to a low of 3.42 ml L⁻¹ (Fig. 2C). The high DO concentrations could be due to air sea gaseous exchange and the primary production. The oxygen saturation ranged from a high of 106.5% to a low of 52.1% (Fig. 2C). The high oxygen saturation indicates the production is more dominant than respiration. The variations in the oxygen saturation indicates that for most of the period respiration is more dominant than the production except only a couple of occasions where either production is dominant than the respiration or both processes are almost equal.

The measurement of water transparency using secchi disc and turbidity module revealed significant variations. Secchi disc depth and turbidity measurements ranged from 285 cm to 23.4 cms and 22170 to 154 (au) respectively. Sechi disc depth measurements and turbidity measurements showed a significant inverse relationship. Water transparency was low during the early to mid phase of the season and as the season progressed the transparency level increased.

Such fluctuation in water transparency was due to benthic resuspension caused by water movements such as freshwater influx and strong waves.

The variations in nutrient concentration (NH_4 , $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{PO}_4\text{-P}$, and SiO_3) are shown in figure. The results showed that the nutrient inputs into the study site were in pulses. These nutrient pulses were coincided with low salinity indicating the influx of nutrients from the Zuari River. On some occasions the high nutrients were also associated with high rainfall indicating influx of nutrients through precipitation. Maximum nutrient ($\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$) concentrations were found during the early phase of the monsoon. The variations in $\text{NO}_2\text{-N}$, and NH_4 followed a similar trend. NH_4 ranged from $0.10\ \mu\text{M}$ to $24.61\mu\text{M}$, $\text{NO}_3\text{-N}$, ranged from $0.07\ \mu\text{M}$ to $22.51\mu\text{M}$, $\text{NO}_2\text{-N}$, ranged from $0.02\ \mu\text{M}$ to $0.97\ \mu\text{M}$, $\text{PO}_4\text{-P}$ ranged from $0.39\mu\text{M}$ to $3.96\ \mu\text{M}$ and SiO_3 ranged from $8.06\ \mu\text{M}$ to $82.67\ \mu\text{M}$ (Fig. 3).

3.1. Meteorological conditions

Temporal variations in meteorological parameters presented in Fig. 4 showed large variations. During the monsoon period the study region experiences high wind speed, low solar radiation and sun shine hours due to cloud cover and receives maximum rainfall. The amount of rainfall received during the study period ranged up to 136 mm (Fig. 4). Maximum rainfall was observed on three occasions and started decreasing as the season was ending (Fig. 4). During this period wind speed ranged up to $18\ \text{ms}^{-1}$. Maximum wind speed was during early phase of the monsoon and was decreasing as the season progressed. Solar radiation and sunshine hours followed a similar trend.

3.3. Chlorophyll a, Size fractionation of chlorophyll fluorescence and quantum yield

Larger variations in Chlorophyll 'a' fluorescence were observed during the monsoon period. The chlorophyll fluorescence ranged from a high of 5153 (au) to a low of 322 (au). Chlorophyll fluorescence revealed six peaks indicating phytoplankton blooms (Fig. 5A). Fluorescence data indicated that the maximum length of the bloom to be six days. In addition to the high peaks, small peaks in chlorophyll concentrations were also observed. Size fraction of the phytoplankton fluorescence reveals that the contribution of $<20\ \mu$ (nano- and pico-phytoplankton) and >100 (micron) to the chlorophyll pool ranged from 5% to 99% and 1% to 95% respectively (Fig. 5B). During bloom periods (except first bloom) the contribution of micro-phytoplankton to the chlorophyll pool was maximum ranging from 60-90%.

During most of the period quantum yield remained >0.3 although there were oscillations indicating that the phytoplankton were not under severe stress. The phytoplankton quantum yield (F_v/F_m) ranged from a high of 0.72 to a low of <0.3 (Fig. 5A). Quantum yield was high during the bloom period and the low quantum yield was encountered under very low saline and high turbid conditions. Maximum quantum yield observed during the bloom period decreased with decline in the bloom.

3.4. Phytoplankton analysis from FlowCAM

Daily variations in the total phytoplankton abundance are shown in Fig. 6. The phytoplankton densities ranged from a high of 1216×10^3 cells L^{-1} to a low of 0.3×10^3 cells L^{-1} (Fig. 6A). The phytoplankton densities observed during six chlorophyll peaks (bloom periods) ranged from 0.4×10^3 cells L^{-1} to 1216×10^3 cells L^{-1} (Table 1). The maximum phytoplankton densities were observed during later stages of the monsoon period. Among the phytoplankton community, diatoms ranged up to 1214×10^3 cells L^{-1} , dinoflagellates ranged up to 10×10^3 cells L^{-1} and others (phytoplankton) ranged up to 8×10^3 cells L^{-1} (Fig. 6B-D). On an average diatoms

(82%) followed by dinoflagellates (10%) and others (10%) dominated the total phytoplankton community (Fig. 6E).

The first bloom was observed during 31st May to 4th June. The FlowCAM analysis revealed the dominance of the *Navicula* and *Thalassionema*. However the cell densities were not so high enough to be contributing to the chlorophyll pool. The size fractionation analysis revealed that the chlorophyll peak was due to abundance of <20µ phytoplankton (nano- and pico-phytoplankton). The second bloom (Bloom 2) was observed from 24th – 28th June. This bloom was due to the dominance of micro-phytoplankton such as *Ditylum*, *Odontella*, *Leptocylindrus* and *Thalassionema*. The variation in the phytoplankton abundance coincided with the fluctuations in salinity. The third bloom observed on 14th July was due to the dominance of single species i.e. *Skeletonema*. The fourth bloom was observed from 20th – 23rd August. This bloom was caused by *Asterionella*, *Bacteriastrum*, *Chaetoceros*, *Ditylum*, *Fragilariopsis*, *Leptocylindrus*, *Pseudonitzschia*, *Skeletonema*, *Thalassionema* and Flagellates. The fifth and sixth bloom observed from 1st – 6th and 28th – 30th September was also dominated by multi-species as listed above (except *Asterionella*).

3.5. Relationship between environmental variables and chlorophyll fluorescence, total phytoplankton abundance, dominant phytoplankton groups (diatom and dinoflagellates) and quantum efficiency.

The results of Spearman's rank correlation test are presented in Table 1. The chlorophyll fluorescence showed a significant positive correlation with salinity, sechi disc depth and negative correlations turbidity, rainfall, wind speed, nitrate, phosphate, N:P ratio and silicate. The abundance total phytoplankton, diatoms and dinoflagellates showed a significant positive correlation with sechi disc depth, solar radiation, sunshine hours and significant negative correlations turbidity, rainfall, wind speed, ammonia, nitrate, phosphate and N:P ratio.

Dinoflagellate abundance also showed significant negative correlations with nitrite and silicate. The f_v/f_m which is an indicator of stress showed significant positive correlation with temperature, salinity, dissolved oxygen, sechi disc depth, solar radiation, sunshine hours and significant negative correlations turbidity, rainfall, wind speed, all the nutrients (ammonia, nitrate, phosphate, silicate) and N:P ratio and

4. Discussion

During monsoon season Dona Paula Bay experiences large variations in physico-chemical conditions. During these period surplus amounts of river discharge from the Zuari River, as well as precipitation is added to the bay results in marked changes in the physico-chemical nature of the water (Qasim and SenGupta, 1981). However the freshwater discharge and precipitation are not continuous. The oscillations in freshwater discharge and precipitation resulted in large variations in the salinity, water transparency and nutrient influx. In this study the frequencies of oscillations were large and the duration for each oscillation varied (few to several days). Such drastic changes in hydrodynamic characteristics bring in the complete transformation of the phytoplankton community structure and production (Devassy and Goes, 1988; Bhattathiri et al. 1976).

Earlier studies through low resolution sampling have revealed contrasting results on phytoplankton. The work carried out earlier (Devassy and Goes, 1988; Krishnakumari et. al., 2002) who worked in the same system, slightly away from the present station, revealed the lowest cell counts/abundance during monsoon season in the year 1980 and 1998. They reported that poor growth conditions (like lowered light and salinity regimes caused by monsoon events) and the absence of species, which can bloom under such conditions, are the cause for the low counts. Whereas, some studies reported the *Skeletonema* blooms during monsoon under low

saline condition in the same system as well as elsewhere along the west coast of India (Subrahmanyam, 1959; Gopinathan, 1974; Mitbavkar and Anil, Patil and Anil, 2005; Patil and Anil 2008). Patil and Anil (2008) also reported a mixed species blooms of diatoms and harmful dinoflagellates during break period in monsoon. Such contrasting results indicated there could be lot more information (eg. number of bloom events, duration of the bloom, bloom causing species and the environmental factors causing blooms) available in such a dynamic season which can be captured only through high resolution sampling.

Through daily observations we found six phytoplankton blooms, resulting in high chlorophyll values, caused by different species under different environmental settings (Figs. 2, 5 and 6). These blooms were observed during different time scales of the monsoon. Based on fluorescence data it is possible to perceive the maximum length of a bloom to be 6 days. It was also found that the magnitude of last three blooms was much larger than the first three blooms. The first and last (6th) bloom was mainly due to the dominance of nano- + pico-phytoplankton (<20 μ) whereas, the rest of the blooms were mainly due to the micro-phytoplankton (>20 μ). These blooms were observed under different salinity regimes: saline (>30; 1st and 6th bloom), moderately low saline (20-30; 2nd and 5th bloom) and low saline (14-20; 3rd and 4th bloom). Mostly the species (*Asterionella*, *Bacteriastrum*, *Chaetoceros*, *Ditylum*, *Fragilariopsis*, *Leptocylindrus*, *Pseudonitzschia*, *Skeletonema*, *Thalassionema*) belonging to diatom group were responsible for the blooms (single as well as multispecies).

Spearman's rank correlation test has revealed that the salinity, rainfall, wind speed, water transparency (secchi disc depth and turbidity), light and nutrients are the major factors influencing the phytoplankton composition (Table 2). Interestingly all the blooms coincided with increase in water transparency (or reduced turbidity) and nutrient influx. The secchi disc

depth measurements and the phytoplankton and chlorophyll data revealed that the secchi disc depth of at least 50 cm is essential for bloom formation in nutrient rich waters. In all the six cases the decline in the bloom was associated with the decline in the nutrients especially nitrate indicating the utilization of nutrients by the bloom. The ratio of maximum and minimum fluorescence (F_v/F_m ; quantum yield) was high and decrease gradually with the decline in the bloom. Such decline in blooms ensuing nitrate depletion and salinity change might result in an increased abundance of benthic propagules and fugitive cells (Patil and Anil, 2008) which can act a seed source for subsequent blooms. Benthic propagules repopulate waters if resuspended and exposed to suitable light, temperature and nutrients (McQuoid et al., 2002). Since the study side is shallow, freshwater discharge, which is rich in nutrients, can cause resuspension of diatom benthic propagules. The resuspended benthic propagules might then seed the subsequent blooms. Probably this process could be the reason for the occurrence of multi-species (*Bacteriastrum*, *Chaetoceros*, *Ditylum*, *Fragilariopsis*, *Leptocylindrus*, *Pseudonitzschia*, *Skeletonema*, *Thalassionema*) bloom thrice on different occasions (August 20 to 23, September 1-6 and 28-30). These three blooms were observed under different salinity regimes (15-20, 27-32 and 28-30) because of euryhaline characteristics of the organisms. Phytoplankton blooms were not observed when the water transparency (sechi disc depth <50 cms) and salinities (<14) are very low. The F_v/F_m was low under these conditions indicating low photochemical efficiency.

Dinoflagellates, forming the next dominant group, are also influenced by salinity, rainfall, wind speed, water transparency (secchi disc depth and turbidity), light and nutrients are the major factors influencing the phytoplankton composition. In this investigation the dinoflagellate blooms were not observed even though their contribution to the total

phytoplankton was more than diatoms on certain occasions. In this study, even though harmful dinoflagellates (eg. *Gymnodinium* and *Cochlodinium*) are reported, their blooms were not observed as seen in the earlier study. In the earlier study the break period (July 2000) in monsoon coincided with bloom of these organisms under high-saline, nutrient-poor and transparent water-column (sechi disc depth - 245 cm) (Patil and Anil, 2008). However such conditions did not prevail during this study indicating that inter annual variations and monsoon influenced perturbations to play an important role. To elucidate such missing links further long term studies are essential and are underway.

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Table 1. Results of Spearman's rank correlation test between 16 environmental variables and chlorophyll fluorescence, total phytoplankton abundance, dominant phytoplankton groups (diatom and dinoflagellates) and quantum efficiency. Only the combinations with significance level >95% are shown in bold.

Parameters	Chl. fluorescence		Diatoms		Dinoflagellates		Total		Quantum efficiency	
	R	p	R	p	R	p	R	p	R	p
Temperature	0.13	0.141	0.12	0.202	0.05	0.581	0.05	0.555	0.46	0.000
Salinity	0.32	0.000	0.07	0.442	0.07	0.419	0.04	0.648	0.32	0.003
Secchi disc depth	0.31	0.001	0.52	0.000	0.52	0.000	0.56	0.000	0.65	0.000
Turbidity	-0.27	0.003	-0.54	0.000	-0.56	0.000	-0.56	0.000	-0.65	0.000
Dissolved oxygen	0.14	0.120	0.13	0.142	0.03	0.767	0.11	0.234	0.24	0.032
Oxygen saturation	-0.04	0.635	0.02	0.833	-0.05	0.572	0.05	0.593	-0.01	0.947
Rainfall	-0.24	0.007	-0.27	0.003	-0.30	0.001	-0.23	0.011	-0.37	0.001
Wind speed	-0.33	0.000	-0.41	0.000	-0.42	0.000	-0.44	0.000	-0.41	0.000
Solar radiation	0.14	0.116	0.32	0.000	0.30	0.001	0.29	0.001	0.29	0.008
Sunshine hours	0.14	0.127	0.31	0.001	0.25	0.006	0.26	0.004	0.29	0.008
Ammonia	-0.26	0.099	-0.43	0.006	-0.48	0.002	-0.40	0.010	-0.44	0.005
Nitrate	-0.57	0.000	-0.41	0.008	-0.55	0.000	-0.55	0.000	-0.61	0.000
Nitrite	-0.24	0.140	-0.25	0.116	-0.54	0.000	-0.27	0.088	-0.36	0.022
Phosphate	-0.34	0.032	-0.44	0.004	-0.69	0.000	-0.52	0.001	-0.49	0.001
Silicate	-0.29	0.067	-0.08	0.614	-0.34	0.031	-0.14	0.376	-0.39	0.013
N:P	-0.48	0.002	-0.43	0.006	-0.32	0.044	-0.38	0.017	-0.54	0.000

Legends to the figure

Fig.1. Geographical location of the time series station

Fig.2 Daily variations in the water parameters. A) Temperature and Salinity, B) Secchi disc depth (cm.) and Turbidity (arbitrary units), and C) Dissolved oxygen and Oxygen saturation. Shaded region represents chlorophyll peaks

Fig.3 Daily variations in the dissolved nutrients. A) Ammonia, B) Nitrate ($\text{NO}_3\text{-N}$), C) Nitrite ($\text{NO}_2\text{-N}$) D) Phosphate (PO_4) and E) Silicate (SiO_3). Shaded region represents chlorophyll peaks

Fig.4 Daily variations in the meteorological parameters. A) Rainfall, B) Wind speed, C) Solar radiation and sun shine hours, D) Air temperature and Relative Humidity and E) Atmospheric pressure. Shaded region represents chlorophyll peaks

Fig.5 Daily variations in the A) Chlorophyll fluorescence (arbitrary units) and DCMU based quantum yield, and B) Percentage composition of different phytoplankton size fraction. Shaded region represents chlorophyll peaks

Fig.6 Daily variations in the abundance of (A) total phytoplankton, (B) diatoms, (C) dinoflagellate, (D) other phytoplankton and (E) their percentage composition. Shaded region represents blooms and numerical indicates number of blooms

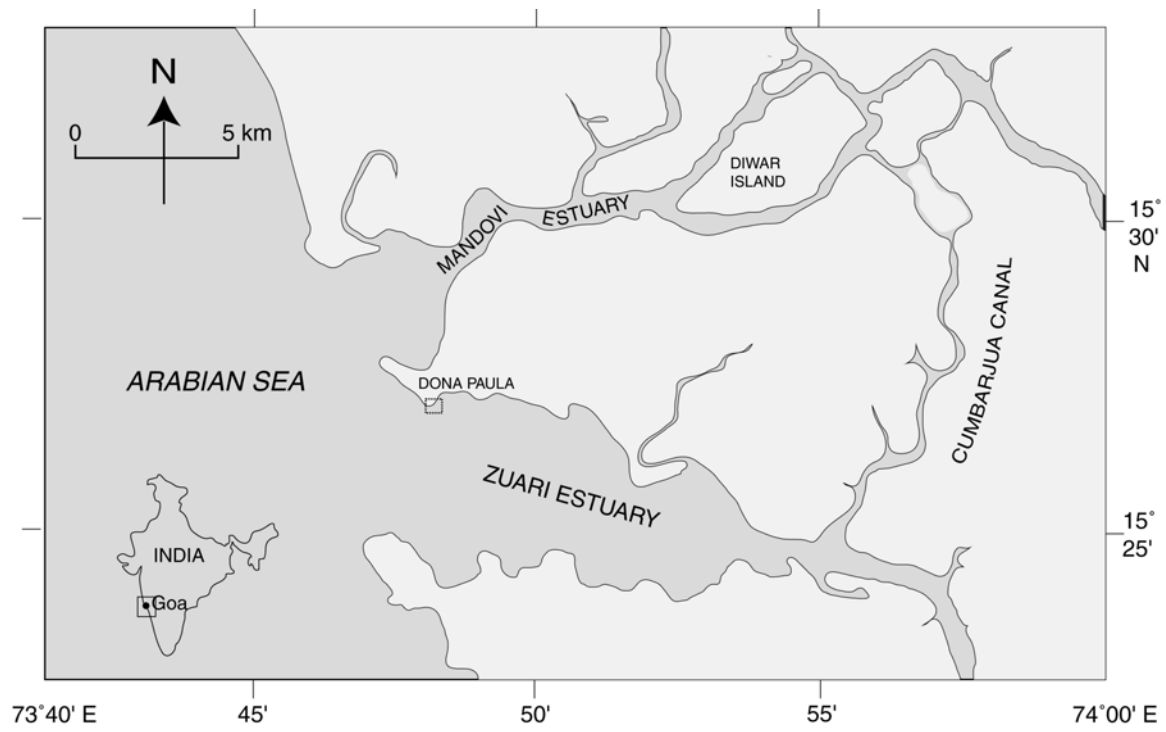


Fig. 1

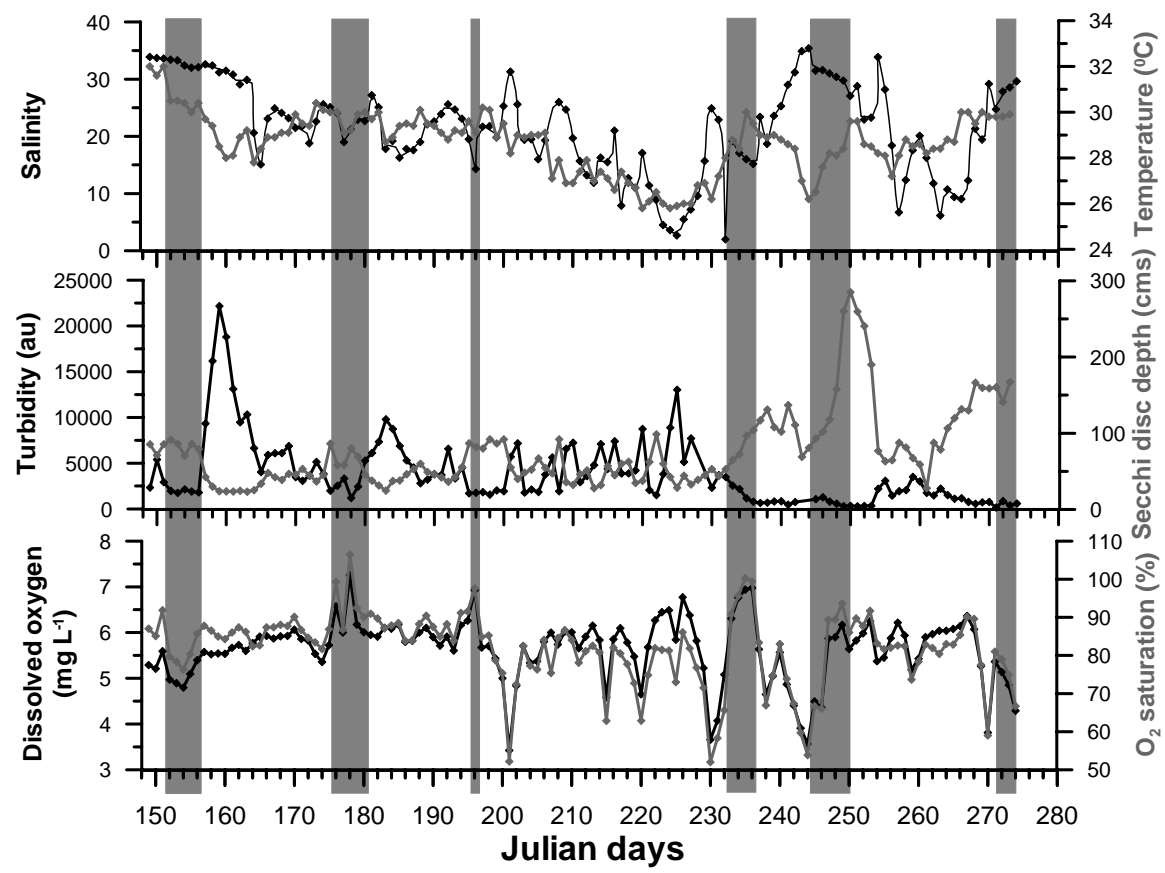


Fig. 2

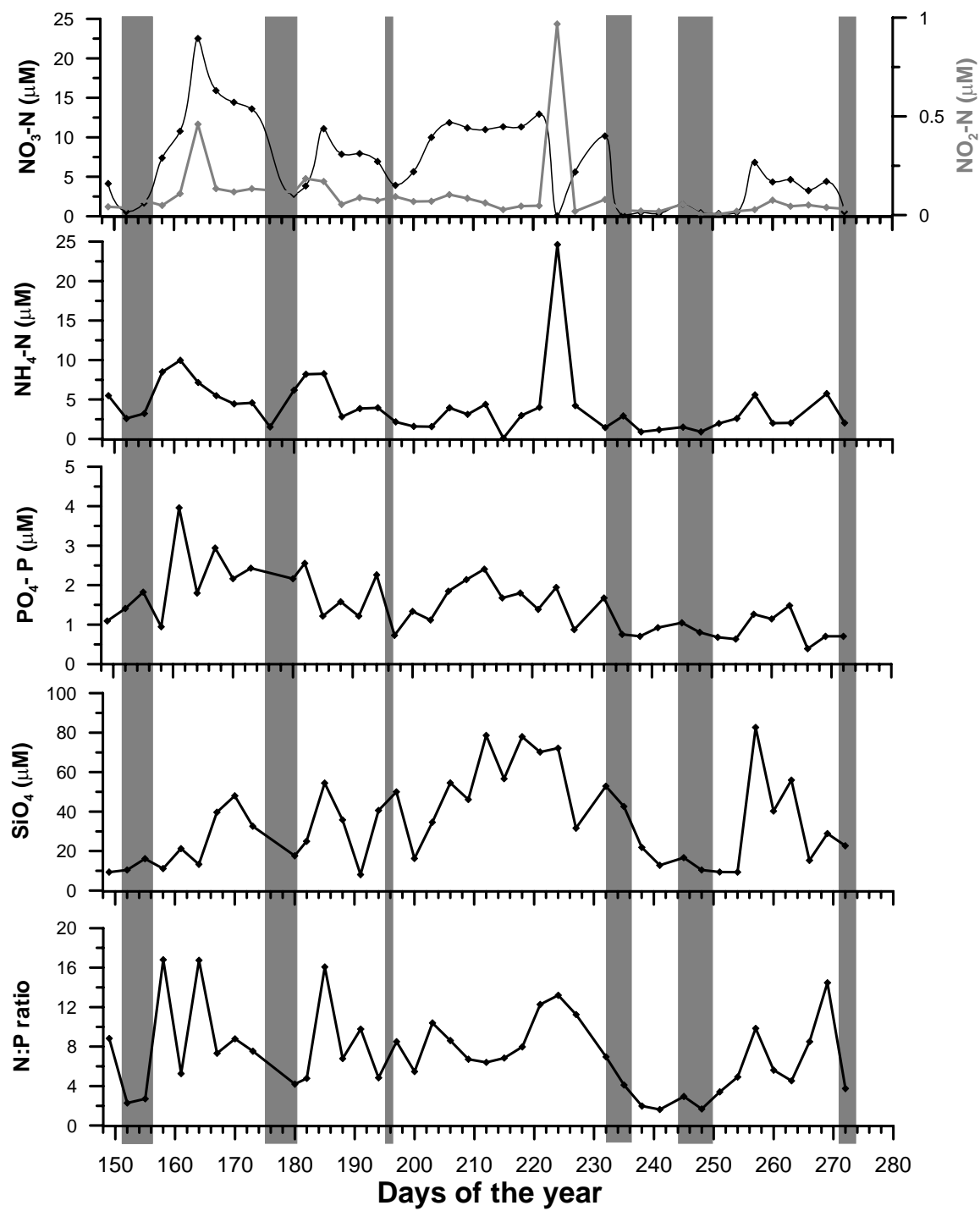


Fig. 3

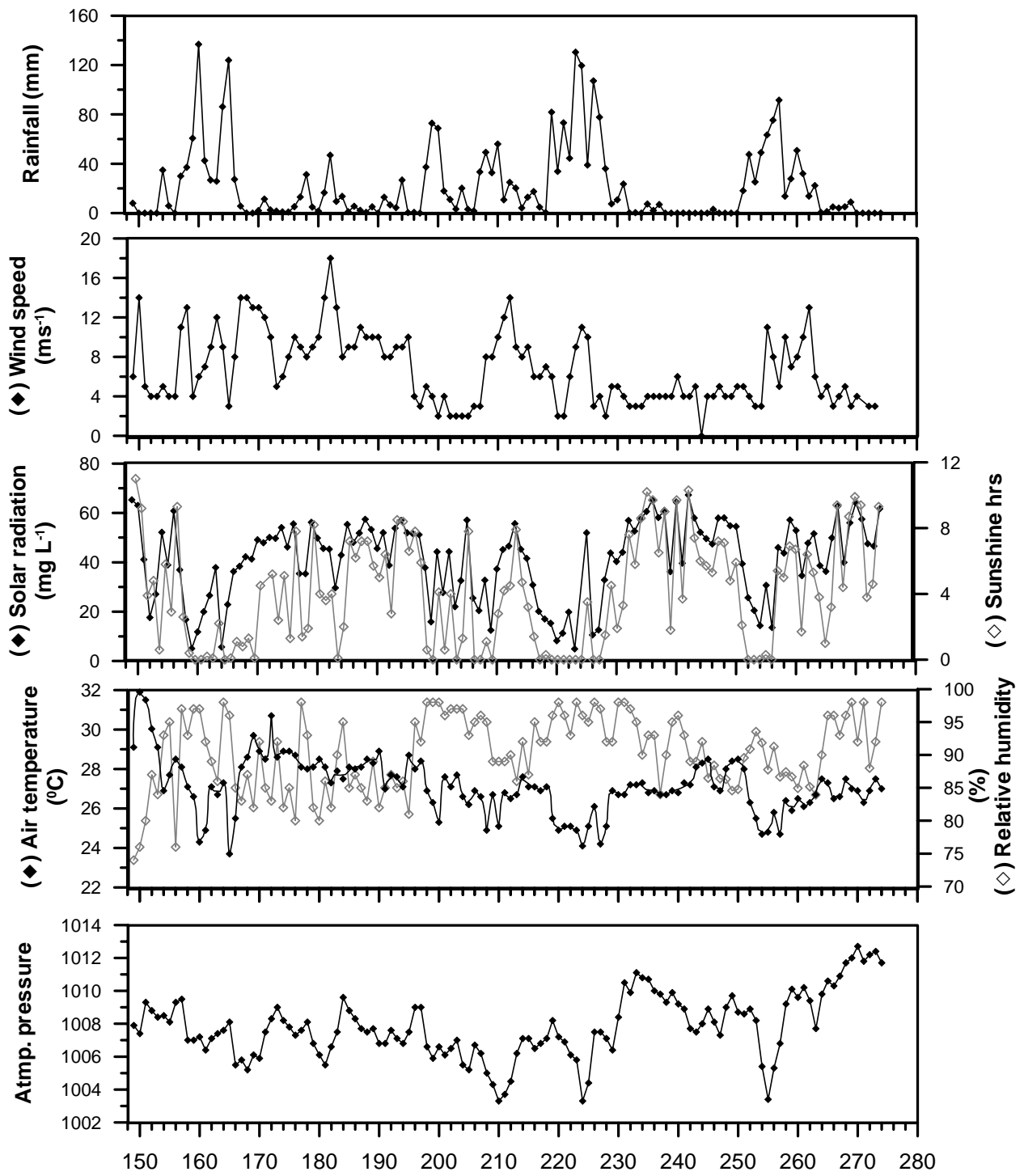


Fig. 4

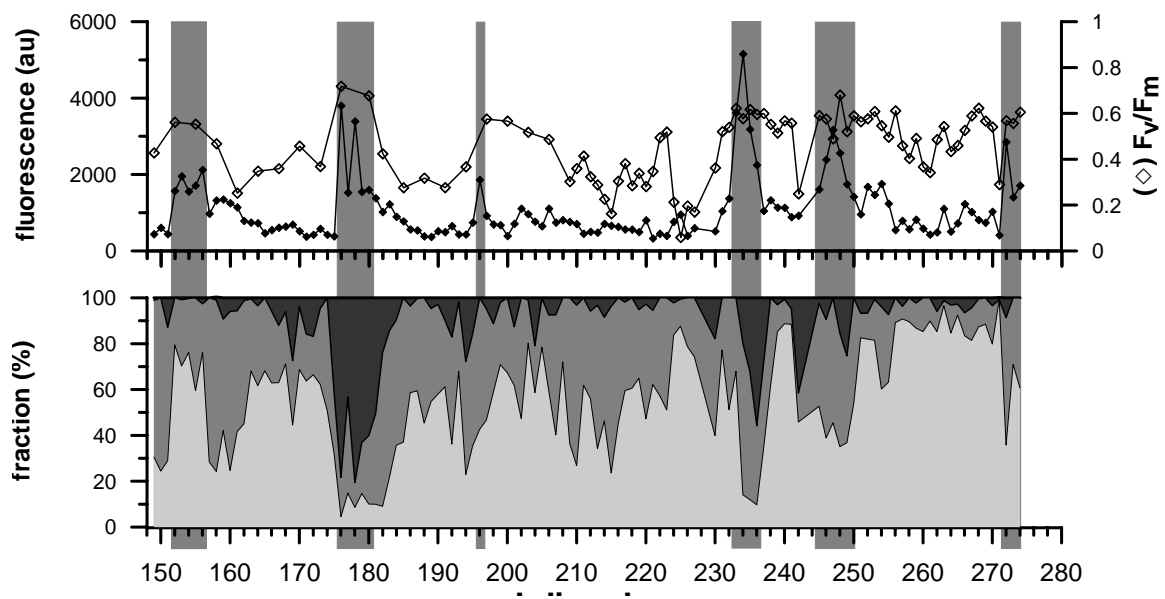


Fig. 5

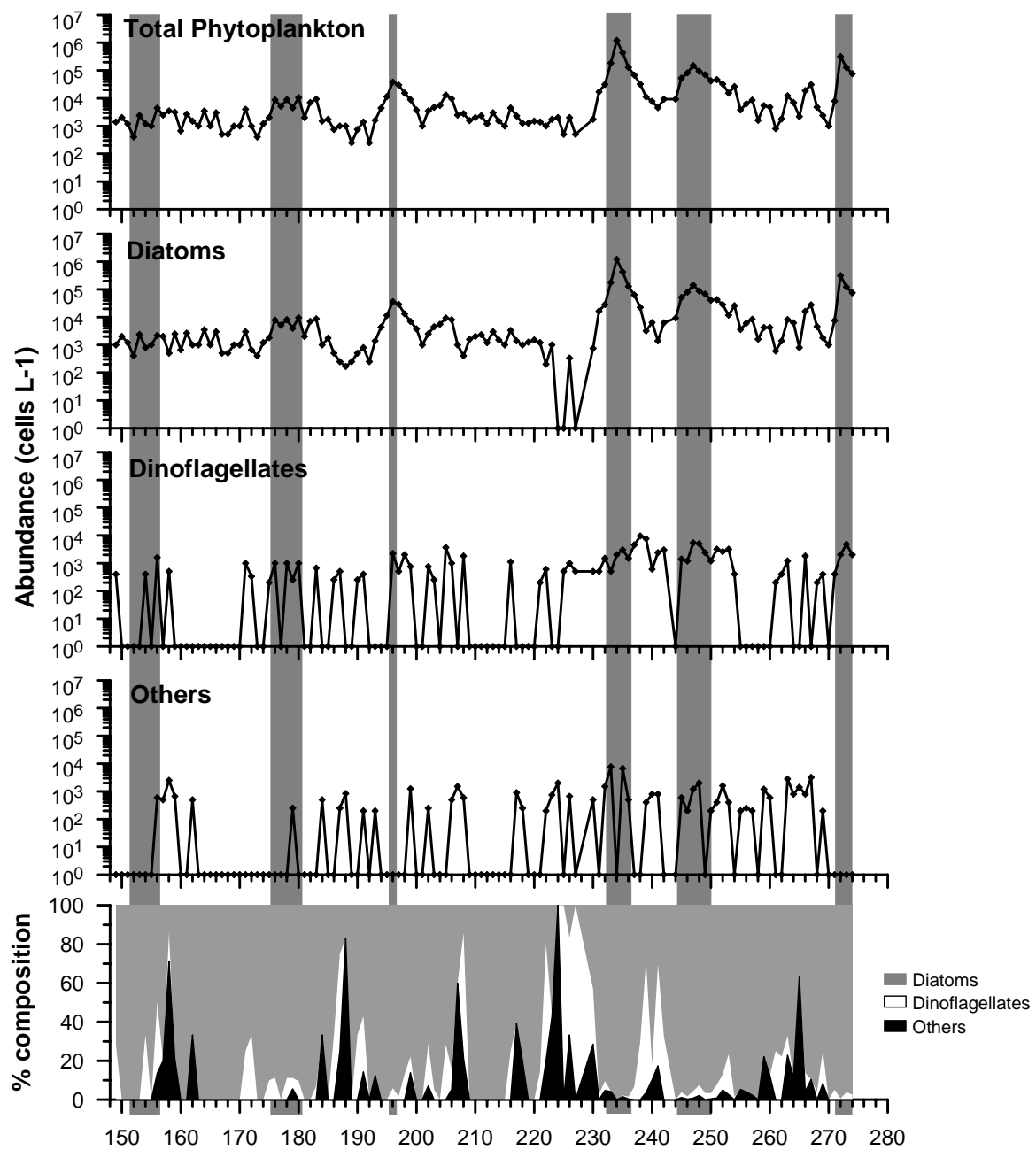


Fig. 6

