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Response of phytoplankton to nutrient enrichment with high growth rates in a tropical monsoonal estuary - Zuari estuary, India

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Present study is about the impact of macronutrient enrichment on phytoplankton biomass and community structure in a tropical monsoonal estuary. *In situ* experiments carried out during the pre-monsoon period (February-March 2006), showed that the response time of phytoplankton to nutrient enhancement was 24-32 h. Phytoplankton biomass increased sizably, indicating nitrate and silicate limitation for phytoplankton growth. An increase in 23µg chl *a* l^{-1} resulted in an uptake of 10µM nitrate, 0.6µM phosphate and 17µM silicate. Phytoplankton showed high growth rates with an average value of 1.36µg chl l^{-1} d^{-1}.This phytoplankton community was largely dominated by diatoms (>96%), particularly chain forming species. Relative preference index (RPI) value for nitrate was >1, suggesting that, irrespective of the ambient ammonium concentration, estuarine autotrophs preferred nitrate. Few species like *Skeletonema costatum and Thalassionema nitzschioides* exhibited the ability to withstand hypoxic condition.

[**Keywords:** Zuari estuary, Premonsoon, Nutrient uptake, Phytoplankton, Hypoxic]

Introduction

Phytoplanktons are responsible for nearly half of global primary production[1]. Diatoms, dinoflagellates, cyanobacteria and coccolithophores are among the most important groups of phytoplankton in the world ocean. A substantial proportion of the coasts include highly productive estuaries[2]. These estuaries, besides supporting a wide variety of animals and plants, act as an important linkage and buffer zone between the ocean and land. They are also sites of high rates of production of organic matter, which not only sustain a secondary food chain internally, but also influence biological productivity of adjacent coastal water in turn sustaining fisheries[2,3]. The extent to which estuaries exchange dissolved and particulate nutrients with adjacent marine ecosystems depends upon several factors, including geomorphology, tidal regime, climate and fresh water inputs. Light and nutrients are the primary factors regulating phytoplankton growth[4,5] followed by temperature and salinity[6]. Major (macro) nutrients essential for plant growth are nitrogen, phosphorous and silicon[7]. Phytoplankton preference for reduced N compounds, primarily as ammonium and urea, is an almost universal phenomenon in marine systems, including estuaries[8,9,10]. Nutrient availability in an estuary is strongly influenced by freshwater flow (river runoff and ground water inputs), atmospheric deposition and exchange with the open ocean. Fixation of dinitrogen is yet another phenomenon of ecological significance known to naturally fertilize tropical waters[11]. In stratified waters, phytoplankton production is primarily enhanced by nitrate supply i.e. new production[12], to the euphotic zone during the active stages of upwelling[13] but, as this supply decreases during the relaxation of upwelling, production is mainly supported by regenerated forms of nitrogen such as ammonium and urea[14,15]. Therefore, phytoplankton must be able to adapt to the changing physical and chemical conditions in these areas[16,17]. This is achieved by storing nitrogen (N) compounds in intracellular pools during periods of N excess, when luxury uptake exceeds growth rates, allowing for the continuation of growth after depletion of external nutrients[18].

In many coastal waters, increasing eutrophication, due to human activities has greatly perturbed the phytoplankton community. With an overwhelming

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majority of the human population living in the coastal zone and with runoff from the continents funneling through estuaries and continental margins, coastal systems are among the most heavily anthropologically impacted ecosystems on the globe. The consequences of eutrophication can only be minimized by identifying the specific nutrient that is limiting to algal growth and primary production. In case of fresh water systems, it is phosphorus[19], whilst in marine system it is generally nitrogen[20]. However, a seasonal shift from phosphorus to nitrogen limitation is observed in coastal transition areas, such as estuaries[21] while limitation in bio available silica has been reported from the subtropical estuary of Taiwan[22].

West coast of India is one of the regions that experiences upwelling bringing low oxygen[23,24], nutrient rich waters eg. NO_3^- enhanced upto 12µM, enters into this estuary[25,26]. In the present study, this effect is simulated by artificially enriching the near mouth estuarine water with inorganic nutrients, in order to understand the dynamics of algal nutrient uptake and its growth and to determine nutrient limitation, if any.

Materials and Methods

Mandovi-Zuari estuarine system is a well-mixed coastal-plain monsoonal estuary situated between latitudes 15° 25' to 15° 31' N and longitudes 73° 45' to 73° 59' E in Goa, along the west coast of India (Fig. 1). Before the incubation experiment, ambient water samples were collected and analyzed for a range of parameters such as pH, temperature, salinity, dissolved oxygen, nutrients, microzooplankton and phytoplankton counts. All samples collected were processed following the JGOFS protocol[29] .

Estimation of phytoplankton biomass (chl *a*) was done by flurometer (Turners Design 10AU). For qualitative studies of phytoplankton, water samples were fixed with 2% acid Lugol's iodine (1% w/v) and preserved in 3% buffered formalin. The sample was then allowed to settle. Abundance and composition was determined using a Sedgwick rafter plankton counting chamber by means of an inverted microscope (magnification 100-400X). Generic and species identification was done according to taxonomic key[30]. While microzooplankton samples were preserved in 2% acid Lugol's solution, Strontium sulphate solution (2mg l^{-1}) and 1% hexamine buffered formaldehyde and analyzed upto group level under an inverted microscope with phase contrast optics following[31] at 200-400X magnification.

Nutrient samples were collected through BD plastic syringes and immediately stored at -20 °C until analysis. After defrosting, the water samples were analyzed for NO_3^-, NO_2^-, NH_4^+, PO_4^{-3} and SiO_4^{-4}, using a SKALAR segmented flow autoanalyser. Samples for oxygen were collected in gas tight Hamilton glass syringes and were fixed immediately by adding Winkler A and Winkler B solution. The precipitate was subsequently dissolved by acidification and the absorbance of developed color was measured at 456 nm[32] using a Shimadzu UV–visible spectrophotometer.

Two nutrient enrichment experiments were conducted in February and March 2006. Nalgene bottles (25.5 l capacity) were modified for this purpose by drilling two holes through the cap of the bottle through which nylon tubes were inserted, one reaching the bottom of the bottle to draw the sample, and the other to replace the volume of the water removed with helium. The outer ends of each tube were fitted with a three way valve and the entire system was ensured to be airtight (Fig. 2).

Water samples from 1m depth were drawn using a Niskin sampler and screened slowly and

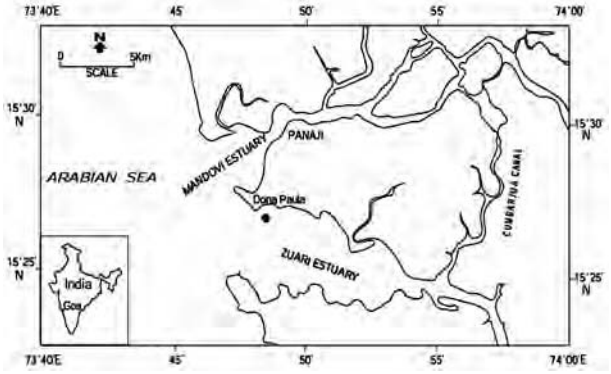

Fig. 1—Map showing study location (solid circle), near the mouth of the Zuari estuary.

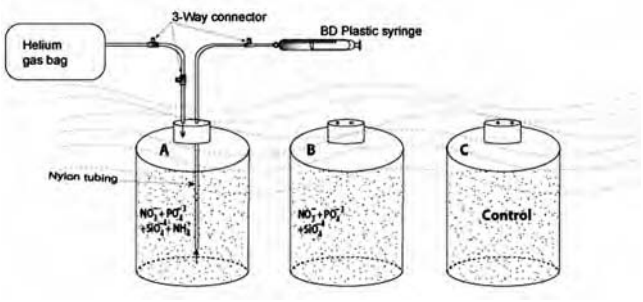

Fig. 2—A schematic diagram of the experimental-1 setup, Bottle (A): enriched with NO_3^-, PO_4^{-3}, SiO_4^{-4}, and $NH4^+$; Bottle (B): enriched with NO_3^-, PO_4^{-3} and SiO_4^{-4} and Bottle (C): acts as a control without any additional nutrients.

carefully through a 200µM nylon mesh to exclude macrograzers, without creating much turbulence to avoid damage to delicate organisms such as ciliates. The first bottle (bottle-A) was enriched with NO_3^-, PO_4^{3-}, SiO_4^{-4} and NH_4^+; the second bottle (bottle-B) was spiked with NO_3^-, PO_4^{-3}, and SiO_4^{-4} and the third bottle (bottle-C) was used as a control without any addition of nutrients. Ambient concentrations of nutrients (µM) before incubation were NO_3^- = 0.51, NO_2^- = 0.09, PO_4^{-3}= 0.57, SiO_4^{-4} = 10.92 and NH_4^+ = 2.28. The concentrations were enhanced to 11.7µM NO_3^- , 0.93µM PO_4^{3-}, 18.9µM SiO_4^{4-} and 4.5µM NH_4^+. Bottles were deployed at 1m depth by hanging from a moored floating raft. Depth of incubation was chosen based on previous measurement (T. Suresh, personal communication) showing on an average 330.18 Mmol photons $m^{-2}s^{-1}$, which is equivalent to the near surface PAR value of SW monsoon period (391.34 Mmol photons $m^{-2}s^{-1}$). Amount of light available at the incubation depth is ca. 50-60%. The first sampling was done after 16h of incubation and subsequently after every 24h. Samples from each bottle were drawn almost at the same time using plastic BD syringes. Care was taken to ensure that the bottles were uniformLy mixed prior to sampling. Volume of water drawn was replaced simultaneously with helium from air tight gas bags. The incubation lasted for ~11 days.

The second experiment (experiment-2) was conducted in March, wherein bottle-A was enriched with NO_3^-, PO_4^{-3}, and SiO_4^{-4} and the second bottle, bottle-B, was enriched with nutrients similar to bottle-A, but deoxygenated by purging helium gas. This bottle was initially maintained at hypoxic level (<2 mL O_2 l^{-1}), while bottle-C served as a control. Physico-chemical characteristics of the estuary were quite similar to those in February and the ambient nutrient concentration of NO_3^-, NO_2^- , NH_4^+, PO_4^{-3}, and SiO_4^{-4} were 0.35, 0.12, 0.46, 0.67 and 8.41µM respectively. Unlike experiment-1, samples were collected at much closer time intervals i.e. every 4 hours during the first 32h; and subsequently at 24 hourly interval for a period of 5 days.

Results

Experiment –1 (2-13 February 2006)

Nutrient uptake

This experiment was initiated during high tide (2.07 m, 13:40 h), and the bottles were incubated *in situ* at 16:00 h. The first sampling (T1) was done after 16h of incubation. Due to the delayed incubation on the first day, only 3 hours sunlight was available for photosynthesis, hence there was no significant decrease in nutrient concentrations. Bottles A and B showed a drastic drop of NO_3^-, PO_4^{-3} and SiO_4^{-4} levels between 16 and 40h of incubation. The decreases in nutrient concentrations coincided with a sharp increase in chlorophyll *a* concentration. In the control bottle (bottle-C) the chlorophyll concentration decreased with time (Fig. 3). The phytoplankton biomass which showed an increase to 23µg chl *a* l^{-1} resulted in the utilization of 10µM nitrate, 17µM silicate, and 2.2µM ammonium in bottle-A. Similarly, in bottle-B chlorophyll increased to 22.5µg chl *a* l^{-1} with concomitant utilization of 8µM NO_3^-, 0.6µM PO_4^{-3}, 15µM SiO_4^{-4} and 0.8µM NH_4^+, after 16h of incubation. After 40h of incubation 21µg chl *a* l^{-1} resulted in the utilization of 12µM, 0.6µM, 19µM and 2.4µM of NO_3^-, PO_4^{-3}, SiO_4^{-4} , and NH_4^+, respectively, from the initial concentrations in bottle- A. While, in bottle-B an increase of 20µg chl *a* l^{-1} resulted in utilization of 11µM, 0.34µM, 19µM and 1µM of NO_3^-, PO_4^{-3}, SiO_4^{-4} and NH_4^+ , respectively.

Thus, after 16h of incubation (up to 64h) concentration of nutrients in bottle-A decreased by 84-98% for NO_3^- , 58-66% for PO_4^{-3} , 88-100% for SiO_4^{4-} and 50-54% for NH_4^+ and in bottle- B, NO_3^- decreased by 65-94% , 34-61% for PO_4^{3-} , 77-100% for SiO_4^{-4} and 30-45% for NH_4^+ of the original values while chlorophyll *a* increased by a factor of three. It clearly indicates that NO_3^- and SiO_4^{-4} were nearly exhausted after 40h of incubation (Fig. 3). Every 1 µM decrease in NO_3^- resulted in 2.3-2.9 (avg. 2.6) µg L^{-1} chl *a* gain in phytoplankton biomass. This gain was particularly seen between 16 and 40h of incubation. Though, NH_4^+ was also taken up by phytoplankton along with NO_3^- (and NO_2^-), the uptake rate was comparatively much lower. However, after 3.5 days when NO_3^- had been depleted, NH_4^+ was still available in the medium that perhaps supported secondary chlorophyll peak in bottle-A with 17µg chl *a* L^{-1} and in bottle-B with 8µg chl *a* l^{-1} coinciding with the decline in NH_4^+ concentration after 136 and 88h of incubation in bottle-A and bottle-B, respectively.

The enclosed water remained well oxygenated (>4mL O_2 l^{-1}) throughout the experiment. Hence, the decrease in NO_3^- should be entirely due to the uptake by phytoplankton. Significantly, NO_3^- was preferred over NH_4^+. Difference in NO_3^- uptake pattern in bottle

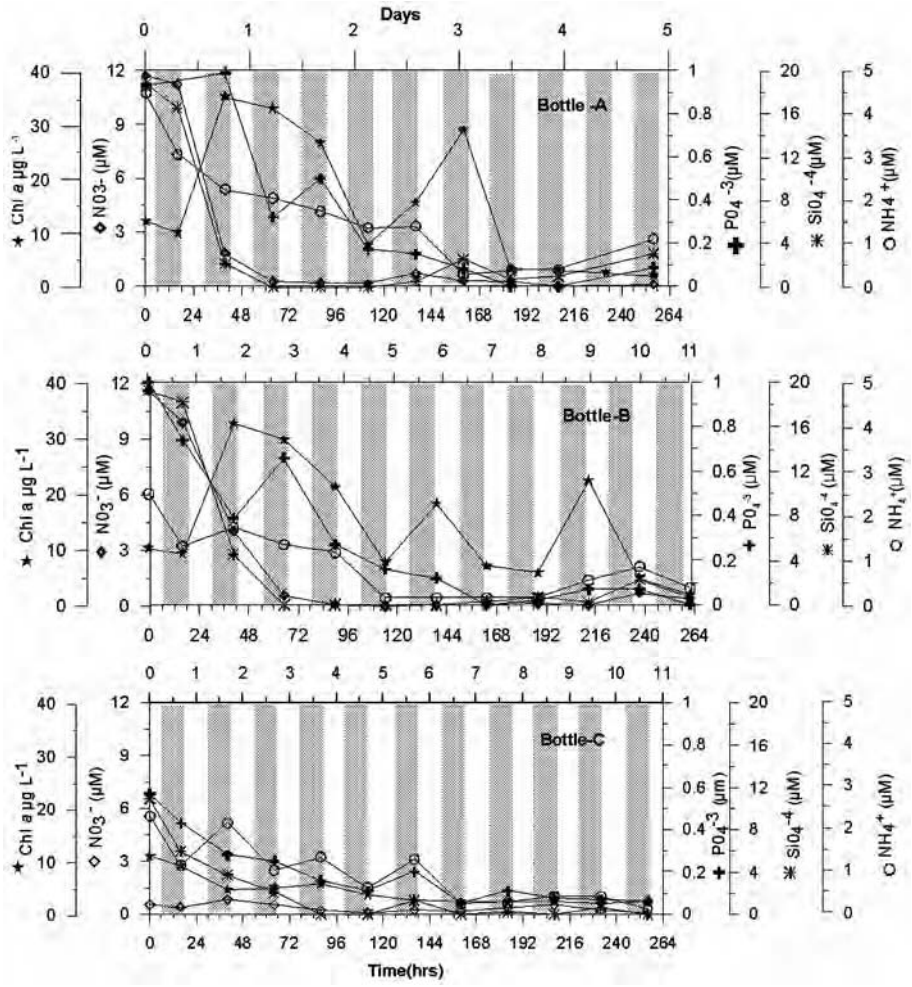

Fig. 3—Comparative variations in chl *a* and nutrient concentrations with time of incubation in February (experiment-1). Bottle (A): NO_3^-, PO_4^{-3}, SiO_4^{-4}, and $NH4^+$; Bottle (B): NO_3^-, PO_4^{-3} and SiO_4^{-4}, and Bottle (C) without any additional nutrients (control). Shaded portions represent dark periods.

A and B was found to be insignificant (ANOVA, p= 0.9) indicating that the uptake pattern of nutrients and biomass growth were similar in both the bottles. Variations between the data sets for all the parameters between the two bottles were insignificant ($p>0.05$) indicating bottle-A and bottle-B behaved almost as duplicates. Net growth rates (µ) from changes in the chlorophyll biomass[33] were calculated to be 1.24 and 1.23µg chl L^{-1} d^{-1} in bottle A and B, respectively. This closeness of µ in both bottles indicates that presence of NH_4^+ did not cause any significant change in algal biomass as long as NO_3^- was available, and at the same time NH_4^+ did not suppress uptake of NO_3^- in bottle-A. While bottle-C showed -0.67µg chl L^{-1} d^{-1} due to high grazing pressure exerted by the microzooplankton, tintinnids in particular and lack of nutrients to support further build up of phytoplankton biomass (Fig. 8).

Phytoplankton composition

It was observed that chlorophyll *a* in both the treated bottles responded in similar pattern. Likewise, the phytoplankton abundance also showed similar trends except at 40h when there was a relative increase in abundance in bottle-B as compared to bottle-A (Fig. 4a). This high value in bottle-B was due to the outburst of *Thalassionema nitzschioides*, *Thalassiosira subtilis*, *Asterionella japonica* and *Chaetoceros curvisetus* (Fig. 4b). The phytoplankton assemblage was composed of 42 species (diatoms-35; dinoflagellates-6, and silicoflagellate-1) in the experimental bottles. Cell density varied from 1.4×10^5 to 3.0×10^6 cells l^{-1} in bottle-A and from 1.1×10^5 to 5.1×10^6 cells l^{-1} in bottle-B. The control bottle showed a range from 1.2×10^5 to 8.3×10^5 cells l^{-1} in 40h. The diatoms accounted for 99% of the total phytoplankton community. The dominant species

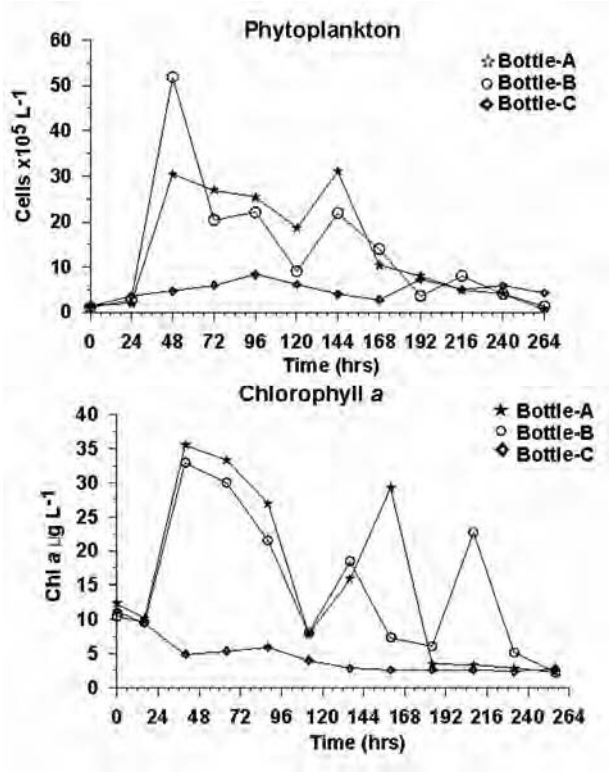

Fig. 4(a)—Variations in phytoplankton abundance and biomass, in Bottle (A) and Bottle (B) during February (experiment-1).

were *Thalassionema nitzschioides*, *Thalassiosira subtilis*, *Asterionella japonica*, *Chaetoceros curvisetus, Melosira* sp*, Skeletonema costatum, Chaetoceros lorenzianus, Guinardia striata, Guinardia delicatula, Leptocylindrus danicus, Pseudo-nitzschia seriata, Dactylisolen* sp and *Nitzschia closterium.* Among these, *Thalassionema nitzschoides, Asterionella japonica, Pseudo-nitzchia seriata,* and *Nitzschia closterium* are chain-forming pennates while others are centric diatoms. However, dinoflagellates like *Ceratium* sp*, Gymnodinium* sp*, Prorocentrum* spp, and *Protoperidinium* spp were negligible in the total community. *Dictyocha fibula* was the only representative of silicoflagellates.

Experiment –2 (25-30 March 2006)

Nutrient uptake

In the first experiment, the bottles were exposed to sunlight only for ~3 h after their deployment. The reason for low uptake of nutrients during the first 16h whether it was due to lack of photosynthesis at night or a time lag in phytoplankton response could not be ascertained. Therefore, the experiment was repeated to resolve this issue. In addition, one bottle,

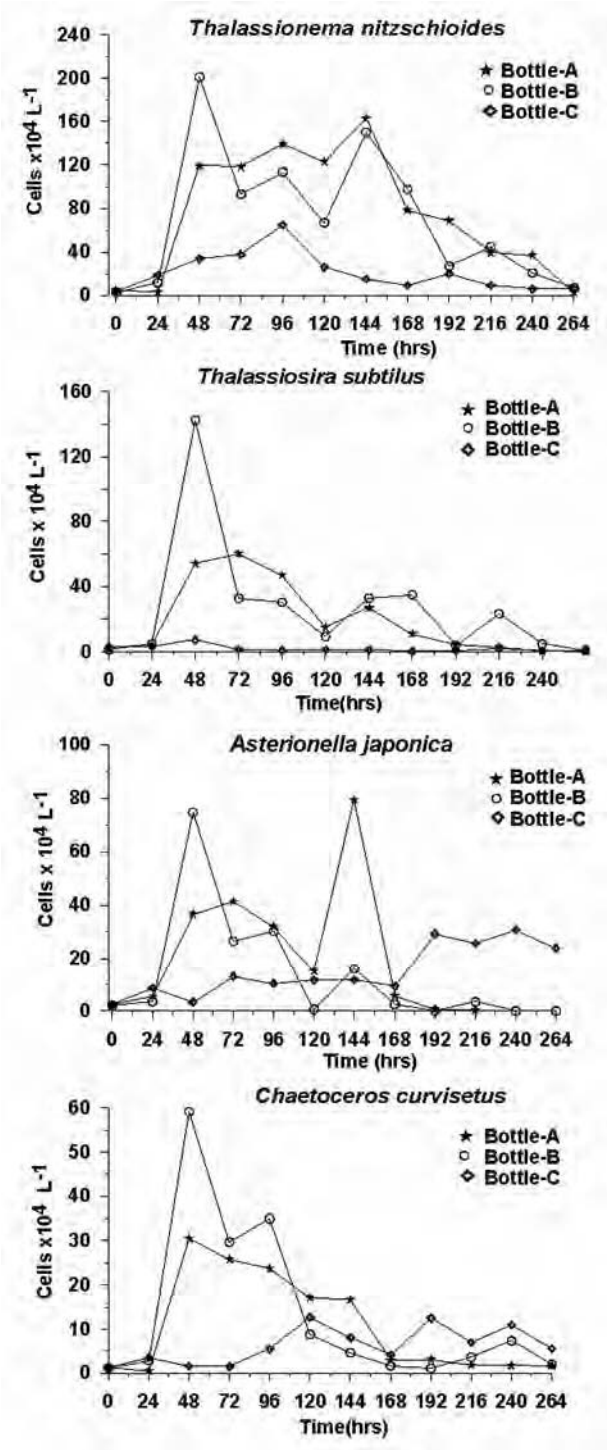

Fig. 4(b)—Variations in abundance of some diatom species viz. *Thalassionema nitzschioides*, *Asterionella japonica*, *Chaetoceros curvisetus* and *Thalassiosira subtilis* with incubation time in Bottle (A), (B) and (C) during February (experiment-1).

bottle-B was kept of O_2 <2 mL L^{-1} (hypoxic) but enriched with nutrients. This was to determine the response of phytoplankton cells when exposed to

deoxygenated waters, as happens naturally during the late phase of southwest monsoon when the study area experiences incursion of upwelled waters[24]. In this experiment, the bottles were exposed to sunlight for the whole day light period (11 h). All the bottles were deployed early in the morning and sampling was done at much shorter time intervals (every 4 hours) within the first 32h to closely monitor nutrient uptake pattern and thereafter at 24 h intervals.

During this experiment, all bottles were incubated well before sunrise after enriching with nutrients. Sharp decreases in nutrients (NO_3^- by 9µM; PO_4^{-3} by 0.3µM and SiO_4^{-4} by 12µM) with a concomitant increase of 19 µg l^{-1} chl *a* in the bottle-A at ~32h of incubation was observed (growth rate = 1.4µg chl *a* l^{-1} d^{-1}). As expected, changes in these parameters were negligible in the control, bottle-C (Fig. 5), and the difference between the initial and final nutrient and chlorophyll concentrations were still found to be statistically insignificant (ANOVA, p=0.9).

Bottle-B was gradually subjected to low oxygen hypoxic conditions, attained within 2 hr, by purging with helium gas. Dissolved oxygen was maintained low with <2 mL O_2 l^{-1} but, after 4-8 h of incubation oxygen level increased to ~3 mL O_2 l^{-1} and remained consistent throughout the experiment except at nights. A close coupling between chlorophyll *a* and dissolved oxygen due to photosynthetic activity is shown in Figure 6. The NH_4^+ concentration in bottle-B dropped by 50% while in other bottles it showed only 5% decline, while NO_3^- and SiO_4^{-4} decline was negligible with only 8 and 3% respectively of their initial concentration in bottle- B. On the whole, substantial nutrient decline and chlorophyll buildup (i.e. 5 to

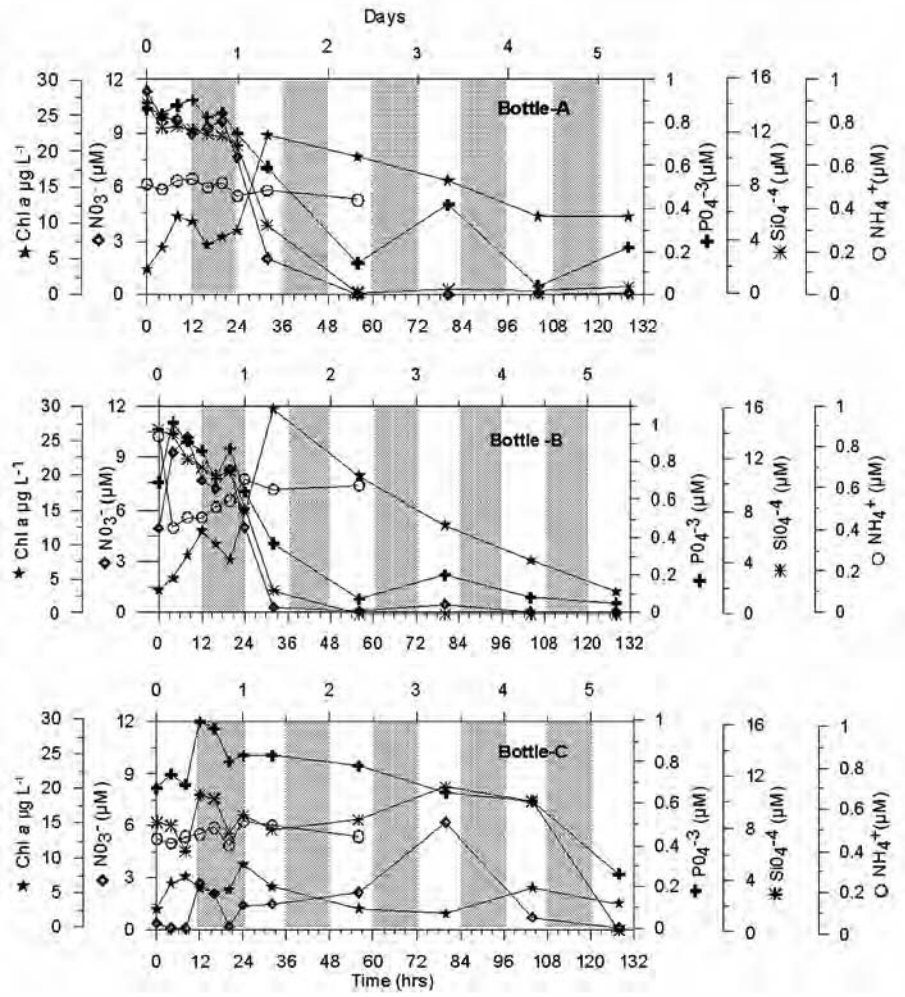

Fig. 5—Comparative variations in chl *a* and nutrient concentrations with time in March (experiment- 2). Bottle (A): with added nutrients NO_3^-, PO_4^{-3}, SiO_4^{-4}; Bottle (B): with nutrients, as in bottle (A), but deoxygenated (< 2 mL O_2L^{-1}) and Bottle (C): without any additional nutrients as control.

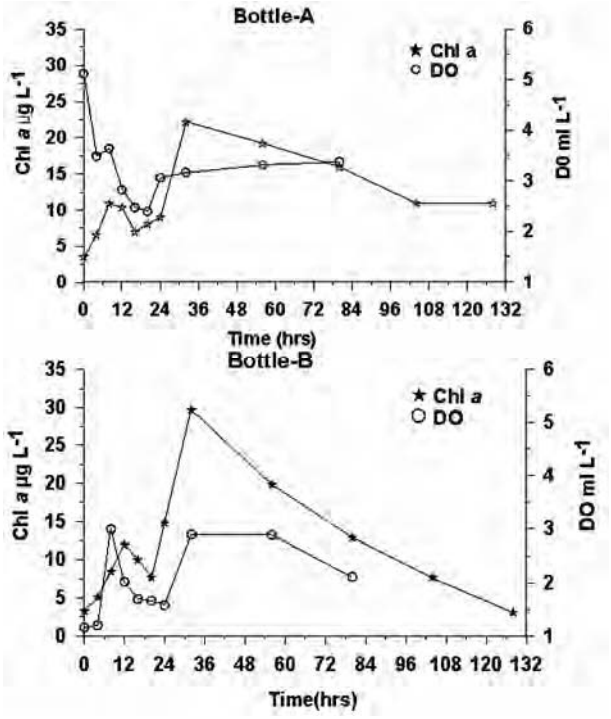

Fig. 6—Co-variations in chlorophyll *a* and dissolved oxygen in Bottle (A) and (B) in March (experiment- 2).

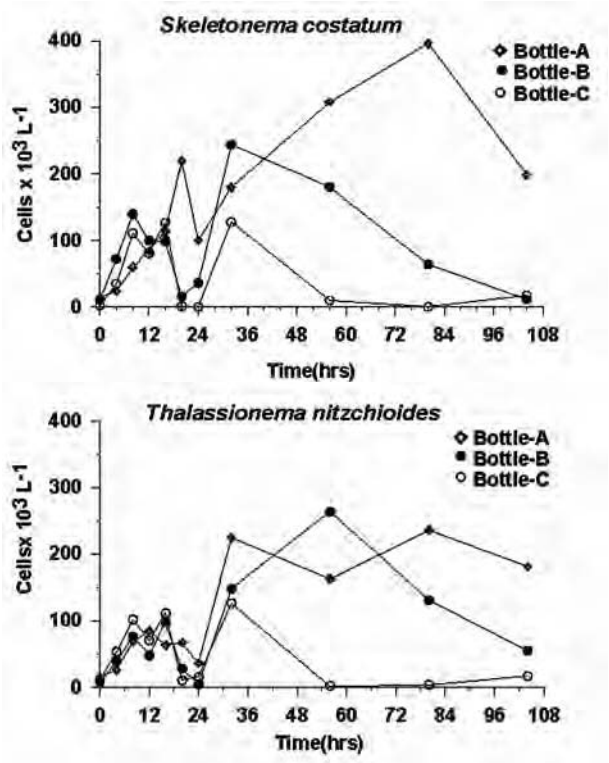

Fig. 7(a)—Variations in cell abundance of some diatom species viz. *Skeletonema costatum* and *Thalassionema nitzchioides* with incubation time in Bottle A, B and C during March (experiment-2).

30µg chl *a* l^{-1}) with rapid uptake occurred only after 20h of incubation (earlier by 4h) in deoxygenated bottle as compared to other bottles. The growth rate was found to be comparatively higher with 1.6 (µg chl *a* L^{-1} d^{-1}) in bottle- B than in control bottle (0.6).

Phytoplankton composition

The phytoplankton composition was similar to experiment-1 and composed of 45 species (diatoms-28; dinoflagellates-7, silicoflagellate-1 and blue green algae-1). Cell density in bottle- A varied from 5.7×10^4 to 7.7×10^5 cells l^{-1}. The control bottle showed a range from 3.5×10^4 to 0.8×10^5 and in bottle-B counts ranged from 7.5×10^4 to 8.6×10^5 cells l^{-1} in 32h. In general, diatom accounted for 98% of the total algae community. These high values in bottles-A and B were again due to the dominance of fast growing chain forming diatoms viz. *Skeletonema costatum, Thalassionema nitzschioides*, *Chaetoceros curvisetus, Chaetoceros lorenzianus, Leptocylindrus danicus and Guinardia striata.* Further, some species showed gradual increase from 4-12 h which coincided with the rise in chlorophyll *a* and oxygen production particularly, *Skeletonema costatum* and *Thalassionema nitzschioides* in bottle-B (Fig. 7a). Conversely, some forms viz. *Thalassiosira subtilis, Ditylum brightwellii, Melosira* sp *and Rhizosolenia crassispina* were low in abundance in bottle-B as compared to the control bottle and showed increase only after 24 hrs. (Fig. 7b).

In general, the phytoplankton composition did not vary much among the bottles. However, numerically cells were higher by 70% in bottles-A and B compared to the control bottle. Similar to the first experiment, maxima in phytoplankton density, was observed at 32h of incubation, clearly coinciding with high chl *a*, which decreased gradually with time and was comparable to control bottle after 182h. Diatoms always remained the dominant group comprising >96% of the algal community. Dinoflagellates, silicoflagellates and diazotroph *Trichodesmium erythraeum* collectively formed <5 % of the total phytoplankton community.

Discussion

Results of incubation experiments provide valuable insights into the response of natural phytoplankton assemblages to nutrient enrichment. Previous results

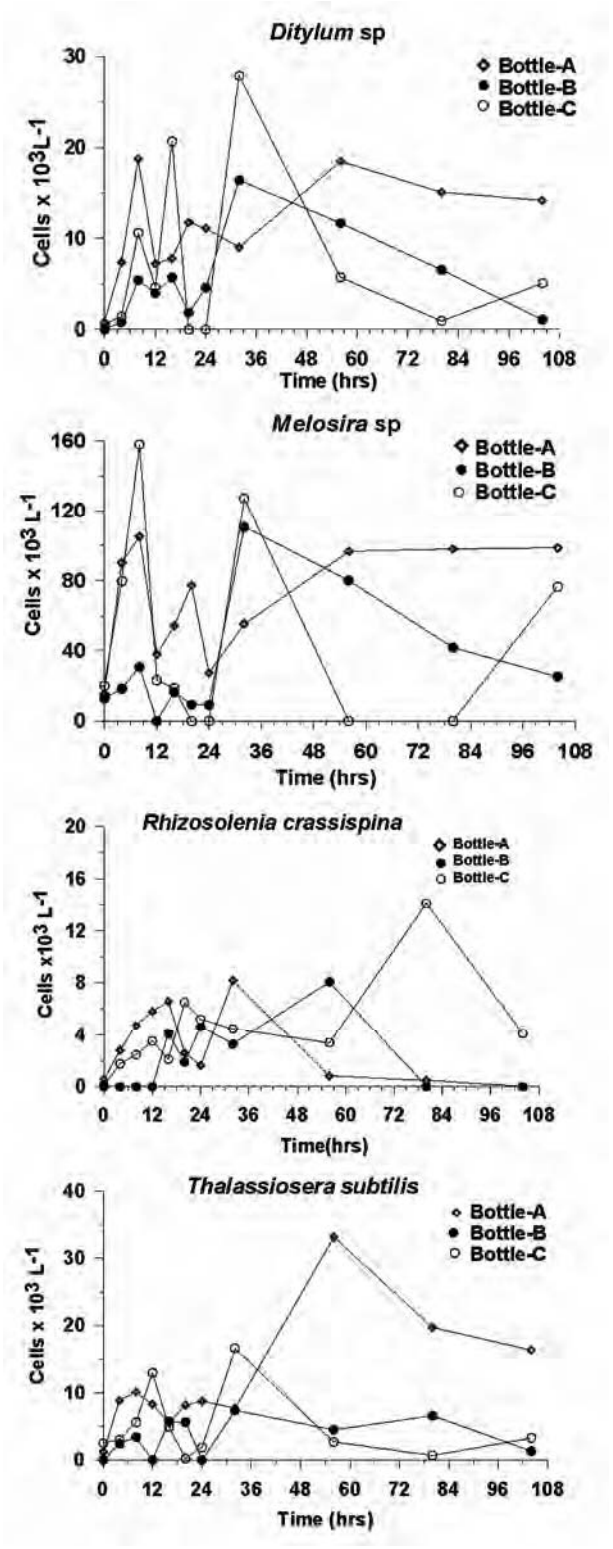

Fig. 7(b)—Variations in cell abundance of some diatom species viz. *Ditylum* sp; *Melosira* sp; *Rhizosolenia* sp; *Thalassiosira* sp with incubation time in Bottle (A), (B) and (C) during March (experiment-2).

from enclosure experiments have been useful to describe processes operating in natural conditions[34]. Such experimental approach has revealed how nutrient limitation may affect algal growth rate and net biomass accumulation. Nutrients are largely assimilated by phytoplankton during the day for photosynthesis. From the above two experiments, it is apparent that the response of estuarine phytoplankton to nutrient enrichment is almost immediate. An increase of 19-26 (avg. 23) µg chl *a* l^{-1} resulted in loss of 8-10 (avg. 9) µM NO_3^- , 0.3-0.6 (avg. 0.45) µM PO_4^{-3} and 9-17 (avg. 13) µM SiO_4^{-4}. The observed rapid phytoplankton uptake within 24h of nutrient enrichment in the study region may be true for other tropical estuaries. There does, however, appear a period of few hours when the uptake is relatively slow, as observed particularly during the experiment-2 conducted in March. The lower initial uptake rate may be due to physiological adaptation of phytoplankton to enrichment.

Studies with unialgal laboratory cultures and artificially enriched coastal seawater have shown that marine phytoplankton prefer NH_4^+ over NO_3^- as a nitrogen source[35]. Several other authors[36,37] have also reported the inhibitory effect of NH_4^+ on NO_3^- which severely reduces the rate of NO_3^- uptake. Further, some studies found a threshold ammonium concentration of 1 µM, above which NO_3^- uptake is largely inhibited despite high concentration of ambient NO_3^-[18,38,]. Similar conclusion was drawn based on the theoretical consideration of the relative energy requirement for the utilization of NO_3^- and NH_4^+ [39,40]. Some reports have shown simultaneous uptake of NO_3^- and NH_4^+ [41,42]. But, unlike others, a preference for NO_3^- over NH_4^+ was also observed[43]. In nitrogen replete cultures, studies have shown enhanced metabolism of NO_3^- with increase in irradiance[44]. Other studies have found that, the half saturation constant for nitrate uptake was related to temperature[45] where higher temperatures enhance NO_3^- utilization[46]. In our study, experiment-1, NH_4^+ concentration was >2µM, but no inhibition was observed in the bottles A and B. Instead, we found that NO_3^- was taken up before NH_4^+ and the uptake pattern of nutrients and biomass growth appeared to be similar in bottle-A and bottle-B. No significant difference was found between these two data sets ($p>0.05$). These results suggest that the phytoplankton community of monsoonal estuary preferred NO_3^- over NH_4^+ and possibly have a higher NH_4^+ threshold.

Though in the experiments NH_4^+ did not seem to contribute directly to the growth of biomass, as NO_3^- was the preferred species, still its conversion to NO_3^- via nitrification, can indirectly meet the N requirement of phytoplankton. Further, NH_4^+ can also be made available through re-mineralization, as seen after 5 days of incubation in experiment-1 which boosted the secondary chlorophyll peak. It also leads to speculation that NH_4^+ is taken up significantly when ambient NO_3^- concentration is low. This speculation arises from the fact that although NO_3^- remains low (<1µM) as compared to PO_4^{-3} (>0.5µM) or SiO_4^{-4} (>8µM) in the non monsoon seasons, maximum primary production occurs during the premonsoon (March-April) and post monsoon (Oct-Nov) periods [47,48] being supported by regenerated nutrients such as NH_4^+. However, the DIN in the Zuari estuary was observed to remain low unless there are some episodic inputs of nutrients. This was clearly seen through higher uptake rates in the experimental bottles. Molar ratios of nutrients in the water at time of experiment-1, (DIN/DIP= 5.05; and DIN/Si= 0.26) and in experiment-2 (DIN/DIP= 1.39; and DIN/Si= 0.11) was much lower than the Redfield values suggesting N limitation in this region during the pre-monsoon season. The nutrient uptake of nitrate, phosphate and silicate by the phytoplankton community was taken up close to the Redfield ratio in February and March (Table 1).

However higher DIN: DIP (>16) in March indicates that nitrogen is assimilated at slightly faster rate (Table 2) possibly due to enhanced solar radiation. NH_4^+ has been found to be present at a concentration ~4µM in post monsoon season (Oct-May), which may enter the system from nearby mangrove swamp, sewage discharge or re-suspension of sediment and benthic regeneration[49]. Further, nitrogen fixation by *Trichodesmium* that occurs every year starting from late January to May, makes a substantial contribution to the total nutrient budget in the region[11]. Following the decay of this bloom, large amount of NH_4^+ (up to 3.3 µM) is released into the medium[11], which leads to proliferation and succession of other planktonic organisms[50]. *In situ* measurements of benthic fluxes[49] have shown that the estuarine sediment is a sink for NO_3^- whereas NH_4^+ remains the dominant N form that is released from sediments in a significant quantity in premonsoon months. Only during the monsoon season (June-Sept) the estuarine waters get enriched with NO_3^- (~8µM) along with PO_4^{-3} (2.5µM) and SiO_4^{-4} (>60µM)[51] but even then, NO_3^- remains unutilized, because of cloud cover and turbidity which results in low algal productivity (61.7mmol C m^{-2} d^{-1})[48]. Thus, NH_4^+, probably supports the estuarine productivity in non monsoon period.

Large sized phytoplankton cells are known to preferentially assimilate NO_3^- over NH_4^+ [52,53]. Studies on nitrogen uptake by size-fractionated plankton showed that the NO_3^- was utilized by net plankton (20-200µM size) and NH_4^+ by nanoplankton (0.8-20µM size)[46,54] .This shifts in N-uptake from NO_3 to NH_4 was also seen during the present study (see Fig. 3), possibly due to the community shift to picoplankton. Hence, most of the dominant taxa were the diatoms (>10µM size) that preferred NO_3^- while other smaller forms must have preferred NH_4^+which is evident from the secondary chlorophyll peak. Several authors[9,55] have used relative preference index, RPI to study the preferential N uptake. A

Table 2—Comparative uptake ratio of DIN, DIP and Si by estuarine phytoplankton

	Experiment-1 (February)		Experiment-2 (March)	
Bottle	DIN/DIP	DIN/Si	DIN/DIP	DIN/Si
A	15.85	0.99	18.78	1.23
B	13.07	0.51	16.20	0.85
C	12.36	0.38	18.29	1.11

Table 1—Comparative uptake of nitrate and ammonium by estuarine phytoplankton. Exp.1-Bottle (A) enriched with NO_3^- and NH_4^+ ; Bottle (B) with nitrate and Bottle (C) as control . Exp.2- Bottle (A) and (B) enriched with only NO_3^-

N-Nutrient	Experiment-1(February)					Experiment-2 (March)				
	Bottle	% Nutrient	% Nutrient utilized	uptake rate (µmol $L^{-1}h^{-1}$)	RPI	Bottle	% Nutrient	% Nutrient utilized	Uptake rate (µmol L^{-1} h^{-1})	RPI
NO_3^-	A	71	84	0.39	1.19	A	93	87	0.78	1.04
NH_4^+	A	27	49	0.01	0.19	A	0.04	14	0.003	0.06
NO_3^-	B	81	65	0.18	1.16	B	84.15	93.9	0.377	1.02
NH_4^+	B	17	30	0.01	0.41	B	14.48	30.58	0.008	0.5
NO_3^-	C	17.7	65.33	0.39	1.172	C	37.63	89.3	0.311	1.18
NH_4^+	C	79.1	30.27	0.033	0.036	C	49.46	6.5	0.02	0.3

consistent value of RPI (>1) for NO_3^- in both experiments indicates that NO_3^- is the preferred N substrate for phytoplankton in the estuary irrespective of ambient NH_4^+ concentration (Table 1).

Diatoms have evolved a multitude of morphologies, which serve as protection against grazing or affect sinking[56]. The impact of cell shape and chain forms can be used to predict uptake of nutrients under turbulent environments[57]. Silicate, an essential nutrient for diatoms, was in surplus to support their build up. But high silicate and low nitrate as in this estuary might limit the phytoplankton growth. Therefore, in this experiment, silicon and nitrogen were added in equal proportions as most diatoms incorporate them in their molar ratio of about 1:1 [58]. It is also evident that along with NO_3^- , SiO_4^{-4} is also limiting for diatoms in this monsoonal estuarine system. This was apparent in these experiments where, fast growing dominant diatoms, viz. *Thalassionema nitzschioides*, *Thalassiosira subtilis, Asterionella japonica*, *Chaetoceros curvisetus, Chaetoceros lorenzianus, Guinardia striata, Guinardia delicatula, Skeletonema costatum* and *Leptocylindrus danicus* showed momentous increase and accounted for >96% of phytoplankton. However, the subsequent decrease in the chlorophyll after 32h possibly occurs due to grazing pressure exerted by the micro-grazers such as ciliates in particular (Fig. 8), which followed the chlorophyll peaks.

Some phytoplankton species viz., *Melosira* sp., *Rhizosolenia crassispina* and *Ditylum brightwellii,* in bottle-B of experiement-2 were found to be initially low in abundance during hypoxia (Fig. 7b), but showed an increase after 24h indicating that as oxygen level built up they had potential to bloom given the right conditions.While *Thalassiosira* sp remained invariably low throughout the incubation period w.r.t. bottle-A and C (Fig. 7b). The buildup of oxygen in the bottle-B coincided with the increase in chlorophyll, although cell counts were low (Fig. 6). This suggests that, larger forms (>10µm) were under stress, while smaller fractions were perhaps efficient under low oxygen conditions utilizing NH_4^+ , which dropped to 50% of its initial value after 4h. Thereafter the oxygen level was restored through rapid photosynthesis.

Interestingly, the species *Skeletonema costatum* and *Thalassionema nitzschioides* in bottle-B of experiment-2 remained consistent with Bottle-A (Fig. 7a). These species picked up after 4 h of

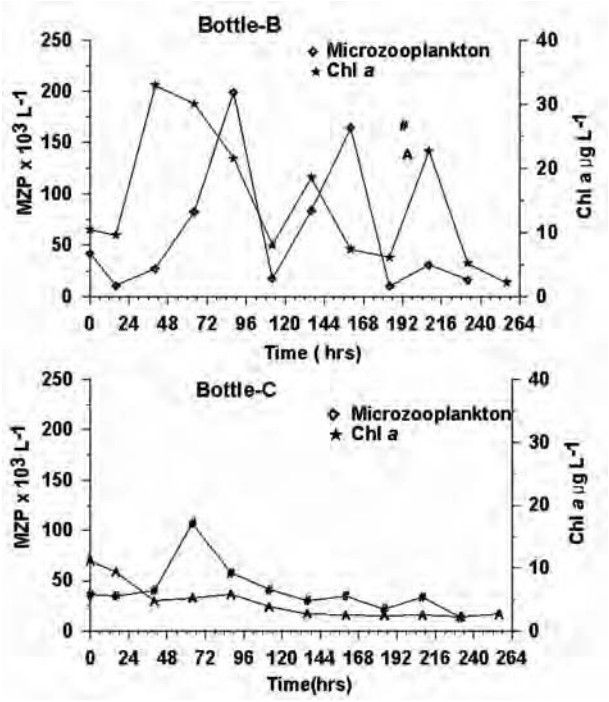

Fig. 8—The grazing effect of microzooplankton on phytoplankton biomass (chl *a*) with time in Bottle (B) and control Bottle (C) in February (experiment-1).

incubation unlike other diatoms signifying that these species have acclimatized under low oxygen conditions and may be thriving in harsh environments such as along the western continental shelf of India, which experiences seasonal oxygen depletion[23]. In support of this view, there is data reported from this coastal region showing few phytoplankton like *Asterionella japonica, Pseudo-nitzschia* sp. *Navicula* spp., *Thalassiothrix* sp., *Thalassionema* spp, *Pleurosigma* sp., and *Skeletomema* sp prevailing during such conditions[59].

Conclusions

Nutrient enrichment experiments were carried out to understand the interaction between the phytoplankton growth and nutrient uptake. Results reveal that the estuarine autotrophs were nitrogen limited during premonsoon period and that the addition of nitrate greatly stimulated the growth leading to biomass accumulation. Presence of considerable amount of NH_4^+ did not show any inhibitory effect on NO_3^- uptake. Rapid uptake of nutrients was observed after a lag phase of 24-32 h and the uptake was significantly dependent on the fast growing diatom taxa that showed high growth rates. *Thalassiosira sp* was one species most sensitive to

low oxygen throughout the incubation period while some species viz. *Melosira sp, Rhizosolenia crassispina, and Ditylum brightwellii* were found to show potential to revive from hypoxic conditions. However, this experiment does not account for an uptake of a regenerated nutrient, which possibly led to an overestimation of N-uptake rates. This warrants more experiment studies to address this issue.

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