Measurement of marine productivity using ^{15}N and ^{13}C tracers: Some methodological aspects

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Various experiments involving the measurement of new, regenerated and total productivity using ^{15}N and ^{13}C tracers were carried out in the Bay of Bengal (BOB) and in the Arabian Sea. Results from ^{15}N tracer experiments indicate that nitrate uptake can be underestimated by experiments with incubation time <4 hours. Indirect evidence suggests pico- and nano-phytoplankton, on their dominance over microphytoplankton, can also influence the f-ratios. Difference in energy requirement for assimilation of different nitrogen compounds decides the preferred nitrogen source during the early hours of incubation. Variation in light intensity during incubation also plays a significant role in the assimilation of nitrogen. Results from time course experiments with both ^{15}N and ^{13}C tracers suggest that photoinhibition appears significant in BOB and the Arabian Sea during noon. A significant correlation has been found in the productivity values obtained using ^{15}N and ^{13}C tracers.

1. Introduction

There is a need to differentiate between new and regenerated production in measurements of marine productivity (Dugdale and Goering 1967) because only the former, a precursor of export production, is relevant to the sequestration of carbon (Eppley and Peterson 1979) from the atmosphere to the deep ocean. The Joint Global Ocean Flux Study (JGOFS) (UNESCO 1994) set a protocol for the ^{15}N tracer method for measuring new and regenerated productivity. The protocol suggests carrying out *in situ* ^{15}N incubations in parallel with *in situ* ^{14}C incubations and ^{15}N deck incubations as well; time course measurements to monitor potential problems associated with extended ^{15}N incubations (i.e., non-linearity of the uptake after a few hours, particularly with the $^{15}NH_4$ tracer) along with routine nitrogen uptake rate measurements for better understanding and interpretation of data when possible. However, such studies are meagre particularly in BOB.

The climatology, hydrography and circulation of the northern Indian Ocean have been described by many authors (e.g., Swallow 1984; Shetye 1999; Schott and McCreary 2001; Balachandran *et al* 2008; Jyothibabu *et al* 2010). Here we give a very brief description. The Arabian Sea and BOB are basins of the northern Indian Ocean that come under the influence of strong monsoonal wind reversal. The upper ocean circulation and hydrography show strong seasonality with the monsoonal forcing (Prasanna Kumar *et al* 2002). Northern Indian Ocean is a very special region from the biogeochemical perspective as both the regions have distinct biogeochemical characteristics. For example, the Arabian Sea is one of the most productive regions of the world's ocean (Madhupratap

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et al 1996; Prasanna Kumar *et al* 2001) with intense oxygen minimum and denitrification zones (Naqvi *et al* 1990; Olson *et al* 1993). In contrast, productivity in BOB is lower (Prasanna Kumar *et al* 2002), and exhibits a relatively thinner and less intense oxygen minimum zone with no evidence of denitrification (Rao *et al* 1994; Sardesai *et al* 2007). On a seasonal time scale, considerable variation in the concentration of nutrients in the surface layers has been observed (Prasanna Kumar *et al* 2002). Availability and intensity of sunlight is also different in both the regions: BOB remains cloudy most of the year, whereas cloudiness in the Arabian Sea is largely confined to 3 months of the summer monsoon (Qasim 1977). BOB receives a large quantity of freshwater from the rivers draining into it. Also, BOB is a cyclone prone region and these events churn up the area, injecting nutrients into the surface layer during the post-monsoon season (Prasanna Kumar *et al* 2004). Unlike the BOB, the Arabian Sea does not receive a large amount of fresh water from the rivers. Its surface nutrient dynamics is mainly governed by upwelling and winter mixing during summer and winter monsoons, respectively. In general, nutrient supply and solar intensity vary seasonally in the northern Indian Ocean.

In this paper, we address the following questions:

- what is the effect of the duration of incubation on the uptake rate of nutrients by phytoplankton? Are there significant variations within the time of 2–4 hours recommended by the JGOFS protocol for ^{15}N experiments?
- What is the effect on uptake rate if the substrate concentration increases while keeping the incubation time fixed?
- What is the effect on uptake rate if the 4-hour deck incubation experiments were done during different times of the day?
- f-ratio, the ratio of new to total production (Eppley and Peterson 1979), has been calculated by different workers (Watts and Owens 1999; Wafar *et al* 1995) for different oceans but what happens to the f-ratio in the first three cases?

For ^{15}N uptake experiments, the JGOFS protocol suggests simulated *in situ* incubation of 2–4 hours. Longer incubation times might lead to increased regeneration of ammonium and urea, which may also be taken up along with nitrate. Slawyk *et al* (1977) showed the importance of the coupled ^{13}C and ^{15}N tracer technique. They also found a consistency between the results obtained by the ^{13}C and ^{14}C methods. The main advantage of using ^{13}C isotope is that it can be combined with the already existing ^{15}N technique to get a better insight into the relationship between photosynthesis and inorganic nitrogen uptake by marine phytoplankton. Therefore, in one of the experiments of the present study, ^{13}C with ^{15}N tracers are combined to test this coupled technique, incidentally the first, in this region.

This study forms a part of the Bay of Bengal Process Study (BOBPS), a programme intended to estimate the biogeochemical fluxes in BOB (Prasanna Kumar *et al* 2002; Madhupratap *et al* 2003), similar to JGOFS in the Arabian Sea. In addition, this paper also includes some results obtained from the Arabian Sea using ^{15}N and ^{13}C tracers.

2. Methodology

In the present study, five different experiments were carried out. Sampling in BOB was done during September–October 2002 and April–May 2003 onboard ORV *Sagar Kanya*. Samples for the first two experiments were collected during September–October 2002 and for the third experiment during April–May 2003. The ambient nitrate concentration was measured by column reduction technique (Strickland and Parson 1972). Ammonium and urea concentrations were taken from Kumar *et al* (2004a), which were estimated indirectly using zooplankton biomass. Nutrient concentrations were also used in the N uptake rate calculations. We checked that the uncertainties associated with ammonium and urea concentrations, in the worst case, if ∼100%, could only introduce less than 15% error in the estimation of the specific uptake rate of nitrogen. Sea surface temperature, salinity and other chemical and biological data were obtained from BOBPS database. The tracers used for experiments were 99 atom % ^{15}N enriched sodium nitrate, ammonium chloride and urea procured from Sigma-Aldrich, USA. All the above-mentioned ^{15}N tracers and 99 atom % ^{13}C enriched sodium bicarbonate (procured from Cambridge Isotope Laboratory, USA) were used during sampling in the Arabian Sea onboard *Sagar Sampada* in March 2007 for the last two experiments. The individual experiments are discussed in detail below.

2.1 *Experiments*

2.1.1 *Experiment 1*

The aim of this experiment was to observe the variation in the uptake rates of different N-species with varying durations of incubation. The JGOFS protocol was followed: surface water samples were collected (at 17°56.55′N, 87°54.64′E) in one litre polycarbonate bottles (procured from Nalgene,

USA), pre-washed to avoid trace metal contamination. Samples were divided into three sets of four bottles each for nitrate, ammonium and urea tracers. In each bottle, a constant amount of the respective tracer was added with the final concentration of 0.01 μM. After the tracer was added, the samples were kept for incubation for 10 h in a deck incubator with flowing surface sea water. Every hour one bottle from each set was taken out of the incubator and filtered on precombusted (4 h at 400°C) Whatman GF/F filters (procured from Sigma Aldrich, USA) under low vacuum. We might have lost some pico-plankton (size range 0.2–2 μm) of size less than 0.7 μm by choosing GF/F filters of pore size 0.7 μm. However, GF/F filters of pore size 0.7 μm are recommended for isotopic analyses (UNESCO 1994). The samples were dried and kept for further mass spectrometric analysis.

2.1.2 *Experiment 2*

This experiment was intended to monitor variations in the uptake rates of different nitrogenous species by phytoplankton, caused by varying concentrations of the relevant substrate. For this experiment too, surface water samples were collected (at 20°0.25′N, 87°59.6′E) in one litre bottles and divided into three sets of four each. The concentration added in different bottles of each set was different. The tracers were added with final concentrations of 0.01, 0.02, 0.03 and 0.04 μM in different bottles of the respective sets. These amounts were 9%, 18%, 27% and 36%, respectively of the nitrate concentration in the surface waters. For ammonium and urea, these were in excess or comparable to the ambient concentrations (see section 3). Incubation was done on the deck for 4 h symmetrical to local noon, i.e., from 10:00 to 14:00 h. Running seawater maintained the temperature during incubation. After incubation, the samples were filtered and preserved for analysis as described above.

2.1.3 *Experiment 3*

To check the effect of incubation during different 4-hour intervals during a day, the experiment was performed at two different locations (12°N, 88°E and 17°N, 83°30′E). At both the stations, the surface water samples were collected early in the morning (before sunrise) in nine one-litre polycarbonate bottles. The three bottles were incubated from 06:00 to 10:00 h after adding each tracer (nitrate, ammonium and urea) to the final concentration of 0.01 μM. The rest six were kept in dark. At 10:00 h, another three were incubated till 14:00 h followed by the next three from 14:00 to 18:00 h. The samples were filtered immediately after the completion of incubation time and dried and stored for further analyses.

2.1.4 *Experiment 4*

Similar to Experiment 3, the effect of incubation during different 4-hour intervals during a day was tested using the ^{13}C tracer at a location (18°N, 70°E) in the Arabian Sea. Surface water samples were collected early in the morning (before sunrise) in six one-litre polycarbonate bottles. Two samples were incubated from 06:00 to 10:00 h after adding $NaH^{13}CO_3$ tracer with the final concentration of 0.2 mM. The rest were kept in the dark. At 10:00 h, another two were incubated till 14:00 h followed by the next two from 14:00 to 18:00 h. The samples were filtered as soon as the respective incubations ended and then preserved for mass spectrometric analysis.

2.1.5 *Experiment 5*

To estimate the effect of different light levels (depths) on the assimilation of carbon and nitrogen, samples were collected (at 21°50′N, 66°E) from surface of 2, 5, 17, 31 and 50 m depths. These depths were chosen to correspond to 100, 80, 64, 20, 5 and 1% of surface irradiance, measured using a hyperspectral radiometer (Satlantic Inc., Canada). For experiments with nitrate and ammonium tracers, water samples were collected in 2-litre polycarbonate bottles whereas for urea and carbon experiments 1-litre bottles were used (all in duplicates). Nitrate and carbon tracers were added at 10% of the ambient concentration, a very small, constant amount of ammonium and urea were added for all depths (to the final concentration of 0.01 μM). Incubation was done on deck for 4 h symmetrical to local noon, i.e., from 10:00 to 14:00 h. Neutral density filters were used to simulate the light conditions for the deck incubation and also the continuous flow of seawater from 5-m depth was maintained to regulate the temperature. As in experiments 1–4, samples were filtered and preserved for mass spectrometric analysis. In all the five experiments, duplicate analyses were made wherever possible (table 1).

2.2 *Analysis*

Analysis of samples was performed in the laboratory using a Carlo Erba Elemental Analyser interfaced via ConfloII to a Finnigan Delta Plus mass spectrometer. For ^{15}N tracer experiment, major parameters measured were PON and ^{15}N atom%, whereas, for ^{13}C tracer experiment, major parameters measured were POC and ^{13}C atom% in

Table 1. *Comparison of specific uptake (h^{-1}) and uptake rates ($\mu mol\ N\ m^{-3}h^{-1}$) at two different stations for 4 hours of incubation for 0.01 μM of tracer addition. Uncertainty based on duplicate measurements given in parentheses.*

	Experiment 1		Experiment 2	
Tracer	Specific uptake rate $\times 10^3(h^{-1})$	Uptake rate (μmol N $m^{-3}h^{-1}$)	Specific uptake rate $\times 10^3(h^{-1})$	Uptake rate (μmol N $m^{-3}h^{-1}$)
Nitrate	1.57 (0.1)	1.5 (0.1)	3.2 (0.05)	3.8 (0.1)
Ammonium	0.48 (0.1)	0.38 (0.1)	1.4 (0.3)	1.3 (0.2)
Urea	1.86 (0.2)	1.56 (0.07)	2.4 (0.3)	2.3 (0.2)

post-incubation samples. PON was measured following Owens and Rees (1989) with a modification in oxygen injection time to reduce the effect of any contaminant N introduced with oxygen used for combustion, just as a precaution. In this method, the integration of ion beam areas (m/z 28+29+30), after calibration against standard material (IAEA-NO-3, KNO_3), provided a quantitative measure of PON. The advantage of the technique was the simultaneous measurement of PON and isotope ratio in the same sample. The maximum difference in PON measurements for duplicate samples was found to be around 10%. The coefficient of variation for ^{15}N atom% measurement was less than 1% for nitrate and urea samples while it was found to be 3% in the case of ammonium. The $\delta^{15}N$ measurement for standard material (IAEA-NO-3, KNO_3) yielded 4.91±0.30‰ (n=13) against the IAEA quoted value of 4.7‰. More details can be found in (Kumar *et al* 2004b, 2005). Similarly, both POC and ^{13}C atom% were measured simultaneously in the same sample. Sucrose from Australian National University (ANU) was used for calibration of the mass spectrometer for the carbon measurements. The maximum difference in POC measurements for duplicate samples was found to be around 11%. The coefficient of variation for ^{13}C measurements was less than 1%.

We have used the equation given by Dugdale and Wilkerson (1986), which accounts for the presence of detrital nitrogen in the filter and is also insensitive to simultaneous uptake of labelled and unlabelled nutrients. The specific uptake rate (N taken up per unit particulate N) is calculated based on the isotope ratio of sample measured at the end of the incubation,

$$V_t^{\mathrm{N}} = \frac{{}^{15}\mathrm{N_{xs}}}{[({}^{15}\mathrm{N_{enr}} - {}^{15}\mathrm{N_N}) \times t]}, \tag{1}$$

where $^{15}N_{xs}$ is atom% excess in the sample after incubation, $^{15}N_{enr}$ is atom% ^{15}N in the initially labelled fraction, t is the incubation time, $^{15}N_N$ is the natural abundance of ^{15}N. The uptake rate ρ_t^{N} (N taken up in concentration unit) is calculated using V_t^{N} and PON at the end of incubation (PON_t),

$$\rho_t^{\mathrm{N}} = V_t^{\mathrm{N}} \times \mathrm{PON}_t. \tag{2}$$

The total N-uptake rate is calculated as the sum of nitrate, ammonium and urea uptake rates. New productivity is equivalent to nitrate uptake rate and regenerated productivity is equivalent to the sum of ammonium and urea uptake rates; f-ratio is the ratio of new productivity to total productivity, calculated as the ratio of nitrate uptake to the total N-uptake rate, following Sambrotto (2001).

Similar to the nitrogen uptake rate estimation, the specific uptake rate of carbon taken up per unit particulate carbon is calculated based on the isotope ratio of sample measured at the end of the incubation:

$$V_t^{\mathrm{C}} = \frac{{}^{13}\mathrm{C_{xs}}}{[({}^{13}\mathrm{C_{enr}} - {}^{13}\mathrm{C_N}) \times t]}, \tag{3}$$

where $^{13}C_{xs}$ is atom% excess in sample after incubation, $^{13}C_{enr}$ is atom% ^{13}C in the initially labelled fraction, t is the incubation time, $^{13}C_N$ is the natural abundance of ^{13}C. The uptake rate ρ_t^{C} (C taken up in concentration unit) is calculated using V_t^{C} and POC at the end of incubation (POC_t),

$$\rho_t^{\mathrm{C}} = V_t^{\mathrm{C}} \times \mathrm{POC}_t. \tag{4}$$

3. Results and discussion

Sea surface temperature (SST) and other meteorological and environmental parameters at the experimental sites are listed in table 2. During Experiments 1 and 2, SST varied between 29.0 and 30.0°C from south to north in BOB while sea surface salinity (SSS) decreased from south to north (33.9–28.2 psu). The variations could be due to the combined influence of eddy pumping, fresh water discharge by the major rivers (Ganga, Brahmaputra, Irrawady, Godavari, Krishna and Cauvery) and large oceanic precipitation, which exceeds evaporation during the season (Prasanna Kumar *et al* 2007). Nitrate concentrations were lower during

Table 2. *Meteorological and environmental parameters at the experimental locations. NA denotes absence of POC measurements not required for the ^{15}N tracer experiments; only PON needs to be measured for these.*

Parameter	Experiment 1	Experiment 2	Experiment 3	Experiment 4–5
Latitude (°N)	~ 18	~ 20	12	18
Longitude (°E)	~ 88	~ 88	~ 88	70
Wind speed (ms^{-1})	6	4	0.8	4.6
Pressure (mbar)	1008	1008	1009	1012
Air temperature (°C)	> 31	29	29.5	26.7
SST (°C)	29.1	29	30.4	27.5
Surface salinity (psu)	292	28.2	32.8	35.9
MLD (m)	~ 14	~ 10	~ 15	72
Chlorophyll *a* (mg m^{-3})	0.12	0.13	0.06	0.16
Surface nitrate (μM)	0.08	0.11	0.20	1.86
PON (μmol N L^{-1})	1.04	1.2	0.67	1.01
POC (μmol C L^{-1})	NA	NA	NA	179
Surface light intensity (Wm^{-2})	~ 230	~ 280	~ 300	~ 400
Dominant diatom species (more than 5% of the total cells counts)	*Navicula spp, Thalassionema nitzschoides, Chaetoceros lorenzianus*	*Navicula spp, Thalassionema nitzschoides, Chaetoceros lorenzianus*	*Chaetoceros didymus, Bacteriastrum cosmosum, Bacteriastrum frucatum, Navicula spp*	*Navicula macunosa*

the period and varied from 0.08–0.21 μM. Molar ratios of nitrate to phosphate (N:P) and nitrate to silicate (N:Si) varied between <1 and 2.5, while molar ratio of silicate to phosphate (Si:P), was always more than 6 and reached as high as >25 at the third site in the surface waters (Paul *et al* 2008). This suggests higher silicate concentration relative to nitrate and nitrate limited conditions (Paul *et al* 2008). Total microphytoplankton cell count varied from ~2000 to ~10000 cells L^{-1} (Paul *et al* 2008). Diatom dominated (>90% of the total microphytoplankton) the microphytoplankton population. Predominant species (more than 5% of the total cell count) were *Navicula spp*, *Thalassionema nitzschoides* and *Chaetoceros lorenzianus* (Paul *et al* 2008).

During the locations of Experiment 3, SST was found to be ~30.0°C at both the locations. SSS varied between 32.2 and 32.5 psu from central to western BOB. During this season, eddies played a significant role in deciding SST, SSS and surface nutrient concentrations (Prasanna Kumar *et al* 2007). However, unlike in September–October 2002, SSS during this season was higher. This high surface salinity could be due to the positive E–P coupled with marginal river discharge during the season (Prasanna Kumar *et al* 2007). Nitrate concentration was lower (~0.20 μM) at both the locations. Molar ratios N:P were >10 and >2 in the central and western BOB locations, respectively. N:Si was found to be less than 1 at both the locations while Si:P was very high (>100) in the central and >10 in the western BOB (Paul *et al* 2008). Total microphytoplankton cell count varied from ~640 to ~1080 cells L^{-1} from central to western BOB (Paul *et al* 2008). During this season, diatom dominated (>90% of the total microphytoplankton) the microphytoplankton population. Predominant species (more than 5% of the total cells counts) were *Chaetoceros didymus*, *Bacteriastrum cosmosum, Bacteriastrum frucatum* and *Navicula spp* (Paul *et al* 2008).

Lower cell concentrations at most of the sampling locations suggest the oligotrophic conditions during both the sampling periods in BOB. Ammonium and urea concentrations were ~0.04 and ~0.01 μM, respectively, at experiment sites 1–3 (Kumar *et al* 2004a).

At experiment sites 4 and 5, surface nitrate was 1.86 μM (table 2). Although the nitrate values were higher, chlorophyll (0.16 mg m^{-3}) and microphytoplankton concentrations were lower, possibly due to lack of other macro and/or micronutrients. In general, this region is diatom dominated during winter and late winter (Madhupratap *et al* 1996). In the present study also, microphytoplankton population was dominated by diatom (>60% of the total cells counts) with the dominance of *Navicula macunosa* (>22%).

A few hundred diatom cells L^{-1} (as found in some of the sampling locations in BOB and the Arabian Sea) would contribute only a negligible proportion to the chlorophyll concentration (>0.1 mg m^{-3}). While diatom might indeed represent most of the microplankton biomass (between 20 and 200 μm), the bulk of the chlorophyll was

probably in smaller (<20 μm, i.e., nano and pico) size classes. One could speculate, in the absence of detailed studies in this region that the latter groups, most likely represented by many different phyla of flagellated algae and cyanobacteria, could have been responsible for most of the uptake of isotopes recorded in the experiments. Their composition would likely differ between stations. Their physiological characteristics, such as preference for nitrogen species, could vary considerably, even within the groups, which would probably also explain the differences, e.g., in urea uptake rates. Distribution and composition of nano- and pico-plankton are sufficiently documented. Until detailed studies on different nano- and pico-plankton communities are carried out, the interpretations presented here should be taken as preliminary and not as conclusive.

3.1 *Experiment 1*

3.1.1 *Urea*

Results from Experiment 1 suggest that the specific uptake rate and uptake rate for urea are the highest (figure 1) in the nitrogen-limited waters of the BOB. This observation is similar to that of Rees *et al* (2002) who observed urea to be the most preferred substrate for the *Emiliania huxleyi*-dominant plankton community in the North Sea. Casey *et al* (2007) have also reported dominancy of uptake of reduced forms of nitrogen (ammonium and urea) for the *Prochlorococcus* populations in the Sargasso Sea. Urea uptake rate was the highest, followed by ammonium uptake. The sum of ammonium and urea uptake contributes more than 90% of the total N-uptake for *Prochlorococcus* (Casey *et al* 2007). Both the studies mentioned above were carried out in oligotrophic conditions, quite similar to the location of the present study. However, the value for the average urea uptake in our study is only one-third of the value obtained by Rees *et al* (2002) for a similar concentration of substrate. Also, the average specific uptake rate of urea lies in the lower limit of the values obtained by Casey *et al* (2007). The plausible reason for the difference could be the presence of different dominant species during their studies or the difference of length of incubation period [6–8 h for Casey *et al* (2007) and 24 h for Rees *et al* (2002) *vs.* 4 h here]. Uptake rates in the present experiment range from a maximum of 2.48 μmol N m^{-3} h^{-1} to a minimum of 1.56 μmol N $m^{-3}h^{-1}$. These values are comparable with values reported for other regions, e.g., the North Atlantic (Varela *et al* 2005) and the Arabian Sea (Watts and Owens 1999).

The specific uptake rate of urea increased in the first three hours of incubation, but declined afterwards. This significant decline is also exhibited by the uptake rate of urea. The decline might be due to the rapid recycling and subsequent isotope dilution, which results in an underestimate of the urea uptake (Bronk *et al* 1998). On the basis of concentration of transparent exopolymer particles (TEP), Kumar *et al* (1998) reported that TEP

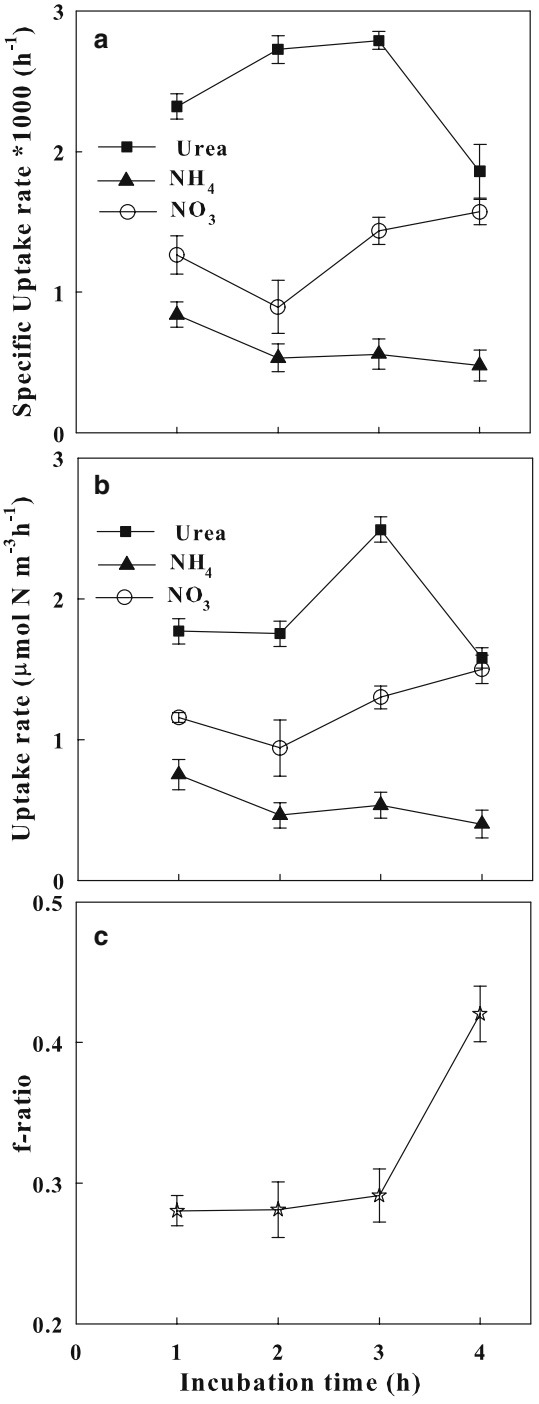

Figure 1. The result of Experiment 1 showing the variation in (**a**) specific uptake rate (h^{-1}), (**b**) uptake rate (μmol N $m^{-3}h^{-1}$) and (**c**) f-ratio with the increase in the duration of incubation from 1 to 4 h.

gets removed efficiently in BOB from the water column by scavenging of mineral particles (Ittekkot *et al* 1991). Therefore, the residence time of organic matter is much less for its complete oxidation, despite the presence of higher bacterial biomass (Sarma 2002). In addition, respiration rates are lower in BOB than in the Arabian Sea (Naqvi *et al* 1996). All these evidences suggest that recycling and respiration rates are lower in BOB. Therefore, isotope dilution is expected to be minimal in BOB. In addition, isotope dilution is significant only for longer periods of incubations (>6 h) and remains minimal for ≤4 h incubation (Bronk *et al* 1998). The other plausible reason could be the exhaustion of added tracer in the dissolved form. We calculated the amount of tracer present in the dissolved form at different intervals during incubation on the basis of incorporated atom% ^{15}N into particulate form. The calculation showed that ∼55%, ∼42% and ∼10% of the added urea tracer remained available in the dissolved form after the first, second and third hours, respectively. This suggests that the depletion of the available pool of urea might limit the specific uptake rate and uptake rate.

3.1.2 *Ammonium*

In the case of ammonium, where constant addition of 0.01 μM was made, both specific uptake rate and uptake rate decreased slightly for the incubation time >1 hour, and remained constant for higher incubation times. The uptake rate for ammonium showed a maximum of nearly 0.74 μmol N $m^{-3}h^{-1}$ and a minimum of 0.38 μmol N $m^{-3}h^{-1}$. These values are comparable to those reported by Rees *et al* (2002) for ammonium uptake rate in the North Sea, extrapolated to the same substrate concentration. Again, values of specific uptake rates here are comparable to those reported by Casey *et al* (2007) for *Prochlorococcus* populations. It is known that in ammonium poor waters, ammonium is taken up as soon as it becomes available (Gilbert *et al* 1982). It could also be possible that higher uptake in the initial hour simply represents storage rather than growth. Collos *et al* (2006) showed that the growth of natural populations of *Alexandrium catenella* in the Thau Lagoon, southern France, is limited by nitrogen and exhibits storage rather than a growth response to an ammonium pulse. The decline in the specific uptake rate and uptake rate after 1 h could be due to excretion of organic nitrogen (Collos *et al* 2006). The release of fixed (assimilated) labeled $^{15}NH_4$ in the form of organic nitrogen leads to isotope dilution and hence the underestimation of ammonium uptake rate (Gilbert *et al* 1982). As in the case of urea uptake, isotope dilution for ammonium uptake is significant only when incubation duration is longer than 4 h. Therefore, isotope dilution is less likely to be responsible for the observed decline in the uptake of ammonium after the first hour; the other plausible reason could be the exhaustion of the added tracer. Like in the case of urea, the amount of dissolved ammonium present at different times during the course of incubation was calculated on the basis of the incorporated atom% ^{15}N in particulate form. The calculation showed that ∼58%, ∼51% and ∼37% of added ammonium tracer remained available in the dissolved form after the first, second and third hours, respectively. This suggests that the depletion of the available pool of ammonium is not as significant as was found in the case of urea.

It appears that when a pulse of ammonium is provided to the nitrogen-poor water, plankton immediately absorb a good amount of ammonium and use this stored pool for uptake/growth later. As plankton uptake excess amount of ammonium in the initial hour, their demand reduces later, possibly resulting in an apparent decline in the uptake rate.

3.1.3 *Nitrate*

The specific uptake rates and uptake rates for nitrate lie between those of urea and ammonium. The uptake rate remains nearly the same for incubation durations of up to 2 h, but for 3- and 4-hour durations, it is slightly higher. The uptake rate varies within a narrow range of 0.92–1.5 μmol N $m^{-3}h^{-1}$. These values are comparable to those obtained in the North Sea waters (Rees *et al* 2002) and the North Atlantic Subtropical Gyral Province (Varela *et al* 2005). The changes in the uptake rates of different N-species as a function of time are reflected in the f-ratio as well. The f-ratio almost follows the pattern of nitrate uptake rate. There is a significant increase in the f-ratio for incubation times greater than 3 h, from 0.29–0.42. This is mainly due to the significant decrease (2.48–1.56 μmol N $m^{-3}h^{-1}$) of the urea uptake rate and the slight increase in the nitrate uptake rate.

It is seen in nitrogen-poor waters that the reduced form of nitrogen preferred to nitrate, which is energetically more difficult to assimilate, as the nitrogen substrate for phytoplankton (Harrison *et al* 1996; Mulholland and Lomas 2008). The nitrate assimilation during the 4-h incubation in our experiment indicates lower assimilation in the initial hours and an increase in the later hours (figure 1). Variation in light intensity from 10:00 to 14:00 h can also account for such variations. In general, light intensity increases during 10:00 to 12:00 h and decreases in the later hours (Suresh *et al* 1996). It could explain the increase in assimilation

during initial hours of ncubation. However, it cannot explain the higher assimilation of nitrate in the later hours during which light intensity decreases. Indeed, variation in the light intensity during 10:00 to 14:00 h are much less than that was before 10:00 h (sharp increase) and after 14:00 h (sharp decrease). Therefore, as in the earlier cases of urea and ammonium, we calculated the amount of nitrate tracer present in the dissolved form during the course of incubation. The calculation showed that more than 50% tracer remains in the dissolved form for further uptake by plankton even after 3 h, thus supporting higher nitrate assimilation in the later hours. Diatom prefer nitrate as the nitrogen source in the natural environment and they are often the dominant taxa in upwelling systems, where nitrate concentrations are often high (Kokkinakis and Wheeler 1987; Wilkerson and Dugdale 2008). Although nitrate concentration levels were lower in our experiment, *Navicula spp* was the dominant species during the experiment. Therefore, specific uptake rate and uptake rate of nitrate increased after the first hour and reached a maximum after 4 h (figure 1). These values are comparable to the values obtained from surface waters of the Norwegian fjords for other diatoms (mainly *Skeletonema costatum*, Fernandez *et al* 1996). The average specific uptake rate values are comparable to the values obtained in the Gulf of Riga, Baltic Sea, for a mixed population of diatoms, cryptophytes, dinoflagellates and filamentous cyanobacteria with the dominancy of the diatom (Berg *et al* 2003).

The effect of these variations on f-ratio is noticeable. It appears that f-ratio may be underestimated if incubation is done only for 2 h, f-ratio at this stage in this water was found to be 0.28. However, the result after 4 h of incubation shows f-ratio of 0.42. This may be because of higher uptake rate for nitrate and decline of urea and ammonium uptakes in later hours of incubation. The underestimation of f-ratio in the first 2 h could be because of higher assimilation of reduced forms of nitrogen (ammonium and urea), which are energetically easier to assimilate. Examining the amount of dissolved tracer left after 3 h of incubation, urea is the first to be exhausted in the nitrogen-poor BOB waters. Assimilation of reduced forms of nitrogen might simply represent storage rather than actual uptake in the first 1–2 h of incubation. On other hand, uptake measurements are underestimated due to the isotope dilution effect and tracer dilution for longer duration of incubations. In both the cases, f-ratio estimation might be erroneous. Therefore, a minimum of 4 h of incubation is needed to get reasonably reliable results for uptake rates and f-ratios.

3.2 *Experiment 2*

The rate of uptake after 4 h of incubation was found to be the highest for nitrate followed by urea, for additions of 0.01 μM of all the three tracers (figure 2). Higher additions of ammonia and urea tracers increased their respective specific as well as uptake rates.

There is a significant increase in the specific uptake rate from 0.0024 to 0.0062 h^{-1} when added urea tracer is increased (from 0.01–0.04 μM). The uptake rate of urea also increased from 2.3–7.3 μmol N $m^{-3}h^{-1}$.

Ammonium closely follows the pattern exhibited by urea, however, the specific and total uptake rate values are less than that for urea. The specific uptake rate varies from 0.0014 to 0.0044 h^{-1} when ammonium tracer addition is increased (from 0.01 to 0.04 μM). Uptake rate varies from 1.3 to 5.6 μmol N $m^{-3}h^{-1}$.

Nitrate shows a completely opposite trend of what has been observed in the cases of ammonium and urea. The specific uptake rate and uptake rate for nitrate decreases with increase in concentration. It shows maximum values when nitrate addition was 0.01 μM. It shows a marginal change in uptake rate when addition increases from 0.02 to 0.03 μM, however it drops down when higher amount of nitrate is added (0.04 μM).

The f-ratio almost reflects the change in nitrate uptake rate. It shows the maximum value of 0.47 when nitrate uptake rate is at the maximum, i.e., when tracer added to the sample is 0.01 μM. It shows the minimum value of nearly 0.10 for 0.04 μM of tracer, because at the nitrate uptake rate too the value drops down.

At lower tracer additions (with incubation time of 4 h), the uptake rate for nitrate is higher but as more tracer is added, the uptake rates for ammonium and urea become higher. For the lowest tracer concentration, nitrate and urea specific uptakes and uptake rates are comparable, similar to the results of the first experiment with 4-hour incubation. Specific uptake and uptake rates decreased for higher concentrations of the added nitrate tracer (figure 2). At this site, microphytoplankton cell count was the lowest among the three experimental sites during September–October 2002, while chlorophyll concentration was comparable (table 2). This indicates the larger contribution of pico- and nano-plankton to the total chlorophyll concentration at this site. Based on N uptake kinetic studies in the North Atlantic, it has been shown that pico-plankton have high capacity of ammonium uptake and this has been argued as evidence of their physiological preference (Harrison *et al* 1996). The high surface area-to-volume ratio of

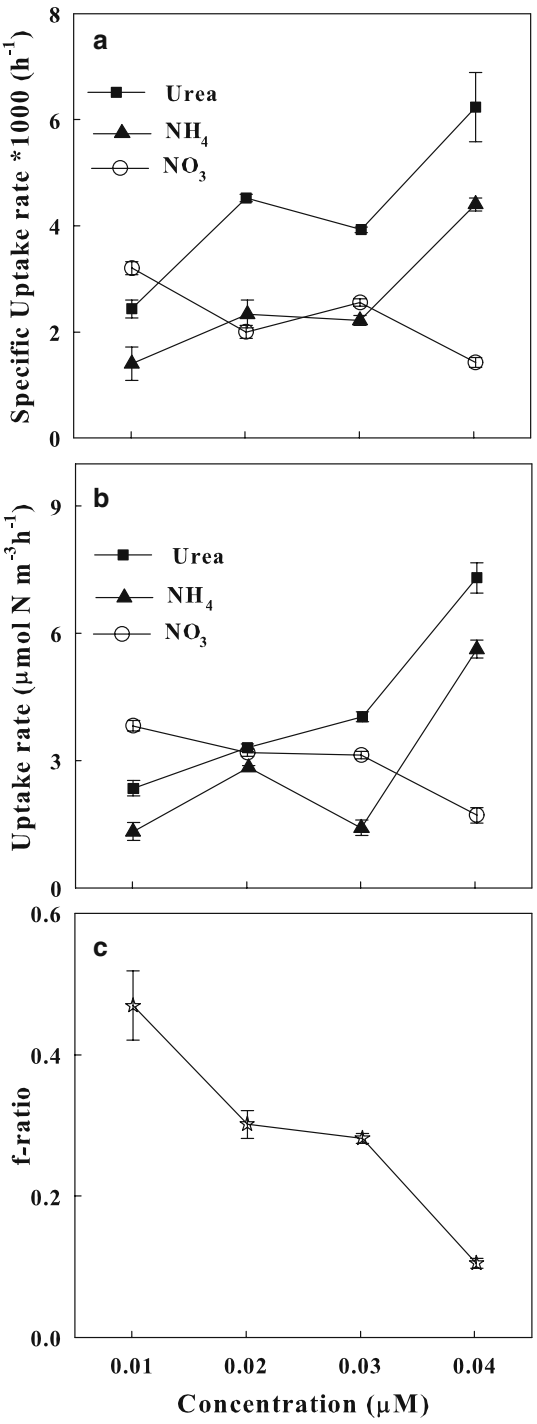

Figure 2. The result of Experiment 2 showing the variation in (**a**) specific uptake rate (h^{-1}), (**b**) uptake rate (µmol N $m^{-3}h^{-1}$) and (**c**) f-ratio with the increase in substrate added.

pico- and nano-plankton may provide them with a competitive advantage over microphytoplankton (Probyn 1992). Several studies have identified a positive relationship between the ratio of surface area to the volume of algae and their uptake rates (e.g., Hein *et al* 1995). Increase in ammonium and urea uptake rates with the added tracer concentrations in our experiment indicates the preference for reduced forms of nitrogen (figure 2). At the same time in the natural environment, the most important ammonium regenerators are in the smaller size classes (Hasegawa *et al* 2005). As the abundance of pico- and nano-plankton appear to be higher at the site, specific uptake and uptake rates of nitrate decreased with the added tracer concentrations (figure 2). Although ammonium regeneration is minimal for $\leq$4 h incubation apparent higher abundance of smaller size plankton do play a significant role. Suppression of nitrate uptake in the presence of ammonium has also been observed for the Arabian Sea (McCarthy *et al* 1999). The observed increase in the uptake rates of reduced form of nitrogen and decrease in the nitrate uptake rate exert a significant control on the f-ratios (figure 2). This is an indirect evidence of the importance of smaller size plankton in nitrogen-limited waters in the estimation of f-ratios. The scenario is limited to the conditions under which nano- and pico-plankton dominates the plankton community. Nevertheless, the result underscores the importance of measurements of interactions among multiple nutrient substrates for mixed plankton populations (species and size) in the natural environment.

3.3 *Experiment 3*

The results show a similar trend at both the stations. The specific uptake rate in the morning (06:00–10:00 h) is higher for all the tracers (except urea at one station) and remains the same for noon (10:00–14:00 h) and evening (14:00–18:00 h) incubations (figure 3). Most striking is the very high specific uptake rate for nitrate during morning at both the stations, which decreases drastically at noon. Photoinhibition due to excess surface light intensity could be a reason for the reduction in the specific uptake rates and uptake rates during noon. It is a well-known phenomenon in the tropical oceans (e.g., Falkowski and Owens 1978; Falkowski 1984). In addition, its effect on N uptake has been observed in different regions, e.g., the central Atlantic (Hu and Smith 1998) and the Ross Sea (Planas *et al* 1999). There is no report of photoinhibition in BOB, known to be cloudy most of the time in a year (Qasim 1977). In this respect, our results are the first to indicate photoinhibition in BOB during noon of a clear day. The ratio of productivities during morning, noon and evening is 4.5:1:1.2. Our analyses suggest that ^{15}N experiments carried out around local noon are likely to underestimate productivity by as much as 30–60%.

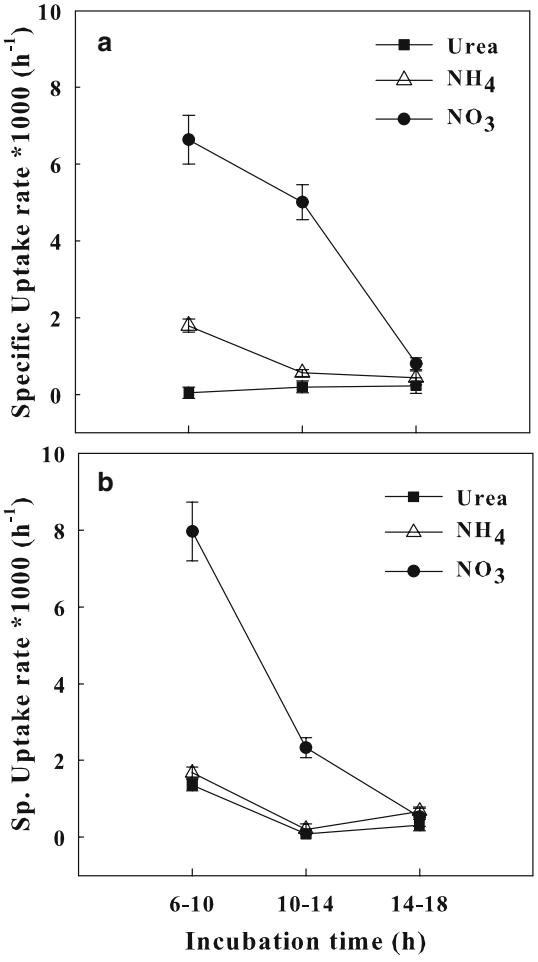

Figure 3. The specific uptake rate (h^{-1}) at (**a**) 17°N, 83°30°E and (**b**) 12°N, 88°E for the incubations at different times of the day.

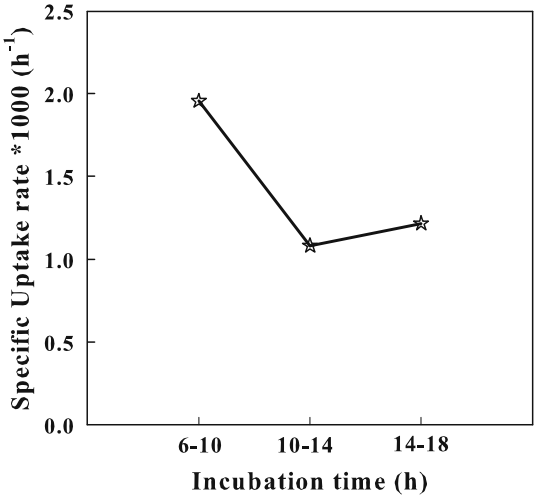

Figure 4. The specific uptake rate (h^{-1}) measured at 18°N, 70°E for incubations at different times of the day.

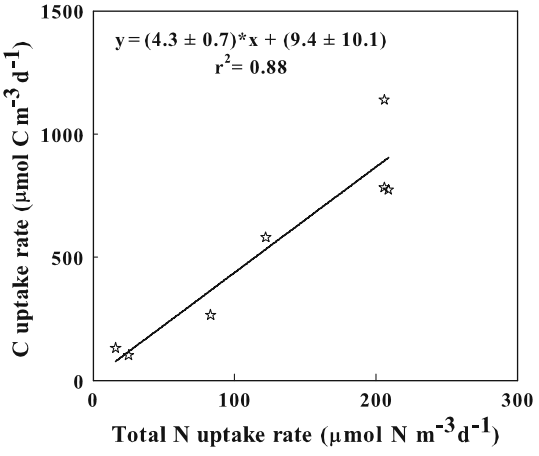

Figure 5. Comparison of carbon and nitrogen (sum of nitrate, ammonium and urea) uptake rates (µmol C (or N) $m^{-3}h^{-1}$) obtained from ^{13}C and ^{15}N experiments.

3.4 *Experiment 4*

This experiment is aimed to verify the results obtained from Experiment 3. The result from the Arabian Sea shows a similar trend as was found at the two BOB stations during April–May 2003. The specific uptake rate of carbon in the morning (06:00–10:00 h) is higher than that for noon (10:00–14:00 h) and evening (14:00–18:00 h) incubations (figure 4). The specific uptake rate does not vary much for the two latter intervals (figure 4). We attribute the suppression of the uptake during noon to the excess surface light intensity (∼400 Wm^{-2}). The ratio of productivities during morning, noon and evening is 1.9:1:1.1. Our results suggest that the experiments carried out around local noon are likely to underestimate productivity by ∼50% in the Arabian Sea.

3.5 *Experiment 5*

The variations between the carbon and nitrogen (the sum of nitrate, ammonium and urea) uptake rates at different depths are shown in figure 5. A significant correlation ($r^2 = 0.88$) is found between the carbon and nitrogen uptake rates (figure 5). The slope (4.3 ± 0.7) of the straight line is slightly off the Redfield ratio (6.6) but such variations (or even more) have also been reported elsewhere, which mainly depends upon the plankton composition (Rees *et al* 2002; Collos *et al* 2006). This method provides a better alternative to measure new and total primary productivities simultaneously on the same sample. This approach definitely minimizes some of uncertainties associated with the nitrogen and carbon assimilation rates, particularly the uncertainty due to the differences in the measurement techniques.

4. Conclusions

Our work emphasises the precautions that need to be taken for ^{15}N experiments in the oligotrophic waters. Urea is the first nitrogen species to be depleted by plankton uptake in the nitrogen-limited waters of BOB. This depletion limits the specific uptake rate and uptake rate of urea in the later hours of incubation, while in the case of nitrate and ammonium, such dilution is not significant. Higher values of assimilation of ammonium during the early hours of incubation may be attributed to storage rather than to growth; lower values during the later hours are possibly controlled by excess assimilation during the early hours. Lower assimilation of nitrate coincides with higher assimilation of reduced forms of nitrogen (ammonium and urea) in the early hours. This is because relatively nitrate is energetically more difficult to assimilate. An overall increase in nitrate assimilation during the 4-hour incubation could be due to the diatom dominated microplankton population. Increasing light intensity in the early hours (10:00–12:00 h) also contributes to the increase in nitrate assimilation in the initial hours. However, the increase in later hours cannot be explained by the variation in light intensity alone. These variations in uptake rates of different N-species lead to change in the f-ratio from 0.28 (after 2 hours) to 0.42 (after 4 hours). Measured f-ratios may be erroneous for less and more than 4 h of incubations. Average results of 4 h of incubation may provide better estimation of uptake rates and f-ratios. Indirect evidence suggests that the estimation of f-ratio is also influenced by the higher abundance of pico- and nano-plankton.

Photoinhibition due to excess surface light intensity could be a reason for the reduction in the specific uptake rates and uptake rates during noon and thus experiments done at noon might significantly underestimate the productivity (∼up to 50%). Photoinhibition in BOB and in the Arabian Sea during noon of a clear day has been observed for the first time. The combined ^{13}C and ^{15}N method seems more promising than the traditional methods. This approach at least minimizes the uncertainty due to the differences in the measurement techniques. These results are indicative of the response of a mixed population of microplankton dominated by diatom to the input of different nitrogenous compounds under nitrogen-limited conditions.

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References

Balachandran K K, Laluraj C M, Jyothibabu R, Madhu N V, Muraleedharan K R, Vijay J G, Maheswaran P A, Mohammed Ashraff T T, Nair K K C and Achuthankutty C T 2008 Hydrography and biogeochemistry of the northwestern Bay of Bengal and the northeastern Arabian Sea during winter monsoon; *J. Mar. Systems* **73** 76–86.

Berg G M, Balode M, Purina I, Bekere S, Bechemin C and Maestrini S Y 2003 Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen; *Aquatic Microb. Ecol.* **30** 263–274.

Bronk D A, Glibert P M, Malone T C, Banahan S and Sahlsten E 1998 Inorganic and organic nitrogen cycling in Chesapeake Bay: Autotrophic versus heterotrophic processes and relationships to carbon flux; *Aquat. Microb. Ecol.* **15** 177–189.

Casey J, Lomas M W, Mandecki J and Walker D 2007 Prochlorococcus contributes to new production in the Sargasso Sea deep chlorophyll maximum; *Geophys. Res. Lett.* **34** L10604, doi: 10.1029/2006GL028725.

Collos Y, Lespilette M, Vaquer A, Laabir M and Pastoureaud A 2006 Uptake and accumulation of ammonium by *Alexandrium catenella* during nutrient pulses; *African J. Mar. Sci.* **28(2)** 313–318.

Dugdale R C and Goering J J 1967 Uptake of new and regenerated forms of nitrogen in primary productivity; *Limnol.Oceanogr.* **12** 196–206.

Dugdale R C and Wilkerson F P 1986 The use of ^{15}N to measure nitrogen uptake in eutrophic oceans; experimental considerations; *Limnol. Oceanogr.* **31** 673–689.

Eppley R W and Peterson B J 1979 Particulate organic matter flux and planktonic new production in the deep ocean; *Nature* **282** 677–680.

Falkowski P O 1984 Physiological responses of phytoplankton to natural light regimes; *J. Plankton Res.* **6(2)** 295–307.

Falkowski P G and Owens T 1978 Effects of light intensity on photosynthesis and dark respiration in six species of marine phytoplankton; *Mar. Biol.* **45** 289–295.

Fernandez E, Maranon E, Harbour D S, Kristiansen S and Heimdal B R 1996 Patterns of carbon and nitrogen uptake during blooms of Emiliania huxleyi in two Norwegian fjords; *J. Plankton Res.* **18** 2349–2366.

Gilbert P M, Lipschultz F, McCarthy J J and Altabet M A 1982 Isotope dilution models of uptake and remineralization of ammonium by marine plankton; *Limnol. Oceanogr.* **27(4)** 639–650.

Harrison W G, Harris L and Irwin B 1996 The kinetics of nitrogen utilization in the oceanic mixed layer: Nitrate and ammonium interactions at nanomolar concentrations; *Limnol. Oceanogr.* **41** 16–32.

Hasegawa T, Fukuda H and Koike I 2005 Effects of glutamate and glucose on N cycling and the marine plankton community; *Aquat. Microb. Ecol.* **41** 125–130.

Hein M, Pedersen M F and Sand-Jensen K 1995 Size-dependent nitrogen uptake in micro- and macro-algae; *Mar. Ecol. Prog. Ser.* **118** 247–253.

Hu S H and Smith W O 1998 The effects of irradiance on nitrate uptake and dissolved organic nitrogen release by phytoplankton in the Ross Sea; *Cont. Shelf Res.* **18** 971–990.

Ittekkot V, Nair R R, Honjo S, Ramaswamy V, Bartsch M, Manginini S and Desai B N 1991 Enhanced particle fluxes in Bay of Bengal induced by injection of fresh water; *Nature* **351** 385–387.

Jyothibabu R, Madhu N V, Habeebrehman H, Jayalakshmy K V, Nair K K C and Achuthankutty C T 2010 Re-evaluation of 'paradox of mezozooplankton' in the eastern Arabian Sea based on ship and satellite observations; *J. Mar. Systems* **81** 235–251.

Kokkinakis S A and Wheeler P A 1987 Nitrogen uptake and phytoplankton growth in coastal upwelling regions; *Limnol. Oceanogr.* **32** 1112–1123.

Kumar M D, Sarma V V S S, Ramaiah N and Gauns M 1998 Biogeochemical significance of transparent exopolymer particles in the Indian Ocean; *Geophys. Res. Lett.* **25** 81–84.

Kumar S, Ramesh R, Sardesai S and Seshshayee M S 2004a High new production in the Bay of Bengal: Possible causes and implications; *Geophys. Res. Lett.* **31** L18304, doi: 10.1029/2004GL021005.

Kumar S, Ramesh R, Bhosle N B, Sardesai S and Seshshayee M S 2004b Natural isotopic composition of nitrogen in suspended particulate matter in the Bay of Bengal; *Biogeosci. (EGU journal)* **1** 63–70.

Kumar S, Ramesh R, Seshshayee M S, Sardesai S and Patel P P 2005 Signature of terrestrial influence on nitrogen isotopic composition of suspended particulate matter in the Bay of Bengal; *Curr. Sci.* **88(5)** 770–774.

Madhupratap M, Prasanna Kumar S, Bhattathiri P M A *et al* 1996 Mechanism of the biological response to winter cooling in the northeastern Arabian Sea; *Nature* **384** 549–552.

Madhupratap M, Gauns M, Ramaiah N, Prasanna Kumar S, Muraleedharan P M, De Souza S N, Sardessai S and Muraleedharan U 2003 Biogeochemistry of the Bay of Bengal: Physical, chemical and primary productivity characteristics of the central and western Bay of Bengal during summer monsoon 2001; *Deep-Sea Res. II* **50(3)** 881–896.

McCarthy J J, Garside C and Nevins J 1999 Nitrogen dynamics during the Arabian Sea northeast monsoon; *Deep-Sea Res. II* **46** 1623–1664.

Mulholland M and Lomas M 2008 Nitrogen uptake and assimilation; In: *Nitrogen in the marine environment* (eds) Capone D G, Bronk D A, Mulholland M R and Carpenter E J (Springer) doi: 10.1016/B978-0-12-372522-6.00007-4.

Naqvi S W A, Noronha R J, Somasundar K and Sen Gupta R 1990 Seasonal changes in the denitrification regime of the Arabian Sea; *Deep-Sea Res. I* **37** 593–611.

Naqvi S W A, Shailaja M S, Kumar M D and Sen Gupta R 1996 Respiration rates in subsurface waters of the northern Indian Ocean: Evidence for low decomposition rates of organic matter within the water column in the Bay of Bengal; *Deep Sea Res. I* **43** 73–81.

Olson D B, Hitchcock G L, Fine R A and Warren B A 1993 Maintenance of the low-oxygen layer in the central Arabian Sea; *Deep-Sea Res. II* **40** 673–685.

Owens N J P and Rees A P 1989 Determination of nitrogen-15 at sub-microgram levels of nitrogen using automated continuous-flow isotope ratio mass spectrometer; *Analyst* **114** 1655–1657.

Paul J T, Ramaiah N and Sardesai S 2008 Nutrient regimes and their effect on distribution of phytoplankton in the Bay of Bengal; *Mar. Environ. Res.* **66** 337–344.

Planas D, Agusti S, Duarte C M, Granata T C and Merino M 1999 Nitrate uptake and diffusive nitrate supply in the central Atlantic; *Limnol. Oceanogr.* **44** 116–126.

Prasanna Kumar S, Madhupratap M, Dileep Kumar M, Muraleedharan P M, De Souza S N, Gauns M and Sarma V V S S 2001 High biological productivity in the central Arabian Sea during the summer monsoon driven by Ekman pumping and lateral advection; *Curr. Sci.* **81(12)** 1633–1638.

Prasanna Kumar S, Muraleedharan P M, Prasad T G, Gauns M, Ramaiah N, De Souza S N, Sardesai S and Madhupratap M 2002 Why is the Bay of Bengal less productive during summer monsoon compared to the Arabian Sea?; *Geophys. Res. Lett.* **29(24)** 2235, doi: 10.1029/2002GL016013.

Prasanna Kumar S, Nuncio M, Narvekar J, Kumar A, Sardesai S, De Souza S N, Gauns M, Ramaiah N and Madhupratap M 2004 Are Eddies nature's trigger to enhance biological productivity in the Bay of Bengal?; *Geophys. Res. Lett.* **31** L07309, doi: 10.1029/2003GL019274.

Prasanna Kumar S, Nuncio M, Ramaiah N, Sardesai S, Narvekar J, Fernandes V and Paul J T 2007 Eddy-mediated biological productivity in the Bay of Bengal during fall and spring intermonsoons; *Deep-Sea Res. I* **54** 1619–1640.

Probyn T A 1992 The inorganic nitrogen nutrition of phytoplankton in the southern Benguela: New production, phytoplankton size and implications for pelagic foodwebs; *S. Afr. J. Mar. Sci.* **12** 411–420.

Qasim S Z 1977 Biological productivity of the Indian Ocean; *Indian J. Mar. Sci.* **6** 122–137.

Rao C K, Naqvi S W A, Kumar M D, Varaprasad S J D, Jayakumar D A, George M D and Singbal S Y S 1994 Hydrochemistry of Bay of Bengal: Possible reasons for a different water-column cycling of carbon and nitrogen from the Arabian Sea; *Mar. Chem.* **47** 279–290.

Rees A P, Malcolm E, Woodward S, Robinson C, Cummings D G, Tarran G A and Joint I 2002 Size-fractionated nitrogen uptake and carbon fixation during a developing coccolithophore bloom in the North Sea during June 1999; *Deep-Sea Res. II* **49** 2905–2927.

Sambrotto R N 2001 Nitrogen production in the northern Arabian Sea during the spring intermonsoon and southwest monsoon seasons; *Deep-Sea Res. II* **48** 1173–1198.

Sardesai S, Ramaiah N, Prasanna Kumar S and de Sousa S N 2007 Influence of environmental forcings on the seasonality of dissolved oxygen and nutrients in the Bay of Bengal; *J. Mar. Res.* **65** 301–316.

Sarma V V S S 2002 An evaluation of physical and biogeochemical processes regulating the oxygen minimum zone in the water column of the Bay of Bengal; *Global Biogeochem. Cycles* **16(4)**, doi: 10.1029/2002GB001920.

Schott F and McCreary J Jr 2001 The monsoon circulation of the Indian Ocean; *Prog. Oceanogr.* **51** 1–123.

Shetye S R 1999 Dynamics of circulation of the waters around India; In: *Ocean Science: Trends and Future directions* (ed.) B L K Somayajulu (New Delhi: Indian National Science Academy), pp. 1–21.

Slawyk G, Collos Y and Auclair J C 1977 The use of the ^{13}C and ^{15}N isotopes for the simultaneous measurement of carbon and nitrogen turn over rates in marine phytoplankton; *Limnol. Oceanogr.* **22** 925–932.

Suresh T, Desa E, Desai R G P, Jayaraman A and Mehra P 1996 Photosynthetically available radiation in the central and eastern Aabian Sea; *Curr. Sci.* **71(11)** 883–887.

Strickland J D H and Parson T R 1972 A practical handbook of seawater analysis; Fisheries Research Board, Canada, pp. 127–130.

Swallow J 1984 Some aspects of the physical oceanography of the Indian Ocean; *Deep-Sea Res.* **25(31)** 639–650.

UNESCO 1994 Protocol for the Joint Global Ocean Flux Study (JGOFS) core measurements; (IOC Manuals and Guides No. 29) SCOR, pp. 130–135.

Varela M M, Bode A, Fernandez E, Gonzalez N, Kitidis V, Varela M and Woodward E M S 2005 Nitrogen uptake and dissolved organic nitrogen release in planktonic communities characterised by phytoplankton size–structure in the Central Atlantic Ocean; *Deep-Sea Res. I* **52** 1637–1661.

Wafar M V M, Le Corre P and L'Helguen S 1995 f-ratios calculated with and without urea uptake in nitrogen uptake by phytoplankton; *Deep-Sea Res.* **42** 1669–1774.

Watts L J and Owens N J P 1999 Nitrogen assimilation and f-ratio in the northwestern Indian Ocean during an intermonsoon period; *Deep-Sea Res. II* **46** 725–743.

Wilkerson F and Dugdale R C 2008 Coastal Upwelling. In: *Nitrogen in the marine environment* (eds) D G Capone, D A Bronk, M R Mulholland and E J Carpenter (Springer) doi: 10.1016/B978-0-12-372522-6.00007-4.

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