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**Effect of incubation
time and substrate
concentration**

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Effect of incubation time and substrate concentration on N-uptake rates by phytoplankton in the Bay of Bengal

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Abstract

We report here the results of three experiments, which are slight variations of the ^{15}N method (JGOFS protocol) for determination of new production. The first two test the effect of (i) duration of incubation time and (ii) concentration of tracer added on the uptake rates of various N- species (nitrate, ammonium and urea) by marine phytoplankton; while the third compares in situ and deck incubations from dawn to dusk. Results indicate that nitrate uptake can be underestimated by experiments where incubation times shorter than 4 h or when more than 10% of the ambient concentration of nitrate is added prior to incubation. The f-ratio increases from 0.28 to 0.42 when the incubation time increases from two to four hours. This may be due to the observed increase in the uptake rate of nitrate and decrease in the urea uptake rate. Unlike ammonium [$y=2.07x-0.002$ ($r^2=0.55$)] and urea uptakes [$y=1.88x+0.004$ ($r^2=0.88$)], the nitrate uptake decreases as the concentration of the substrate (x) increases, showing a negative correlation [$y=-0.76x+0.05$ ($r^2=0.86$)], possibly due to production of glutamine, which might suppress nitrate uptake. This leads to decline in the f-ratio from 0.47 to 0.10, when concentration of tracer varies from 0.01 to 0.04 μM . The column integrated total productions are 519 $\text{mg C m}^{-2} \text{d}^{-1}$ and 251 $\text{mg C m}^{-2} \text{d}^{-1}$ for in situ and deck incubations, respectively. The ^{14}C based production at the same location is $\sim 200 \text{ mg C m}^{-2} \text{d}^{-1}$, which is in closer agreement to the ^{15}N based total production measured by deck incubation.

1. Introduction

Nitrogen isotopes have been very useful in delineating the various marine processes, particularly those related to marine algal production. Nitrogen, along with other major elements like carbon, hydrogen and oxygen, is an indispensable element for phytoplankton growth. Nitrogen is found in many forms depending on the redox condition of ocean water. The species predominant in reducing environments are: NH_4^+ and N_2 ,

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and in oxic environments are: N_2O , NO , NO_2^- and NO_3^- . In addition, urea and dissolved organic nitrogen are also present. The availability of these forms in different oceans depends on chemical, biological as well as physical interactions. In addition, there is considerable variation in the concentration of these species on a seasonal time scale.

5 Seasonal changes are prominent in the northern Indian Ocean (Arabian Sea and Bay of Bengal), which is affected by seasonal reversal of winds. The form of nitrogen incorporated by phytoplankton gives an insight into the new and regenerated productions. New production is defined as the part of primary production supported by external nitrogenous inputs (e.g., nitrate of upwelled, riverine or eolian origin introduced into the euphotic zone), whereas regenerated production is defined as that part of primary production which sustains on recycled nutrients (e.g. ammonium and urea), in the euphotic zone itself (Dugdale and Goering, 1967). However, Dore and Karl (1996) have pointed out that there can be a contribution to the regenerated production from nitrate, due to bacterial nitrification within the photic zone; conceivably, new production could be also
10 due to extraneous sources of ammonium (e.g. aerosols). The new and regenerated productions are traditionally estimated using the ^{15}N tracer technique, where samples are incubated after adding isotopically enriched (>99 atom%) tracers of nitrate (for new production) or ammonium and urea (for regenerated production) salts to assess the uptake of different species of nitrogen. This approach is valid when nitrification in the euphotic zone is minimal or absent and there are no significant external sources of urea and ammonium to the photic zone. Under steady state, the new production is considered equal to export production, which is the part of primary production settling out of the euphotic zone (Eppley and Peterson, 1979). Once it leaves the euphotic zone in the form of export production, it isolates the photosynthetically fixed carbon
15 from the atmosphere for long periods of time. Thus, this process is a significant step in the global carbon cycle (Falkowski et al., 1998).

20 New production estimates have been made in different regions of the world ocean (Dugdale et al., 1992; McCarthy et al., 1999; Watts and Owens, 1999; Sambrotto, 2001; L'Helguen, 2002; Dham et al. 2002; Rees et al., 2002). These are based on the

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incorporation of “trace” addition of ^{15}N -labelled NO_3 into phytoplankton during incubation experiments. The details of the experimental procedure followed in these studies are somewhat variable; e.g. time of incubation could vary between 2 and 24 h (the latter in the case which includes dark incubation). Though in general, the JGOFS protocol (JGOFS, 1996) is followed, a number of questions arise regarding these procedures. These are: (a) what is the effect of duration of the incubation on the uptake rate of nutrients by the phytoplankton? Are there significant variations within the time of 2–4 h as recommended by the JGOFS protocol? (b) What is the effect on uptake rate if the substrate concentration increases while keeping the incubation time fixed? (c) f-ratio, the ratio of new to total production (Eppley and Peterson, 1979), has been calculated by different workers (Wafar et al., 1995; Watts and Owens 1999) for different oceans but what happens to the f-ratio in cases (a) and (b)? (d) The JGOFS protocol suggests simulated in situ incubation for ^{15}N uptake experiments for durations of 2 to 4 h. Longer incubation times could lead to problems such as increased regeneration of ammonium and urea, which will also be taken up along with nitrate. However, primary production (PP) experiments using ^{14}C are preferably done in situ for 12 h (Madhupratap et al., 2003). To facilitate comparison of PP measured and new production estimated from ^{15}N experiments, it is essential to know whether the results of in situ and simulated in situ incubation experiments using ^{15}N from dawn to dusk are comparable.

In this paper we intend to discuss the above questions based on ^{15}N uptake experiments performed in the surface waters of the Bay of Bengal (BOB). This study forms a part of Bay of Bengal Process Study (BOBPS), a programme intended to estimate the biogeochemical fluxes in the BOB (Prasanna Kumar et al., 2002; Madhupratap et al., 2003), similar to JGOFS in the Arabian Sea.

2. Materials and methods

Sampling was done during September–October 2002 onboard ORV Sagar Kanya. The tracers used for experiments were 99 atom% ^{15}N enriched sodium nitrate, ammonium

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chloride and urea procured from SIGMA-ALDRICH. Details of the individual experiments are discussed below.

2.1. Experiment 1

The aim of this experiment was to observe the variation in uptake rates of different N-species with varying durations of incubation. The JGOFS protocol was followed: surface water samples were collected (at 17°56'33.1" N, 87°54'38.6" E) in one litre Polycarbonate NALGENE bottles, 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 0.01 μM of the respective tracer was added. After the tracer addition, samples were kept for incubation at 10:00 hrs, in a deck incubator with flowing surface sea water. No neutral density filters were used as the samples were from the sea surface. 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 under low vacuum. The samples were dried and kept for further mass spectrometric analysis.

2.2. Experiment 2

This experiment was intended to find out the uptake rate variations of different nitrogenous species by the phytoplankton due to varying concentration of substrate. For this experiment too, surface water samples were collected (at 20°0'15.0" N, 87°59'36.4" E) in one litre bottles and divided into three sets of four each. But the concentration added in different bottles of each set was different. The concentrations added were 0.01, 0.02, 0.03 and 0.04 μM of the respective tracers in different bottles of the respective sets. These amount to 9%, 18%, 27% and 36% respectively of the nitrate concentration in the surface waters. For ammonium and urea, these are much in excess of the ambient concentrations (see Sect. 2.5). Incubation was done on deck for 4 h symmetrical to local noon i.e. from 10:00 to 14:00 hrs. Running seawater maintained the temperature during incubation. Neutral density filters were not used as in experiment 1. After the

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incubation the samples were filtered and preserved for analysis as described earlier.

2.3. Experiment 3

To estimate the difference in the uptake rates due to deck and in situ incubations, samples were collected (at 14°0'17.2" N, 80°59'54.9" E) from surface, 20, 40 and 60 m depth and transferred to six one litre bottles from each depth. Three bottles were used for in situ and the other three for deck experiments for each of the three different tracers. Ambient concentration of nitrate was measured by the column reduction method manually. In the case of urea and ammonium, the ambient concentration measurements could not be performed due to logistics problems; however they were estimated indirectly using zooplankton biomass (see Sect. 2.5). The euphotic zone in the Bay of Bengal is well oxygenated; the expected ambient ammonium and urea concentrations here are low, hence, a constant concentration of 0.01 μM for ammonium and 0.03 μM for urea was added for all the four depths. No literature exists for the relationship between oxygen and ammonium concentration for the Bay of Bengal. However, US JGOFS data for Arabian Sea suggests absence of ammonium in surface layers where water is well oxygenated as in the Bay of Bengal. An attempt to add less than 10% of ambient concentrations was made in the case of nitrate, which lead to the addition of 0.03, 0.02, 0.03 and 0.6 μM for surface, 20, 40 and 60 m samples. A SECHHI disk was used to measure the light attenuation with depth. It was found that light was less than 1% of the surface value at ~60 m depth. Further, chlorophyll-concentrations were near zero below this depth. The light conditions for the deck incubation were simulated using well calibrated neutral density filters and also the continuous flow of seawater from 5m depth was maintained in order to maintain the temperature. The neutral density filters used were such that equivalent depths were 4, 41, 55 and 77 m. The incubation was done for 12 h (from dawn to dusk) in both cases and subsequently, the samples were filtered and preserved for analysis.

In all the three experiments above duplicate analysis was made wherever possible (Table 1).

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2.4. Analysis

Analysis of samples was performed in the laboratory using a Carlo Erba Elemental Analyser interfaced via Confloll to a Finnigan Delta Plus mass spectrometer. The major parameters measured were PON and ^{15}N atom% in post incubation samples. PON was measured as described by Owen and Rees (1989) with modification in oxygen injection time to reduce the effect of contaminant N introduced by oxygen injected for combustion. In this method the integration of ion beam areas (m/z 28+29+30), after calibration against standard material (IAEA-NO-3, KNO_3) provides a quantitative measure of PON. The advantage of technique lies in the simultaneous measurement of PON and isotope ratio in the same sample. Due to the difficulty in accommodating the whole 47 mm diameter GF/F filter papers (on which filtration of samples were done) in the carousel of elemental analyser, these were cut into four/two equal parts for analysis. The maximum difference in PON measurements for duplicate samples was found to be around 10%. The coefficient of variation for ^{15}N atom% measurement is less than 1% for nitrate and urea samples while it was found to be 3% in the case of ammonium. The $\delta^{15}\text{N}$ measurement for standard (IAEA-NO-3, KNO_3) yielded $4.91 \pm 0.30\%$ for $n=13$ against the IAEA quoted value of 4.7‰.

For the calculation of uptake rate several equations are in use (Nees et al 1962, Dugdale and Goering 1967, Eppley et al., 1977) and almost all equations rest on several assumptions such as neglecting isotope dilution by remineralization of organic matter producing unlabelled ammonium and exchange of particulate nitrogen during incubation. We have used equation given by Dugdale and Wilkerson (1986). This equation takes care of 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 taken at the end of incubation:

$$V_t = {}^{15}\text{N}_{xs} / [({}^{15}\text{N}_{enr} - \langle F \rangle) * t]$$

where, ${}^{15}\text{N}_{enr}$ is atom% ^{15}N in the initially labelled fraction, t is the incubation time,

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$^{15}\text{N}_{xs}$ is atom% excess in sample after incubation, $\langle F \rangle$ is natural abundance of ^{15}N . The uptake rate $\rho(t)$ (N taken up in concentration unit) is calculated using V_t and PON at the end of incubation [PON (t)]: $\rho(t)=V_t*\text{PON}(t)$.

2.5. Hydrodynamic conditions and nutrients

5 BOB is a semi-enclosed tropical basin and is a part of the northern Indian Ocean which experiences the seasonal changes in oceanography and climatic conditions due to the monsoon system. BOB receives a large quantity of freshwater from the rivers draining into it. This riverine freshwater input causes a considerable variation in surface salinity, which varied from 21 to 35 psu during the study period. The salinities at the sites of
10 experiment 1 and 2 were 29.2 and 28.4 psu and are affected by fresh water influx. However at the site of experiment 3 surface salinity was 33.4 psu. The riverine inputs are a potential source of nutrients like phosphate and silica to the Bay. 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. Sea surface temperature (SST) varied
15 28.2 to 30.5°C. SST along with other meteorological and hydrodynamic parameters at the experimental sites are listed in Table 2.

The ambient nitrate concentration required for the uptake calculation was measured by column reduction technique (Strickland and Parson, 1972). The values are listed in Table 2. Ammonium and urea concentrations have been calculated as follows: The
20 regeneration of ammonium and urea by zooplankton is well known (Mullin et al., 1975; Jawed 1973). Mesozooplankton biomass in this season in BOB ranged from 0.5 to 1.0 mL.m⁻³. However, microzooplankton had poorer biomass than Arabian Sea (avg. 45 org. L⁻¹). Based on the equations given by Wiebe et al. (1975) the zooplankton biomass was converted into dry weight and using average ammonium and urea excretion rates of 0.59 and 0.32 mg at-N (g dry wt)⁻¹ d⁻¹, the release rates were calculated
25 for 12 h residence time of zooplankton in mixed layer (Wafar et al., 1986). According to this calculation, the ammonium and urea concentrations in the site (experiment-3)

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were found to be 0.0136 and 0.0036 μm , respectively. Considering the uncertainties involved in equations used for the calculation, the above values could well be near zero.

3. Results and discussion

3.1. Experiment 1

5 3.1.1. Urea

Results from experiment 1 suggest that both specific uptake rate and the uptake rate are highest for N-uptake from urea (Fig. 1) in the nutrient poor waters of the Bay. This observation is similar to that of Rees et al. (2002), who observed urea to be the most preferred substrate in the oligotrophic North Sea. However our value for the average uptake rate from urea is only one third of the value obtained by Rees et al. (2002) for similar concentration of substrate added. The specific uptake rate for urea increases for incubation time more than 2 h, but declines for incubation time more than 3 h. This significant decline is also exhibited by the uptake rate for urea. Uptake rates range from a maximum of 2.48 $\mu\text{g at-N m}^{-3}\text{h}^{-1}$ to a minimum of 1.56 $\mu\text{g at-N m}^{-3}\text{h}^{-1}$.
15 These values are comparable in magnitude with values obtained by others elsewhere (McCarthy et al., 1999; Cochlan et al., 2001).

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 incubation time >1 h, and remained constant for higher incubation times. The uptake rate for ammonium showed a maximum of nearly 0.74 $\mu\text{g at-N m}^{-3}\text{h}^{-1}$ and a minimum of 0.38 $\mu\text{g at-N m}^{-3}\text{h}^{-1}$. These values are comparable to those reported by Rees et al. (2002) for ammonium uptake rate in the oligotrophic North Sea, extrapolated to the same substrate concentration. It is known that in ammonium poor waters, ammonium is taken up as soon as it becomes

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available (Gilbert et al., 1982). For e.g. phytoplanktons growing in ammonium deprived cultures can assimilate ammonium at a much faster rate compared to their growth rate (McCarthy and Goldman, 1979).

3.1.3. Nitrate

5 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 times upto 2 h, but for 3 and 4 h incubations, it is slightly higher. The uptake rate varies within a narrow range of 0.92 to 1.5 $\mu\text{g at-N m}^{-3}\text{h}^{-1}$, values comparable to those obtained by Rees et al. (2002) for North Sea waters.

10 These 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 (defined as the ratio of the uptake rate of NO_3^- and uptake rates of ($\text{NO}_3^- + \text{NH}_4^+ + \text{Urea}$)), almost follows the pattern of NO_3^- uptake rate. There is a significant increase in the f-ratio for incubation time greater than 3 h, from 0.29 to 0.42. This is partly due to the significant decrease (2.48 to 1.56 $\mu\text{g at-N m}^{-3}\text{h}^{-1}$) of the urea uptake rate.

15 The change in uptake rates of individual N-species within 4 h of incubation indicates the high demand for ammonium in the initial hours so that ammonium may become limited in the third and fourth hours due to rapid initial uptake. In contrast, the uptake of nitrate is less prominent in first 2 h but rises in the third and fourth hours. This may be because unlike reduced species such as urea and ammonium, nitrate has to be reduced in the cells before uptake, which therefore has a larger time constant. The effect of these variations on f-ratio is notable. It appears that f-ratio may be underestimated if incubation is being done 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 in later hours of incubation. The f-ratio after 20 25 4 h of incubation is one and a half times more than that observed after 2 h.

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3.2. Experiment 2

As seen in the case of experiment 1, urea seems to be the most preferred substrate in this water, in general. When concentration added is $0.01 \mu\text{M}$ for all the three tracers, the rate of uptake after 4 h of incubation is highest for nitrate followed by urea (Fig. 2).

5 But when the concentration of substrate added is increased the specific uptake as well as uptake rate for urea becomes higher.

There is a significant increase in sp. uptake rate from 0.0024 to 0.0062 h^{-1} when the concentration of urea added increased from 0.01 to $0.04 \mu\text{M}$. The uptake rate of urea also increased from 2.3 to $7.3 \mu\text{g at-N m}^{-3}\text{h}^{-1}$. There is a significant linear correlation between the urea-N uptake rate (y) and the substrate concentration (x): $y=1.88x+0.004$ ($r^2=0.88$).

Ammonium closely follows the pattern exhibited by the urea, however, the sp. uptake rate and uptake rate values are less than that for urea. The sp. uptake rate varies from 0.0014 to 0.0044 h^{-1} when ammonium concentration added increased from 0.01 to $0.04 \mu\text{M}$. Uptake rate varies from 1.3 to $5.6 \mu\text{g at-N m}^{-3}\text{h}^{-1}$. There exists a significant linear correlation between the ammonium-N uptake rate (y) and the substrate concentration (x): $y=2.07x-0.002$ ($r^2=0.55$). Similar linear correlations for ammonium and urea uptakes have been reported by Rees et al. (2002). Their slopes are lower because their experiments pertain to a plankton bloom, whereas ours do not.

20 Nitrate shows 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 $0.01 \mu\text{M}$. It shows a marginal change in uptake rate when concentration changed from 0.02 to $0.03 \mu\text{M}$, however it drops down when concentration added is increased to $0.04 \mu\text{M}$. There is a significant negative correlation between the nitrate-N uptake rate (y) and the substrate concentration (x): $y=-0.76x+0.05$ ($r^2=0.86$).

25 The f-ratio almost reflects the change in nitrate uptake rate. It shows maximum value of 0.47 when nitrate uptake rate is maximum i.e. when concentration added to

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the sample is 0.01 μM . It shows minimum value of nearly 0.10 for 0.04 μM addition, because at this concentration the nitrate uptake rate drops down.

At lower concentration level (with incubation time 4 h), the uptake rate for nitrate is more but as the concentration of substrate increases, the uptake rates for ammonium and urea are higher. The reason for the decrease in the nitrate uptake for higher concentration may be the build up of ammonium due to its regeneration in the bottle. This ammonium might be preferred leading to increase in concentration of glutamine on reduction. Glutamine is known to suppress the synthesis of enzyme needed for reduction of nitrate and hence suppresses its uptake (Dortch, 1990; Flynn et al., 1997; Flynn, 1998). Suppression of nitrate uptake in presence of ammonium has also been observed for Arabian Sea (McCarthy et al., 1999). The f-ratio decreases drastically with increase in tracer concentration. To circumvent this effect it is important to add less than 10% of the ambient nitrate concentration to get a reasonably correct estimate of the f ratio.

If the above results are extrapolated to in situ conditions, as long as the surface nutrients are close to zero as observed, a small amount of extraneous input can increase the new production. If the extraneous input is used up quickly enough, then the conditions are restored for further uptake as and when nutrient pulses are introduced. On the other and, if the extraneous input is quite large, the initial surge in new production may not be sustained at a high level for prolonged periods. This is in contrast to the observation of Rees et al. (2002), who observed a linear increase in the nitrate uptake rate with substrate concentration. However, it is worth noting that their experiments were conducted on a coccolith bloom, whereas the waters of the Bengal seldom support a bloom.

It is interesting to compare the uptake and specific uptake rates of the 4 h incubations at 0.01 μM nitrate addition from the two experiments above (see Table 1). Values obtained in the second station (experiment 2) are significantly larger than those from the first (experiment 1). However it is to be noted that there is no significant difference in the f-ratios. Table 2 shows that the hydrodynamic conditions are more or less same

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on both the days. The reason for the difference in uptake rates, therefore, may be attributed to the difference in available light levels on these two days. On the day of the first experiment, the sky was intermittently overcast (during the incubation period), whereas on the day of the second experiment, it was bright and sunny. Day to day variations in uptake rates can be quite significant depending on cloudiness, in the Bay of Bengal. Gomes et al. (2000) have observed that column productivity in the Bay is significantly controlled by cloudiness.

3.3. Experiment 3

The nitrogen uptake rates from ammonium (triangle), urea (square) and nitrate (circle) are plotted as a function of depth in Fig. 3 both for deck (filled symbols) and in situ (open symbols) incubations. Results of the deck experiments are plotted such that the depths correspond to the light levels provided, rather than the actual depths from which the water samples were taken. The analytical uncertainties in the calculated uptake rates are shown as error bars corresponding to one standard deviation. It is seen that the surface values (2 to 4 m depth) are the same for in situ and deck incubations, within errors. Ammonium uptake rates are the lowest and do not vary much with depth in both in situ and deck experiments. Urea uptake rates are also in agreement within two standard deviation for the in situ and interpolated deck values for corresponding depths. Only at 20 and 40 m depths the nitrate uptake rates are significantly higher in the in situ case relative to the interpolated deck values. In region such as the Bay of Bengal one would expect a subsurface maximum in the nitrate uptake rate, which is seen in the in situ experiment, but is absent in deck experiment. Two possible reasons for this discrepancy could be (a) The deck incubation were probably carried out at a higher temperature (of water at 5 m depth) than the actual temperature at 20, 40 and 60 m depths (the mixed layer was only 5 m, see Table 2); (b) Sample heterogeneity and (c) The light cut off was more than that required for the depths of incubation of the in situ experiment.

The column-integrated values for the productivity (taking C/N Redfield ratio as 6.6)

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obtained by us were $519 \text{ mg C m}^{-2} \text{ d}^{-1}$ and $251 \text{ mg C m}^{-2} \text{ d}^{-1}$, respectively for in situ and deck incubations. In the same location primary production was measured using ^{14}C method for the same depths during the previous day (same mean solar radiation $\sim 100 \text{ mW/cm}^2$ on both days, N. Ramaiah, personal communication). This value ~ 200 $\text{mg C m}^{-2} \text{ d}^{-1}$, is closer to our deck incubation value. Use of 6.6 for C/N ratio might be questionable as variation from $<0.5\text{--}>40$ have been reported (Rees et al., 2002) for C:N uptake rates. However, here we use 6.6 as average value observed for organic matter in this region (Sambrotto, 2001).

4. Conclusions

This is the first study conducted in the Bay of Bengal using ^{15}N tracer technique. The study shows urea to be the most preferred substrate in this water, which contrasts with the adjacent Arabian Sea, where ammonium was found to be the preferred substrate (Watts and Owens, 1999). Our work emphasises the precautions that need to be taken for of ^{15}N experiment in the waters such as the Bay of Bengal.

Results indicate that the new production may be underestimated if the incubation time is less than 4 h. Incubation done for different time periods (one to 4 h) after adding the enriched tracers for nitrate, ammonium and urea revealed that the uptake rate for nitrate remained the same for the first two hours but increased after the end of fourth hour (from 0.92 to $1.5 \mu\text{g at-N m}^{-3}\text{h}^{-1}$). However, for ammonium, it decreased after one hour and remained the same for higher incubation times (0.74 to $0.38 \mu\text{g at-N m}^{-3}\text{h}^{-1}$). The urea uptake declined after the third hour (2.48 to $1.56 \mu\text{g at-N m}^{-3}\text{h}^{-1}$). These variations in uptake rates of different N-species lead to change in the f-ratio from 0.28 (after two hours) to 0.42 (after 4 h).

Opposite trend has been observed for the case where tracer addition significantly higher than 10% of the ambient concentration was made. When the concentration of tracer was varied (keeping the incubation time 4 h), the uptake rate for both urea [$y=1.88x+0.004$ ($r^2=0.88$)] and ammonium [$y=2.07x-0.002$ ($r^2=0.55$)] showed

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a positive relationship with substrate concentration (x). However, nitrate uptake [$y = -0.76x + 0.05$ ($r^2 = 0.86$)] showed a negative correlation. The f-ratio changed from 0.47 to 0.10 when tracer added was increased from 0.01 to 0.04 μM . This might have happened due to decrease in uptake of nitrate for higher substrate concentrations.

Column productivity from deck and in situ incubation were found to be 519 $\text{mg C m}^{-2} \text{d}^{-1}$ and 251 $\text{mg C m}^{-2} \text{d}^{-1}$ respectively and deck value was found to be in better agreement with ^{14}C based estimate of column productivity ($\sim 200 \text{ mg C m}^{-2} \text{d}^{-1}$).

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Table 1. Comparison of specific uptake and uptake rates at two different stations for 4 h incubation at 0.01 μM concentration. Uncertainty based on duplicate measurements given in parentheses.

Tracer	Experiment 1		Experiment 2	
	Sp. Uptake rate *1000 (h^{-1})	Uptake rate $\text{ugat-N m}^{-3} \text{ h}^{-1}$	Sp. Uptake rate *1000 (h^{-1})	Uptake rate $\text{ugat-N m}^{-3} \text{ h}^{-1}$
Nitrate	1.57 (0.1)	1.5 (0.1)	3.2 (0.05)	3.8 (0.1)
Ammonia	0.48 (0.1)	0.38 (0.1)	1.4 (0.3)	1.3 (0.2)
Urea	1.86 (0.2)	1.56 (.07)	2.4 (0.3)	2.3 (0.2)

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Table 2. Meteorological and hydrodynamic parameters at the experimental locations.

Parameter	Experiment 1	Experiment 2	Experiment 3
Latitude (° N)	~18	~20	~14
Longitude (° E)	~88	~88	~81
Wind speed (m/s)	6	4	4
Pressure (mbar)	1008	1008	1010
Air Temperature (°C)	>31	29	27.5
SST (°C)	29.1	29	30.3
surface Salinity (PSU)	29.2	28.4	33.4
MLD (m)	~10	<5	5
Chlorophyll-a (mg/m ²)	15	13	15
surface Nitrate (μM)	0.08	0.11	0.17
PON (ug at-N/l)	1.04	1.2	1.08

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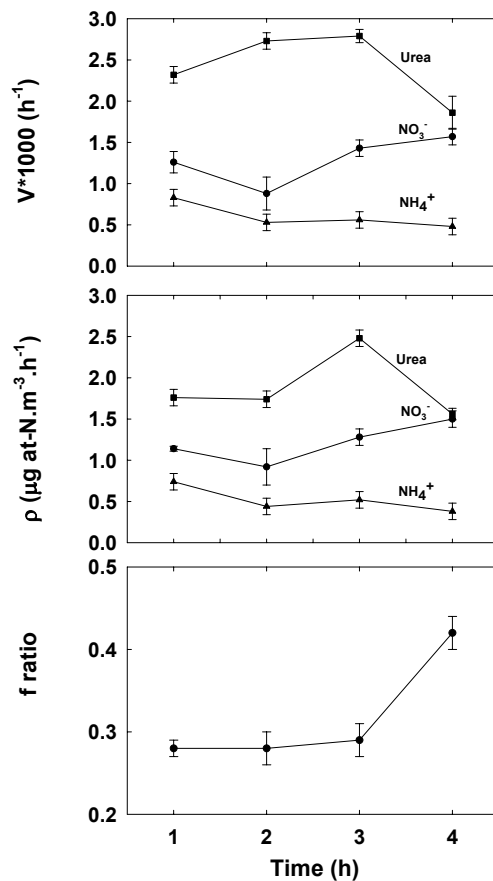


Fig. 1. The result of experiment 1 showing variation in specific uptake rate (top panel), uptake rate (middle panel) and f-ratio (bottom panel) with increase in the duration of incubation from 1 to 4 h.

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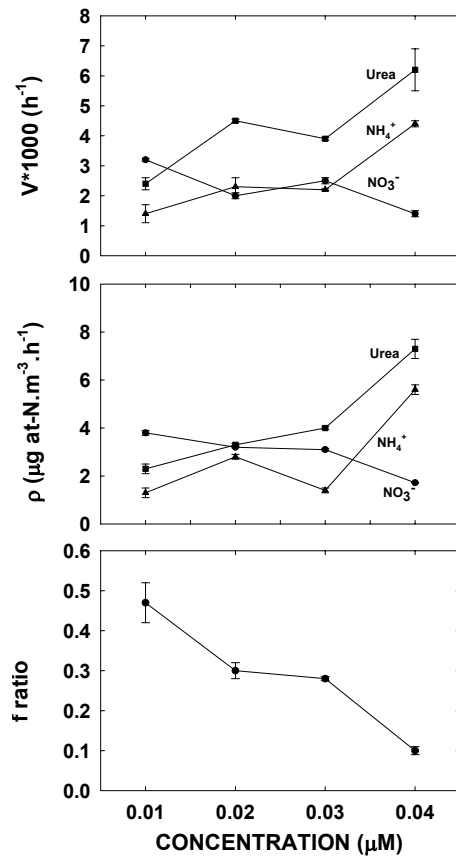


Fig. 2. The result of experiment 2 showing variation in specific uptake rate (top), uptake rate (middle) and f-ratio (bottom) with increase in substrate concentration.

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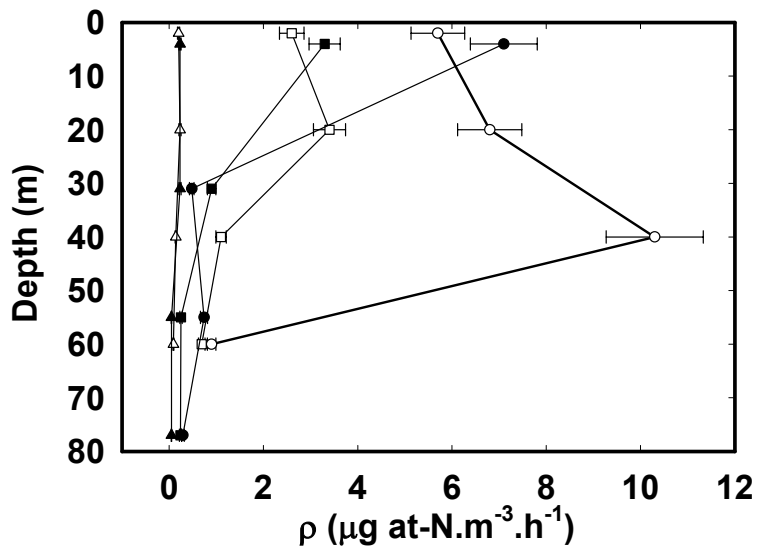


Fig. 3. Comparison of uptake results obtained from in situ and simulated in situ experiments. In situ nitrate, ammonium and urea uptake are indicated by open circle, triangle and square respectively. Simulated in situ nitrate, ammonium and urea uptake are indicated by filled symbols.

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