Chapter 2
Materials and Methods

2.1 Introduction

The main aim of this thesis, as mentioned in the earlier chapter, is to estimate new and regenerated productions along with natural nitrogen isotopic variability in suspended matter of the northern Indian Ocean and evaluate its carbon fixing capability. To carry out the present study, four cruises were undertaken, two each in the Bay of Bengal and the Arabian Sea. The cruise number, seasons, duration, and the ships on which the studies were carried out are listed in Table 2.1.

Table 2.1 Details of the cruises undertaken for the present study.

Bay of Bengal

Cruise No.	Season	Cruise duration	Ship
SK-182	Postmonsoon	17th Sep-11thOct 2002	ORV Sagar Kanya
SK-191	Premonsoon	16th Apr-6th May 2003	ORV Sagar Kanya

Arabian Sea

Cruise No.	Season	Cruise duration	Ship
SK-186	NE monsoon	4th Jan-17th Jan 2003	ORV Sagar Kanya
SS-212	Late NE monsoon	28th Feb-5th Mar 2003	FORV Sagar Sampada

The cruises on which present study was carried out in the Bay of Bengal and in the Arabian Sea had different purposes. The Bay of Bengal study was carried out on cruises intended for a programme known as BOBPS (Bay of Bengal Process Studies), aimed at thoroughly studying the Bay's physical, chemical and biological parameters. BOBPS is a Department of Ocean Development programme mainly carried out by scientists from National Institute of Oceanography (NIO), Goa, India. Present study is a part of BOBPS; it was performed in collaboration with NIO scientists where they mainly contributed nitrate and Chl *a* data required for the present study.

The Arabian Sea cruises were undertaken in collaboration with Space Applications Centre (SAC), Ahmedabad, India. These were basically the biological parameter retrieval and validation cruises for Ocean Colour Monitor (OCM), an ocean colour sensor on Indian Remote Sensing Satellite IRS P4 launched in May 1999. Since the objective, group and logistics were different during different cruises of the present study, different sampling procedure and timings were followed. The information

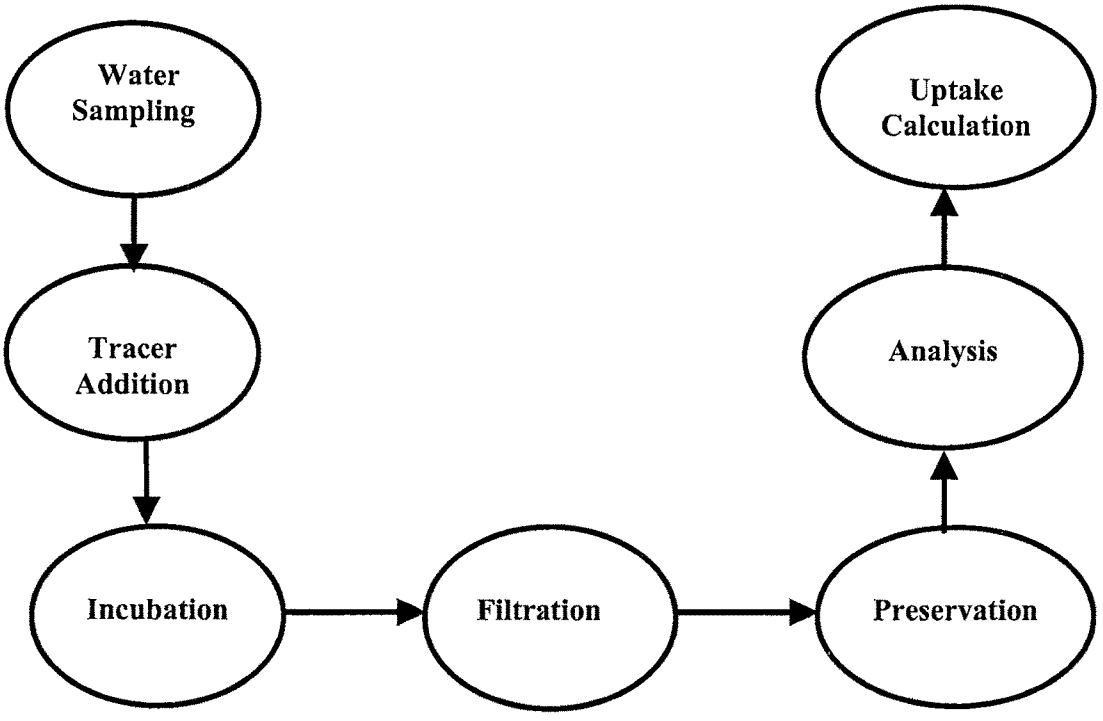
regarding the stations, sampling procedure, nutrient estimation and other physical properties are described in different subsections to follow.

2.2 New and Regenerated production estimation

For new and regenerated production estimation the Joint Global Ocean Flux Study (JGOFS) Protocol was followed. The principle and requirements for the measurements are as follows:

Principle: During the present study the nitrate uptake would be considered as new production whereas sum of ammonium and urea uptakes would be regenerated production. The measurement of nitrate uptake is based on the incorporation of 'trace' addition of ^{15}N-labelled nitrate by the phytoplankton during incubation experiments. Similarly the estimation of regenerated production is based on ^{15}N-labelled ammonium and urea incorporation.

The steps involved in the estimation of new and regenerated production are shown in the flow chart below:



Requirements: The determination of the uptake experiments requires the knowledge of following parameters:

A. The initial substrate concentration (NO_3^-, NH_4^+ or Urea).

B. Final concentration of particulate nitrogen.

C. The final ^{15}N enrichment of particulate matter.

D. The enrichment level of dissolved fraction after tracer addition.

Preparation of tracer solution

The ^{15}N-labelled (99 at% enriched) nitrate ($NaNO_3$), ammonium (NH_4Cl) and urea (NH_2-CO-NH_2) salts in dry chemical form were procured from Sigma-Aldrich (USA). The stock solutions were prepared to contain 0.5mmol of $NaNO_3$, NH_4Cl, or urea in 250ml of solution (0.5 mmol/250ml). For that 43mg of $NaNO_3$ (molecular weight ~85.98 g), 27.24 mg of NH_4Cl (molecular weight ~54.48 g), and 31 mg of urea (molecular weight~62.04 g) were added in three different volumetric flasks containing 250ml of doubled distilled water and mixed thoroughly to make a homogeneous solution and was later transferred to 250ml NALGENE bottles for further use. The working solutions were prepared from the stock solution in two different concentration levels: the first containing 0.01μmol NO_3^- (NH_4 or urea) per ml and other containg 0.1 μmol NO_3^- per ml of solution. To prepare the former, 5ml of the stock solution (contained 0.01mmol of NO_3^-, NH_4^+ or urea) was added to 995ml of double distilled water. This solution contained 0.01mmol NO_3^-, NH_4^+ or urea per 1000ml i.e., 0.01μmol per ml of solution. The latter solution was prepared by adding 12.5ml of stock solution to 237.5ml of distilled water, which lead to the concentration of 0.1 μmol per ml of solution. The weighing of the salts was done using Thomas Scientific weighing paper on Sartorius microbalance (model no: MC-5; Germany).

2.3 Sampling

2.3.1 Bay of Bengal

The locations where the seawater samples were collected in the Bay of Bengal during post and premonsoon are shown in the Figure 2.1. Nine stations, during both seasons, were sampled for new and regenerated production studies referred henceforth as PP1 to PP9. However, surface water samples were collected at 24 locations for natural isotopic variability studies in the suspended matter. The details of sampling locations along with the dates of sampling are listed in Table 2.2.

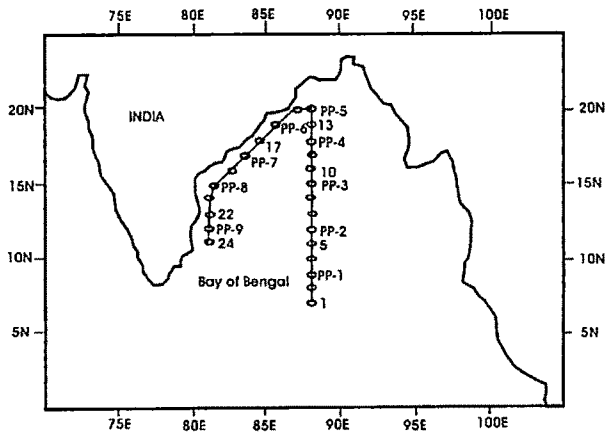


Figure 2.1 Locations showing all the CTD stations as well as new and regenerated production stations (PP) during post (SK-182) and premonsoon (SK-191) in the Bay of Bengal.

Table 2.2 Sampling locations along with dates of sampling during both pre and postmonsoon in the Bay of Bengal.

Stns.	New Production Stations	Latitude (°N)	Longitude (°E)	Premonsoon Sampling Date (D/ M /Y)	Postmonsoon Sampling Date (D/ M /Y)
1		7	88	16/04/2003	17/09/2002
2		8	88	17/04/2003	18/09/2002
3	PP1	9	88	18/04/2003	18/09/2002
4		10	88	19/04/2003	20/09/2002
5		11	88	19/04/2003	20/09/2002
6	PP2	12	88	20/04/2003	21/09/2002
7		13	88	21/04/2003	22/09/2002
8		14	88	21/04/2003	22/09/2002
9	PP3	15	88	22/04/2003	23/09/2002
10		16	88	23/04/2003	24/09/2004
11		17	88	23/042003	24/09/2002
12	PP4	18	88	24/04/2003	26/09/2004
13		19	88	25/04/2003	26/09/2004
14	PP5	20	88	26/04/2003	29/09/2004
15		20	87	27/04/2003	30/09/2002
16	PP6	19	86	28/04/2003	1/10/2002
17		18	84.5	29/04/2003	2/10/2002
18	PP7	17	83.5	1/05/2003	3/10/2002
19		16	82 .5	2/05/2003	5/10/2002
20	PP8	15	81 .5	3/05/2003	6/10/2002
21		14	81	4/05/2003	8/10/2002
22		13	81	4/05/2003	9/10/2002
23	PP9	12	81	6/05/2003	10/10/2002
24		11	81	—	11/10/2002

Postmonsoon (SK-182)

Water samples were collected before dawn (around 4:30 hours) by a rosette sampler fitted with 30L Go Flo bottles (General Oceanics, Miami, Florida, USA). A Sea-Bird Electronics CTD was used with the rosette to obtain the conductivity-temperature-depth profile. The temperature sensor of the CTD was calibrated before the cruise. The software package SEASOFT was used for processing the raw CTD data. Water samples were collected when the rosette was hauled up, by tripping the bottles prefixed for the desired depths. The rosette was allowed around one minute stabilisation before the bottles were closed to ensure that the samples from desired depths were collected (Figure 2.2).

Figure 2.2 Sampling of seawater onboard ORV Sagar Kanya. Left: Rosette attached with 30L Go-Flo bottles and underwater CTD unit being hauled up after temperature-salinity profiling and seawater sampling. Right: deck unit onboard for real time data acquisition and monitoring the sampling operation.

A day prior to the sampling or on the previous station, euphotic depth was estimated using a Secchi disk (also verified on the sampling location later in the day). Euphotic depth was determined as ~2.8*Secchi depth, where Secchi depth was determined by appearance or disappearance of Secchi disk in water column. Interestingly, on an average the euphotic zone during postmonsoon was found to be around 60m. However, at PP-6 the euphotic depth was just 40m. Water samples were collected from four different depths to cover the entire euphotic zone; 0, 20, 40 and 60m at all stations except PP6 where depths were altered to 0, 15, 25 and 40m. Once the water samples from different depths reached the deck, the samples were collected in prewashed two and one litre Polycarbonate NALGENE bottles (New York, USA) in duplicate pairs for each tracer (nitrate, ammonium and urea) and each depth. Two litre bottles were used for nitrate and ammonium experiments while one litre bottles were used for the urea

experiment. The bottles were rinsed thoroughly with the seawater of the particular depth before collecting the samples. Beforehand, the bottles were named in such a manner that they represented the station no, depth of sample collection, tracer information (whether for nitrate, ammonium or urea) as well as for primary and duplicate samples (e.g., 11NP representing first station, first depth i.e., surface for Nitrate Primary sample). Subsequently the bottles were lined up in a big plastic crate to avoid confusion. The water sample was collected directly from the Go-Flo bottles without taking the water into carboys to avoid possible contamination. Apart from taking the water samples for nitrate, ammonium and urea experiments, water samples from any chosen depth was collected in three one litre bottles for blank determination. After the collection of the samples was over, they were covered with a thick black cloth from all the sides to prevent light shock to the phytoplankton. This sample collection procedure was over by 5 A.M (IST), before any trace of daylight appeared.

Premonsoon (SK-191)

The method of sample collection during premonsoon was different from that of postmonsoon. Whereas the sample depth during the postmonsoon was fixed; the sample during the premonsoon was collected based on light levels. Samples were collected from six different light levels of 100, 80, 64, 20, 5, 1 % of surface irradiance to cover the entire euphotic zone. The depths at which the samples had to be taken for the above mentioned light levels were estimated using the formula:

$$Z = (2.8*d/4.6) \ln (100/x)$$

Where, Z = Sample depth (m)

d = Secchi depth (m)

x = % of light level

All other procedures were same as mentioned for premonsoon sample collection. However, the sample depths varied from one station to another depending on the light penetration at a particular station. The Table 2.3 lists the sample depths during premonsoon season (SK-191). Six litres of water samples were also collected at all new production stations (PP stations) at 0, 30, 60, 100, 300 and 500m depths for studying the vertical variability of natural ^{15}N in suspended matter in the Bay of Bengal.

Table 2.3 Details of sample depths during the premonsoon season in the Bay of Bengal.

%Light Used	Sample Depths (m)								
	PP1	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**	**PP7**	**PP8**	**PP9**
100	0	0	0	0	0	0	0	0	0
80	3	3	5	5	3	5	5	5	5
64	6	7	10	10	6	10	10	10	10
20	20	25	35	35	20	20	35	35	35
5	40	50	60	60	40	40	60	60	60
1	60	70	100	100	60	60	100	100	100

2.3.2 Northeastern Arabian Sea

As mentioned in the earlier chapter, the main aim in the northeastern Arabian Sea was to study the changes in new and regenerated production and $\delta^{15}N$ of surface suspended matter during and late northeast monsoon. For this purpose two cruises were undertaken in the year 2003. The first was onboard ORV Sagar Kanya during January and the second one was onboard FORV Sagar Sampada during late February-early March. The cruise tracks were not exactly the same; however, they were concentrated mostly in the same region i.e., off Gujarat. The locations of study are shown in the Figure 2.3. New and regenerated production studies were carried out at seven stations during January and six stations during late February-early March. However, the surface water samples at thirteen stations (except stn.1) during January and five stations (except Stn.1) during late February-early March were collected to assess the natural ^{15}N isotopic variability in the suspended matter.

January

The list of all the stations where the water samples were collected along with new production stations, their exact locations and dates of sampling are shown in Table 2.4. The samples were collected at six different depths at each station to cover the entire euphotic zone (Table 2.5). To know the euphotic depth and the percentage of light at the depth of sampling, a Satlantic radiometer was operated at each station. Apart from the light regime the radiometer also provided the information regarding the water column temperature and chlorophyll concentration. Since the aim of the cruise in the Arabian Sea was not concentrated only on new production and primary productivity measurements, it was not always possible to sample before dawn as in the case of Bay

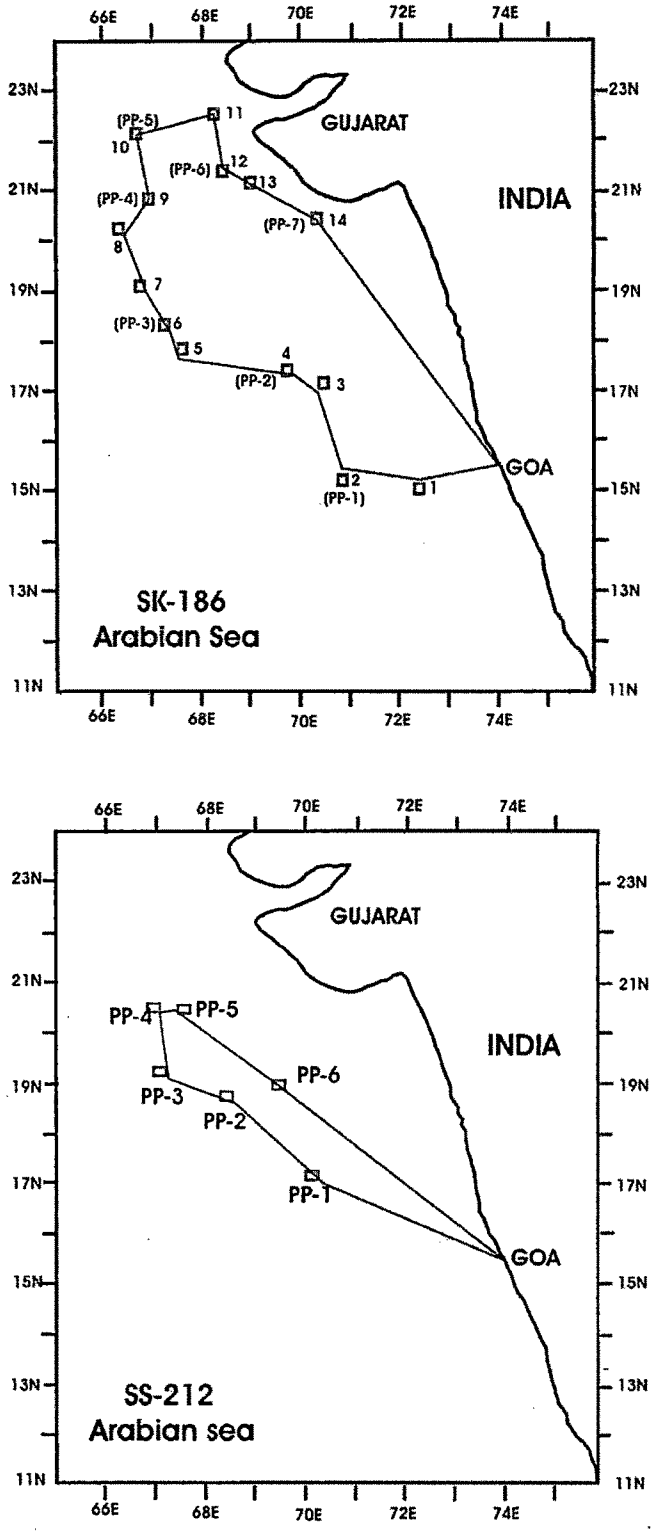


Figure 2.3 Sample locations in the NE Arabian Sea [January, SK-186 (top) and February-March, SS-212 (bottom)]. During January water samples were collected at 13 stations (except stn.1) for studying natural isotopic variability and new production experiments were performed at PP stations only. During February-March the water samples were collected at 5 stn (except stn.1) for natural variability whereas new production measurements were performed at all the stations shown.

Table 2.4 List of stations with locations and dates of sampling during January 2003.

Station	New Production Station	Latitude (N)	Longitude (E)	Date (D/ M/ Y)
1		15° 07' 10"	72° 26' 07"	04/01/2003
2	PP1	15° 14' 03"	70° 46' 01"	05/01/2003
3		17° 13' 49"	70° 32' 48"	06/01/2003
4	PP2	17° 29' 32"	69° 44' 26"	07/01/2003
5		17° 53' 35"	67° 41' 49"	08/01/2003
6	PP3	18° 16' 44"	67° 12' 38"	09/01/2003
7		19° 11' 08"	66° 44' 08"	10/01/2003
8		20° 19' 18"	66° 12' 38"	11/01/2003
9	PP4	20° 57' 40"	66° 54' 25"	12/01/2003
10	PP5	22° 12' 10"	66° 41' 41"	13/01/2003
11		22° 31' 18"	68° 14' 17"	14/01/2003
12	PP6	21° 22' 26"	68° 11' 51"	15/01/2003
13		21° 12' 24"	69° 0' 17"	16/01/2003
14	PP7	20° 26' 15"	70° 23' 35"	17/01/2003

Table2.5 Sampling depths during January 2003 in the Arabian Sea.

Sample Depths (m)						
PP1	**PP2**	**PP3**	**PP4**	**PP5**	**PP6**	**PP7**
0	0	0	0	0	0	0
20	10	20	15	15	10	5
40	30	30	25	30	20	10
50	50	40	40	40	30	15
60	58	60	50	55	45	20
70	65	70	65	65	60	25

of Bengal. Since the expected biomass in the Arabian Sea was higher than that of the Bay, sample was collected in two litre NALGENE bottles for nitrate and one litre NALGENE bottles for ammonium and urea experiments. The method of the sample collection was the same as that followed in the Bay. However, the Go-Flo bottles attached to rosette for the sampling were only of 10 Litre capacity compared to 30 Litre used in the Bay. Hence, sometimes more than one cast was required for the sampling. As in the Bay, special care was taken to prevent the planktons from the light shock; a thick black cloth was used to cover the samples immediately after the collection. The samples for the blanks were also collected at each station at a chosen depth in three one litre bottles, one each for nitrate, ammonium and urea.

February-March

The exact sampling locations along with the date of sampling are listed in Table2.6. The sampling during February-March was different from that of January in the Arabian Sea in a way that it was collected based on light levels as listed in Table 2.7, all other details remains the same.

Table 2.6 List of stations along with locations and sampling dates during late February-early March.

Station	New Production Stations	Latitude (N)	Longitude (E)	Date (D/ M/ Y)
1	PP1	17° 19.02'	70° 11.82'	28/02/2003
2	PP2	18° 48.33'	68° 28.53'	01/03/2003
3	PP3	19° 5.03'	66° 51.82	02/03/2003
4	PP4	20° 44.42'	66° 58.82	03/03/2003
5	PP5	20° 28.88'	67° 30.24'	04/03/2003
6	PP6	18° 57.71'	69° 21.17'	05/03/2003

Table 2.7 Sampling depths during Feb-March 2003 in the Arabian Sea.

% Light Used	Sampling depths (m)					
	PP1	PP2	PP3	PP4	PP5	PP6
100	0	0	0	—	—	0
80	5	20	—	—	—	—
64	12	25	4	2	2	5.5
20	40	40	17	13	16	19
5	60	60	30	27	27	36
1	85	85	42	42	42	58

This was a typical bloom period in the Arabian Sea and the attenuation of light in the water column was very fast with depth, particularly at stations PP3, PP4 and PP5. It was very difficult to differentiate between the corresponding depths for 100 and 80% light levels given the limitation that the length of Go-Flo bottles itself were around 1m. Because of this limitation the sampling at the above mentioned three stations were done only for four light levels as listed in the Table 2.7. Two litre bottles for nitrate and one litre bottles for ammonium and urea were used.

Sampling of surface water for natural isotopic studies during all the cruises were performed using clean plastic buckets.

2.4 Nutrients

The nutrients of interest during the present study were the nitrate, ammonium and urea. In the Bay of Bengal, the nitrate measurements were carried out onboard by Dr. S. Sardesai and her colleagues of National Institute of Oceanography (NIO), Goa, by using column reduction technique (Strickland and Parson 1972). Ammonium and urea measurements could not be performed due to logistic problems. However, indirect estimation of ammonium and urea concentration for a few locations was done for the premonsoon period using the displacement volume of mesozooplankton measured during the cruise. The 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 0.1 to 1.1 $ml.m^{-3}$. From the displacement volume the dry weight was calculated using the relationship given by Wiebe et al. (1975):

Log (Displacement volume) = -1.842 + 0.865 * Log (dry Weight)

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 of ammonium and urea were calculated for 12 hour residence time of zooplankton in mixed layer (Wafar et al. 1986). Table 2.8 lists the displacement volume of mesozooplankton biomass in the Bay of Bengal during the premonsoon period along with the location, depth, calculated dry weight and ammonium and urea regeneration.

Table2.8 The calculated urea and ammonium concentrations using mesozooplankton displacement volume.

Latitude (°N)	Longitude (°E)	Displacement Volume (ml/ $100m^3$)	Depth (m)	Dry Weight (g m^{-3})	Ammonium (μM)	Urea (μM)
9	88	110	0-40	0.14	0.042	0.011
11	88	25	0-60	0.027	0.008	0.002
15	88	10	0-40	0.009	0.003	0.0005
15	81	40	0-20	0.046	0.014	0.004
17	84	110	0-20	0.148	0.043	0.011
19	85	110	0-30	0.148	0.043	0.011

In the Arabian Sea, the nutrient measurements were not done onboard, however, samples were preserved (in deep freeze) to carry out the measurements (nitrate+ammonium) at Dr. S.W.A. Naqvi's lab in NIO, Goa at the end of the cruise using autoanalyzer. The detection limit for nitrate was 0.1μM, in both the methods.

2.5 Tracer addition

Once the samples were collected they were arranged in the dark room. The tracers were added just before the beginning of incubation. During the Bay of Bengal cruises, since the nitrate measurements were available onboard, an attempt was made to add less than 10% of ambient nitrate. In some cases, if the ambient nitrate of the same station was not available due to time constraints, nitrate concentration of the nearest (previous) station was used as the ambient value for tracer addition. Once the nitrate of the same station was estimated, it was used for calculating the actual percentage of tracer added and for the final uptake calculations. Since ammonium and urea could not be estimated, constant amounts of ammonium (0.01μM) and urea (0.03μM) were added.

For the Arabian Sea, since the nutrient measurements could not be carried out onboard, a pre-fixed amount of nitrate, ammonium and urea concentrations were added. Since the nitrate concentration in the region is well studied for the northeast monsoon, an attempt was made to add less than 10% of typical ambient values. However, when the actual nitrate measurements were performed after the cruise, it was found that the addition was sometimes around 50% of the ambient value. Constant amounts of ammonium (0.1μM) and urea (0.1μM) were added in the Arabian Sea following Watts and Owens (1999).

Tracer additions were done using well calibrated micro pipettes. The pipette tips were changed after each addition to avoid cross contamination. After the tracer addition the lid of the bottles were closed tightly to avoid any leakage during incubation.

2.6 Incubation

Incubation during the present study was done for four hours from 1000 to 1400 Hrs. (IST) symmetrical to local noon in deck boxes. The appropriate neutral density filters were put on to the bottles to simulate light conditions inside the bottles, to be the same as the depths from which the samples were drawn. The filters were specially prepared for the purpose and were well-calibrated using lux meter (at Dr. M.V.M. Wafar's lab at NIO, Goa) in dry and wet conditions. Flowing seawater from 5m depth was continuously maintained in the deck boxes to regulate the temperature. However, the flowing water from 5m depth may be inappropriate for deeper samples where the

subsurface Chl *a* maxima are situated within or below the thermocline. Exactly at 1400 Hrs., the samples were taken out from the deck boxes for filtration and kept in dark till the filtration was over.

2.7 Filtration

After the incubation, the samples were filtered onto Whatman GF/F glass fiber filters (47mm diameter and 0.7µm pore size). These filters were precombusted at 400°C for 4hrs after carefully wrapping them in Al-foil in sets of six. The filtration was done under low pressure (<70mm Hg) on a manifold unit procured from Millipore (Figure 2.4). Separate glass cups were used for nitrate, ammonium and urea samples to avoid cross contamination. A separate cup was dedicated for the filtration of natural samples. Once the filtration of the sample was over it was rinsed with filtered seawater to remove residual $^{15}NO_3^-$ from filter interstices. Care was taken not to evacuate the filters to dryness. Once the filtration is over the filter papers were taken out carefully with forceps (separate for nitrate, ammonium and urea) to place into the 47mm diameter petrislides procured from Millipore. Filters were then immediately dried (50°C) for isotopic analysis in the shore laboratory.

Figure 2.4 Filtration unit used during the present study.

As mentioned in the earlier sections, samples for blank estimation were collected at all the stations during different cruises. The idea here was to estimate the zero time enrichment. For that the same concentration of tracer was added to the blank samples as was added to the new and regenerated production samples. Immediately after the addition of tracer, the sample was filtered and dried for isotopic analysis.

2.8 Isotopic Analysis and Instrumentation

The important requirements for the uptake rate estimation were the total nitrogen content and the atom% ^{15}N in the post incubation samples. During the present study, broadly, the method of Owens and Rees (1989) for "Determination of Nitrogen-15 at sub-microgram levels of Nitrogen using automated continuous-flow Isotope ratio mass Spectrometry" was followed. The small amount of samples available (< 1μM) for analysis poses a severe problem for mass spectrometric analysis in marine applications. Emission spectrometry is another method of analysis in which small amounts of sample (< 1μM) can easily be detected but the precision of measurement (typically < 0.05at %) is too low for marine applications. However, the improvement of electronics, vacuum system and ion optics has enabled to analyse the small amount of samples (comparable to emission spectrometry) with high precision using a mass spectrometer. The continuous on-line analysis by automated isotope ratio mass spectrometers interfaced with elemental analyser provide one such option. Although this single inlet continuous on-line method of analysis is not as precise as dual-inlet analysis, this technique is sufficiently precise (± 0.005 at%) for the present kind of study (Owens and Rees 1989). The modern design of nitrogen analyser with helium carrier and oxygen pulse was used during the present study, which has the following advantages:

(1) Oxidation conditions can be optimised by varying oxygen pulse; products can then be checked by mass spectrometer.

(2) Samples are accompanied by helium, there are no problems due to leakage.

(3) The range of sample size may be large.

(4) Memory effects are reduced as the analyser is continuously swept with helium.

(5) Helium does not interfere with nitrogen isotope analysis.

2.8.1 Principle of Analysis

The samples are combusted in a chamber containing oxidising catalysts at high temperature. Due to the oxidation of all major elements like C, H and N present in the sample, oxides like CO_2, H_2O and N_2O are formed. After the complete combustion of the samples, the gases formed are passed through a reduction chamber, where oxides of nitrogen get converted to N_2. The gas mixture is passed through dry absorbants and a gas chromatography column and the purified N_2 is admitted to an isotope ratio mass

spectrometer tuned for masses 28, 29 and 30. Integration of ion beams of the standards and samples enable both the total nitrogen and ^{15}N: ^{14}N ratio to be determined.

2.8. 2 Instrumentation

During present study, Elemental Analyser (Flash EA 1112 Series, CE Instruments, Italy) interfaced with Finnigan Delta^{Plus} Continuous-Flow Mass Spectrometer (Thermo Quest Finnigan, Bremen, Germany) via ConfloIII was used for analysis. The description of the instruments as well as procedure followed is given below.

(A) Elemental Analyser

A Carlo Erba (CE) elemental analyser was used throughout this study. The mode of operation is based on Dumas principle with high temperature "Flash" combustion. The carrier gas used was 5 grade (99.999%) helium (procured from Praxair-Bangalore) at 80ml/min. 5 grade (99.999%) oxygen (procured from Hydragas-Bombay) was used for combustion. The elemental analyser consists of two reactors: the first one acts as an oxidation or combustion chamber for the samples and the second, as a reduction chamber. Before the start of the experiment the reactors are prepared with the necessary chemicals in 47cm long quartz tube with outer diameter of 18mm and inner diameter of 14mm. The first reactor contains the oxidizing catalysts: silvered cobaltous oxide and chromium oxide separated by quartz wool. The reduction chamber contains reduced copper with quart wool on the top and bottom. The exact size of the filling materials in the reactors is shown in Figure 2.5. The chemicals were procured from Courtage Analyses Services (France).

There is a turret on top of the oxidation chamber where samples are loaded, a maximum of 50 at a time. The positions of the samples are numbered from 0 to 49. The sample that has to be analyzed falls in an intermediate zone (well) and is purged with He. After the purging is over, the sample is allowed to fall in the oxidation chamber followed by a pulse of pure oxygen (1 second pulse @ 175ml min^{-1}). The temperature of the oxidation camber is normally maintained at 1060°C. The sample drop and the simultaneous supply of oxygen pulse lead to an increase in the temperature of the chamber to the order of 1800°C. This rise in temperature leads to 'flash' combustion of the sample resulting into the production of CO_2, H_2O and oxides of nitrogen. The flash combustion can be seen through the hole provided for the purpose as a change in colour

during combustion. Since helium is always flowing as a carrier gas, it carries all the oxides produced due to the combustion of the samples

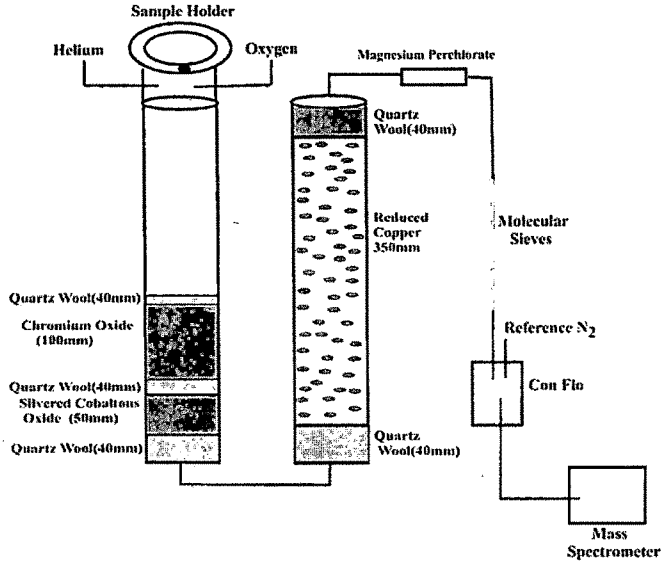


Figure2.5 Schematic diagram showing the elemental analyzer set up.

along with it to the reduction chamber containg reduced copper at 680°C. In the reduction chamber, the oxides of nitrogen get reduced to N_2. After the reduction chamber these gases are passed through magnesium perchlorate (anhydrous) to absorb any water vapor (moisture) that may be present. Subsequently, the gases are passed through a gas chromatographic column (molecular sieve). In the chromatographic column, a mixture of different gases (CO_2, N_2 etc.) gets separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column. The components of the sample move at velocities that are influenced by the degree of interaction of each constituent with the stationary nonvolatile phase. The substances having the greater interaction with the stationary phase are retarded to a greater extent and consequently separate from those with smaller interaction leading to their release at different times from the chromatographic column. The retention time for N_2 is less than that of CO_2. Hence, N_2 comes faster than the CO_2 out of chromatographic column (whose temperature is maintained at 60°C). After the different components elute from the column they pass through Con Flo from where they are allowed into the mass spectrometer for isotopic analysis.

Sample Preparation for elemental analyzer

As mentioned earlier, the samples were filtered on 47mm diameter Whatman GF/F filter paper. An attempt was made to accommodate the whole filter paper in the

carousel of the elemental analyzer by pressing them into a smallest size possible but it was not feasible to accommodate as such. After that the filter papers were cut into two equal halves to make two small pellets. Although it was somehow fitting into the carousel, there was problem in its free fall into the well and subsequently into the oxidation chamber. Because of this the samples were getting stuck into the well (and not falling in the oxidation chamber) falling one over the other and hence the identity of the samples were lost. To avoid these problems and smooth analysis, samples were cut into four equal halves and the small pellets were prepared in thoroughly cleaned silver foils. The silver foils were checked for blank after cleaning. While preparing the samples extra care was taken to not to touch the samples and cleaned silver foils with hand. Two curved forceps were used for the wrapping the samples in silver foil. The smallest possible silver foil (1cm x 1cm) was used to wrap the samples.

(B) Con-Flo

Con-Flo is an interface between elemental analyzer and mass spectrometer. Broadly, it contains three capillaries in a small tubular glass structure: two incoming capillaries bring reference gas and the sample gas to the tubular structure, whereas the third capillary transfers the reference or sample gases from tubular structure to the mass spectrometer.

(C) Mass Spectrometer

The mass spectrometer used for the present study was Finnigan's Delta^{plus} Isotope ratio Mass Spectrometer. The Delta^{Plus} is a single inlet ("online" coupling of gas chromatographs and Elemental Analyzer through Con Flo III) instrument with electron impact ionization source. The energy of ionization electron is 80 eV. The vacuum was maintained by turbomolecular pump at a rate of 240 l s^{-1} (type: TMH 260, manufacturer: Balzers) with fore vacuum provided by rotary pump rated at 1.5 m^3s^{-1} (E2M1.5, manufacturer: Edwards). Ions produced are accelerated at 3kV. The ion beam produced exits the ion source into magnetic field through a slit of fixed width (0.3mm) and enters the magnetic field boundary at an angle of 26.5° and exits at the same angle resulting in a radius of 9cm for this system. The maximum strength of the magnetic field was 0.75 Tesla. The resolution of the mass spectrometer ($m/\Delta m$) was 95. The

collector system of the mass spectrometer was MEMCO (Multi-Element-MultiCollector) system with three Faraday collector cups connected to amplifiers.

During the present study two types of samples were analysed:

(A) Natural samples.

(B) Enriched samples (i.e., new and regenerated production samples).

The main interest of the analysis was to find:

(A) The total Nitrogen content in the natural and enriched samples

(B) Nitrogen isotopic composition ($\delta^{15}N$) in the natural samples and atom% ^{15}N in the enriched samples.

The operation of the whole system (Elemental analyser + mass spectrometer) was fully computerized and was controlled by Finnigan MAT software ISODAT. A method instructing the timing of different events (i.e., on and off time of reference gas, on and off time of elemental analyser and He dilution) was followed for the analysis. Also the information to find the peak such as start and end slope, peak mean height, peak mean width etc were fed. Acquisition time for a sample was 450 seconds and the nitrogen peak used to appear at around 155 sec after the start of the analysis. A typical chromatogram obtained during analysis is shown in Figure 2.6. The parameters of interest during present study for which ISODAT was customized were: amplitude of 28, 29 and 30 mass peaks, ratio of 29/28 and 30/28, individual and total areas under the peaks of masses 28,29 and 30, atom%^{15}N and $\delta^{15}N$ w.r.t. air.

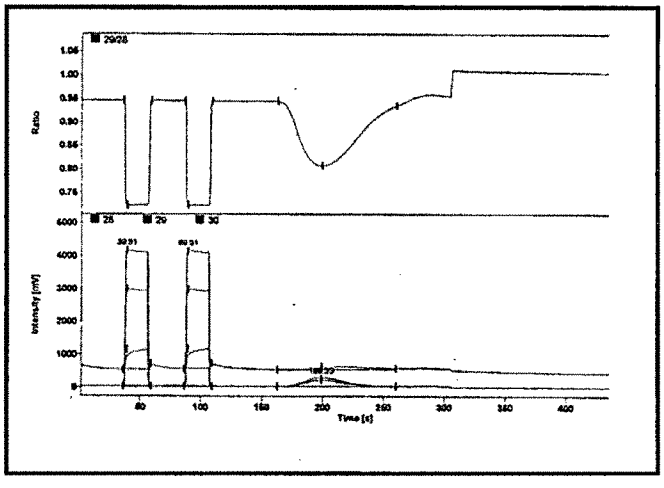


Figure 2.6 A typical chromatogram (for ammonium sulphate ~ 0.053mg) obtained during the present study.

2.9 Standards Used and precision

The isotopic composition of nitrogen ($\delta^{15}N$) is expressed with respect to air ($\delta^{15}N$ = 0 ‰). However, it is common practice to use laboratory standards whose isotopic composition is known w.r.t. air. Throughout the present study three standards of known isotopic compositions (procured from IAEA) were used. The laboratory standards with their quoted isotopic compositions and values and precision obtained during the present study are as listed in Table 2.9.

Table 2.9 Values and precision obtained during the present study for standard materials used.

Standards Used	Isotopic composition (quoted by IAEA) (‰ w.r.t. Air)	Values obtained during present study (‰ w.r.t. Air)
$(NH_4)_2SO_4$ [IAEA-N-2]	20.3	20.3±0.3* (n= 15)
KNO_3 [IAEA-NO-3]	4.7	4.9±0.3* (n=13)
KNO_3 [USGS 32]	180	177±2.3* (n=12)

* standard deviation

2.10 ^{15}N atom% Measurement

The ISODAT software calculates the ^{15}N atom% for each sample. The ^{15}N atom% can be calculated if the ratio of $^{28}N/^{29}N$ is known, as in the case of mass spectrometer, according to following relationship:

$$^{15}N \text{ atom\%} = 1/(2R+1)*100, \text{ where } R = {}^{28}N/{}^{29}N$$

As stated earlier, ^{15}N atom% was one of the important parameters to be estimated for the samples processed to measure new and regenerated production.

2.10.1 ^{15}N atom% for blank samples

The ^{15}N atom% in the blank samples (samples which were immediately filtered after addition of tracers) was also estimated. The average values of the blank samples for nitrate, ammonium and urea uptake experiments are listed in Table 2.10. These are the average values of respective blanks for all the stations where the samples for blank were collected. The blank at% values may be taken as zero time enrichment i, e., it

Table 2.10 The average value of ^{15}N atom% in the blank samples during the present study (natural level: 0.3678197).

	Nitrate	Ammonium	Urea
Arabian Sea			
SK-186	0.3680±0.0009	0.399±0.040	0.387±0.007
SS-212	0.3718±0.0005	0.3932±0.0008	0.406
Bay of Bengal			
SK-182	0.368±0.001	0.397±0.011	0.411±0.036
SK-191	0.373±0.015	0.384±0.011	0.403±0.014

might provide the measure of the affinity for a particular nutrient by the phytoplankton; in other words, it may provide the measure of the capability of the phytoplankton to take up a particular nutrient in a very short time (time of filtration). For example, the at% ^{15}N for urea is maximum in the Bay of Bengal and during SS-212 (Feb-March) in the Arabian Sea whereas it is highest for ammonium during SK-186 (January) in the Arabian Sea probably indicating the immediate preference for these nutrients.

2.10.2 Error in ^{15}N atom% estimation

The percentage error in ^{15}N atom% estimation observed during present study is listed in Table 2.11. The maximum error observed was in the case of ammonium uptake experiment and was found to be 2.8 and 3.5% in the Arabian Sea and the Bay of Bengal respectively. In all other cases it was less than 2% and well within the limits observed elsewhere.

Table 2.11 The percentage error in ^{15}N atom% estimation during the present study.

	Nitrate	Ammonium	Urea
Arabian Sea	1.76	2.8	1.4
Bay of Bengal			
SK-182	0.20	3.50	0.91
SK-191	0.26	1.82	1.43

2.10.3 Linearity for ^{15}N atom%

Since the expected concentration of total nitrogen in marine samples is very less (typically ~1μM or less), it is very important to check for the linearity (Figure 2.7) of

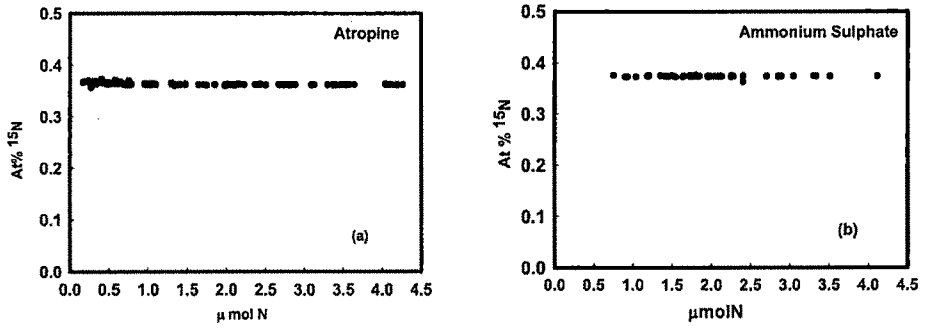


Figure 2.7 The linearity of ^{15}N atom% for (a) atropine and (b) ammonium sulphate over widely varying sample amounts.

the desired parameter (atom% ^{15}N during present study) over the expected sample range. During the present study this exercise was performed using the most commonly used standard material $[(NH_4)_2SO_4]$ and material used for calibration purpose (Atropine) for total nitrogen content. The linearity of the atom% was found up to very small sample amounts (0.2µM). The precision for $[(NH_4)_2SO_4]$ and atropine during analysis was around 0.001 and 0.009 atom% respectively.

2.11 Estimation of Particulate Nitrogen Content

Material which was used mostly for the calibration purpose for estimating the total nitrogen content of the samples was atropine. However, sometimes ammonium sulphate and potassium nitrate were also used to recheck the differences, if any. Atropine is an organic material, which contains only 4.8% nitrogen. This small presence of nitrogen allows the larger quantity to be taken up than potassium nitrate or ammonium sulphate for the same concentration of nitrogen and hence reducing the error in weighing the smaller amounts. Potassium nitrate and ammonium sulphate contain 13.8 and 21.2% nitrogen respectively.

For the calibration for total nitrogen, a range of atropine and ammonium sulphate were weighed (e.g. ranging from 0.10 to 0.80 mg) and corresponding nitrogen contents were calculated by multiplying the percentage of nitrogen in them. For each sample the total area (sum of the areas under the 28, 29 and 30 mass peaks) obtained during analysis was plotted against the nitrogen content and a regression equation was derived to calculate the unknown nitrogen content of samples.

The calibration was performed on day to day basis to take into account any possible sensitivity change. A normal practice during the analysis was to prepare a

standard calibration curve using 10-15 atropine or ammonium sulphate samples after each time reactor of the elemental analyzer was changed (normally reactors were refilled after analyzing 200-250 samples). In addition, each lot of samples (consisting 50 samples) contained at least eight standards, which were used to prepare the calibration curve if any sensitivity change was observed. A typical example of calculation used for the calibration is shown in the Table 2.12 and subsequently its plot

Table 2.12 A typical example for the method used for the calibration for the estimation of total nitrogen. Number of samples presented are smaller than typically used for calibration.

Samples	Weight (mg)	mg N	Total Area (V*sec)
ATROPINE	0.1	0.0048	2.99
ATROPINE	0.28	0.0134	8.83
ATROPINE	0.39	0.0187	12.35
ATROPINE	0.51	0.0245	16.38
ATROPINE	0.61	0.0293	19.75
ATROPINE	0.73	0.0351	25.24
KNO_3	0.12	0.0165	10.24
KNO_3	0.23	0.0317	24.04
KNO_3	0.38	0.0524	39.44
KNO_3	0.64	0.0883	66.60
KNO_3	0.78	0.1076	81.74
$(NH_4)_2SO_4$	0.1	0.0212	14.05
$(NH4)_2SO_4$	0.22	0.0466	33.38
$(NH4)_2SO_4$	0.38	0.0805	61.31
$(NH_4)_2SO_4$	0.57	0.1208	75.23
$(NH_4)_2SO_4$	0.78	0.1653	122.93

and equations in Figure 2.8. The equations derived were normally between milligram of nitrogen (mgN) and total Area obtained. The calibration curves for atropine (mgN= 0.0014*Area + 0.0013; r^2= 0.99), ammonium sulphate (mgN= 0.0013*Area + 0.0021; r^2= 0.99) and potassium nitrate (mgN= 0.0014*Area + 0.0034; r^2= 0.97) suggest almost similar slopes with different intercepts. However the intercept for atropine (0.0013) and ammonium sulphate (0.0021) are closer compared to that of potassium nitrate (0.0034). All the three (atropine, ammonium sulphate and potassium nitrate) put together yielded a calibration equation (mgN= 0.0014*Area + 0.0016; r^2= 0.98) very similar to that of

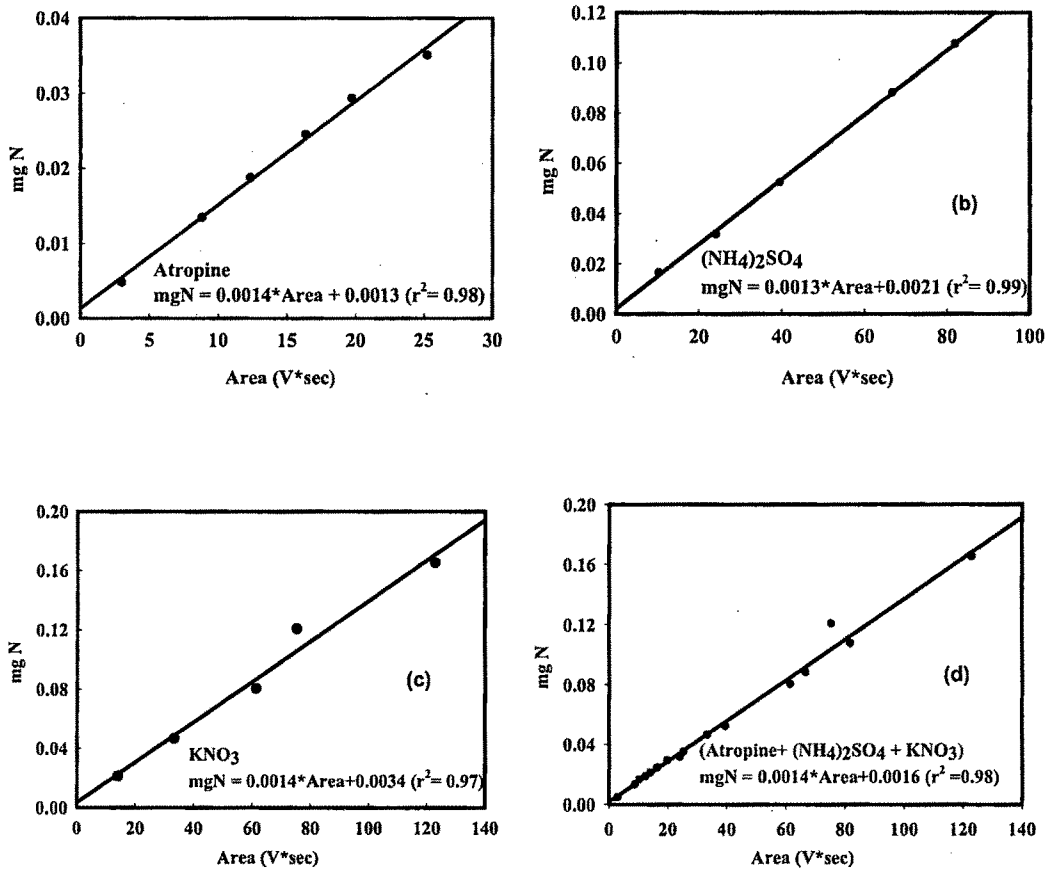


Figure 2.8 A typical example of calibration curves using different materials (atropine, ammonium sulphate and potassium nitrate).

atropine. This trend was reflected in almost all calibrations used for present study. In general, for the estimation of nitrogen content from unknown samples the calibration equations obtained by atropine was used. Table 2.13 lists the calibration equations actually followed for the estimation of total nitrogen content during present study.

Since the filter papers were cut into four equal halves, the nitrogen content obtained represented one fourth of the water filtered. Therefore, the nitrogen contents obtained were multiplied by the required number to get the molar concentration and were subsequently converted into μM N (μg-at N L^{-1}) using formula: μM N = (mg N *1000) / 14.

2.11.1 Error in the Particulate Nitrogen Content estimates

Table 2.14 lists the maximum % error observed during the particulate nitrogen content estimation in the Bay of Bengal and the Arabian Sea. The maximum error of 15% was

observed during the particulate nitrogen estimation for urea samples in the Bay of Bengal, which may be due to small amounts of sample or natural heterogeneities.

Table 2.13 Calibration equations used for the estimation of total nitrogen content during the present study.

Cruise No.	Samples	Calibration Equations used	r^2
Arabian Sea SK-186	Natural	mg N = 0.00286*Area-0.00005	0.96
	Nitrate	mg N = 0.00133*Area+ 0.00355 (up to 5th station samples)	0.99
		mg N = 0.0014*Area+ 0.0013 (5th to 7th station samples)	0.98
	Ammonium	mg N = 0.0013*Area-0.0008	0.99
	Urea	mg N = 0.0014*Area-0.0014 (upto 4th station)	0.99
		mg N = 0.0023*Area-0.0025 (5th to 7th station)	0.87
Arabian Sea SS-212	Natural	mg N = 0.00286*Area-0.00005	0.96
	Nitrate	mg N = 0.00138*Area+ 0.00129	0.99
	Ammonium	mg N = 0.0013*Area-0.0008	0.99
	urea	mg N = 0.0014*Area-0.0014	0.99
Bay of Bengal SK-182	Natural	mg N = 0.0014*Area-0.0006	
	Nitrate	mg N = 0.0014*Area-0.0006 (primary samples)	
		mg N = 0.0009*Area-0.0008 (Duplicate samples)	0.97
	ammonium	mg N = 0.0014*Area-0.0006 (primary samples)	
		mg N = 0.0009*Area-0.0008 (Duplicate samples)	0.97
	Urea	mg N = 0.0009*Area-0.0008	0.97
Bay of Bengal SK-191	Natural	mg N = 0.00286*Area-0.00005	0.96
	Nitrate	mg N = 0.000875*Area-0.000329	0.99
	Ammonium	mg N = 0.000875*Area-0.000329 (primary sample upto 3rd station)	0.99
		mg N = 0.0014*Area-0.0012 (Duplicate samples up to 3rd and both primary and duplicate from 4th station onwards)	0.95
	Urea	mg N = 0.0015*Area-0.0002	0.87

Table 2.14 Percentage error in particulate nitrogen content in the Arabian Sea and the Bay of Bengal.

Sample	Arabian Sea	Bay of Bengal
Nitrate	0.5	4.4
Ammonium	3.6	11
Urea	3.1	15

2.12 Estimation of uptake rate

Since the advent of the ^{15}N labeled nitrogen incorporation method for the estimation of uptake rate, several different equations are in use (Neess et al. 1962; Dugdale and Goering 1967; Eppley et al. 1977). All these equations rest on several assumptions, which become invalid under certain conditions.

2.12.1 Concept of the general equation for uptake

The concept of uptake equation is based on the isotopic mass balance at the end of incubation experiment (Collos, 1987). The number of ^{15}N atoms in final particulate nitrogen is equal to the sum of number of ^{15}N in the initial particulate nitrogen and number of ^{15}N atoms in the nitrogen taken up.

$$C_pN_f = C_0N_0 + C_d\Delta N \quad \text{-------} \{1\}$$

Where, C_p = Atom% ^{15}N in particulate phase after incubation (t = t)

C_0 = Atom% ^{15}N in particulate phase at the start of experiment (t = 0)

C_d = Atom% ^{15}N in dissolved phase at the start of experiment (t = 0)

N_f = Particulate nitrogen at end of incubation

N_0 = Particulate nitrogen at the start of experiment (t = 0)

ΔN = Nitrogen taken up during incubation.

Since, $N_f = N_0 + \Delta N$

$$N_0 = Nf - \Delta N \text{ --------} \{2\}$$

Putting eqn. (2) in eqn. (1)

$$C_pN_f = C_0 (N_f - \Delta N) + C_d \Delta N$$

$$N_f(Cp - C_0) = \Delta N (C_d - C_0)$$

$$\Delta N = N_f(C_p - C_0) / (C_d - C_0)$$

Nitrogen uptake rate

$\rho_N = \Delta N/\Delta t = N_f(C_p - C_0) / \Delta t\,(C_d - C_0)$, where Δt is the incubation time.

Following assumptions are made for simplicity:

1. Single nitrogen source for phytoplankton.
2. No excretion of nitrogen by phytoplankton during incubation.
3. Isotopic discrimination is negligible.
4. ^{15}N atom% in the dissolved phase remains constant.

Collos (1987) argued that the uptake rate estimated using this concept will be closer to the real uptake rate, as bias introduced due to the contribution of unlabeled compound is exactly compensated by biased introduce by final particulate nitrogen and hence uptake rate will be unaffected.

2.12.2 Equation used for the calculation of uptake rate during the present study and involved uncertainties

During present study the equation of Dugdale and Wilkerson (1986) was used for the calculation of uptake rates, where uptake rate was calculated by multiplying the particulate nitrogen content with specific uptake rate (nitrogen taken up per unit particulate nitrogen) at the end of incubation.

Specific uptake rate $V = [^{15}N_{xs} / (^{15}N_{enr} * t)]$ and

Uptake Rate (μmol N m^{-2} d^{-1}) $\rho = V * PON\ (t)$

Where, $^{15}N_{xs}$ = excess ^{15}N in the post incubation particulate samples

= measured ^{15}N atom% - ^{15}N natural abundance (0.36781 atom %)

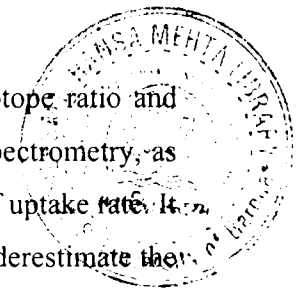
PON (t) = Particulate Organic Nitrogen content of sample after incubation (μmol N L^{-1})

t = incubation time in hours.

$^{15}Nenr$ = ^{15}N enrichment in the dissolved fraction

$= [(I_o * S + I_{tr} * S_t)/(S+S_{tr}) - {}^{15}N \text{ natural}]$

Where, I_o and I_{tr} are the natural ^{15}N atom% and ^{15}N atom% of the added tracer, respectively. S and S_{tr} are the ambient nutrient concentration and concentration of tracer added, respectively.



This formula was chosen for the calculation because it allows the isotope ratio and particulate nitrogen to be measured on the same sample during mass spectrometry, as in the present study, and therefore provides the most accurate estimate of uptake rate. It also cancels out the effect of detrital nitrogen and does not allow it to underestimate the uptake rated because of its presence:

$$\rho_{cells} = V_{cell} * PON_{cell} = (V_{meas.} * PON_{total} * PON_{cells}) / (PON_{cells}) = V_{meas} * PON_{total}$$

For nitrate and urea, daily uptake rate was calculated assuming a day length of 12 hours and negligible uptake during night time. For ammonium hourly rate was multiplied by 18 to get daily uptake rate (Dugdale and Wilkerson 1986). Ammonium uptakes reported here have not been corrected for isotope dilution (Glibert et al. 1982a). The uptake rates determined in terms of nitrogen can be converted in carbon units by using Redfield ratio (C: N= 106:16).

Apart from analytical uncertainties involved in measurement of PON and at% ^{15}N the uncertainty in uptake rates arises due to overaddition of tracer (>10% of ambient concentration) and due to isotopic dilution (Dugdale and Goering 1967). The uncertainty due to over addition arises mainly from lack of analytical precision when the ambient nutrient concentrations are very low (McCarthy et al. 1992), especially in surface water. This results in enrichment of dissolved fraction with ^{15}N from 50 to 100% and hence overestimation of uptake rate (Dugdale and Wilkerson 1986; Harrison et al. 1996; L'Helguen et al. 2002). During present study care was taken to minimise this uncertainty by adding the tracer as recommended by JGOFS protocol (~10nM range). However, the enrichment during the first station of SS-212 was more than 90% as there was no nitrate present in the surface layer. The addition of tracer causes the substantial increase in dissolved N concentration and in turn overestimate the uptake rate (Dugdale and Wilkerson 1986). The extent of overestimation for nitrate uptake at discrete depths during present study was estimated as suggested by MacIsaac and Dugdale (1972). The half saturation constant for Bay of Bengal was taken as 0.05μM which is a characteristic value of nitrogen poor oceanic waters (Kanda et al. 1985; McCarthy et al. 1992; Harrison et al. 1996) whereas for the Arabian Sea, it was taken as 1μM, typical for eutrophic waters (MacIsaac and Dugdale 1969; half saturation constant of 1.7μM has been reported for northwestern Arabian Sea by McCarthy et al. 1999). This exercise suggests that on an average the percentage of overestimation in the Bay of Bengal is <3% during SK-191 and <10% during SK-182. However, the

overestimation during Arabian Sea has been found to be relatively more (<5% during SK-186 and around 20% during SS-212). The effect of isotope dilution on uptake rate (Dugdale and Goering 1967) has not been considered as the incubation period was short (less than four hours) enough not to have effective dilution of initial isotopic enrichment, so as to affect the uptake rate significantly (Dugdale and Wilkerson 1986).

2.13 Quality Control

Quality control was of utmost importance during the experiments. Go-Flo bottles were used to avoid trace metal contamination. The NALGENE bottles used for experiments were filled directly from the Go-Flo bottles. No carboys were used to store the water samples in order to avoid possible contamination. Bottles were thoroughly rinsed with the same water before collecting the samples. The samples were collected in low light conditions. Samples were covered with thick black cloth after the collection. During the tracer addition new pipette tips were used for each addition to avoid cross contamination. The samples were always kept in dark and were not exposed suddenly to light when taking out for incubation. These measures were taken to avoid possible light shock to the phytoplankton. Filtration was also done in an almost dark environment. During the filtration of the samples, only the sample to be filtered was taken out and rest of them were kept in dark. Different filtration cups and forceps were used for different tracers and natural samples. These cups were thoroughly rinsed with filtered seawater after filtering one sample. Once the whole filtration was over the polycarbonate NALGENE bottles were cleaned with 0.25N HCl and three times with Milli-Q water (Fitzwater et al. 1982) before the next experiment.

2.14 Physical and Chemical parameters

Onboard measurements of various physical properties were carried out during both Arabian Sea as well as Bay of Bengal cruises. The primary physical properties measured were pressure, temperature and salinity using conductivity-temperature-depth profiler (CTD). The chemical components in the seawater such as dissolved oxygen and total dissolved inorganic carbon were measured by NIO, Goa during Bay of Bengal studies. The chemical constituent studies were not a priority in the Arabian Sea and hence no such measurements were carried out. However, Dr. S.G.P. Prabhu Matondkar of NIO, Goa, carried out the chlorophyll *a* measurements using fluorometric technique.

A Satlantic radiometer was operated during both cruises of Arabian Sea to monitor the upwelling and downwelling irradiance at different depths. Continuous profiles of temperature and chlorophyll was also obtained from sensors attached to the radiometer for the purpose. All sensors were well calibrated before the starting of the cruise. Quality of chemical and chlorophyll *a* measurements was monitored, by analysing suitable standard materials and repeat analyses.