Chapter Two

Sampling Locations, Seasons and Experimental Techniques

2.1 Introduction

The main aim of this thesis is to characterize the Indian Ocean on the basis of new production and *f*-ratio and to evaluate its role in the Global Carbon Cycle. To achieve this goal, three different methods were used:

1. Satellite based Ocean color studies of northeastern Arabian Sea
2. ^{15}N measurements in the same region of the Arabian Sea. Measurements were also done in the equatorial and the southern Indian Ocean
3. Small scale iron enrichment experiment in the southern Indian Ocean

Recent observations based on ocean colour show that the summer productivity in the western Arabian Sea has been increasing during the last seven years, reportedly due to the warming of the Eurasian landmass (Goes et al., 2005). Ocean color data from SeaWiFS (Sea-viewing Wide Field of view Sensor) for the Arabian Sea were analyzed to asses the role of global climate change on the productivity of the eastern Arabian Sea.

For ^{15}N measurements four cruises were undertaken: two cruises in the eastern Arabian Sea and one each in the equatorial Indian Ocean and the Southern Indian Ocean. The details of the cruises with the region of study, cruise number, duration and the ships on which studies were carried out are listed in Table 2.1.

Region	Cruise No.	Duration	Ship
Arabian Sea	SS- 222	20th Feb to 11th March-2004	FORV *Sagar Sampada*
Arabian Sea	SK- 214	4th Dec to 16th Dec-2004	ORV *Sagar Kanya*
Equatorial Indian Ocean	SK- 220	10th May to 6th June-2005	ORV *Sagar Kanya*
Southern Ocean	ABP- 15	25th Jan to 1st April- 2006	*Akademik Boris Petrov*

Table 2.1 Details of the cruises undertaken for this study

Iron is believed to be a limiting nutrient in the southern ocean. In the southern Indian Ocean small scale iron enrichment experiments were also done, for the first

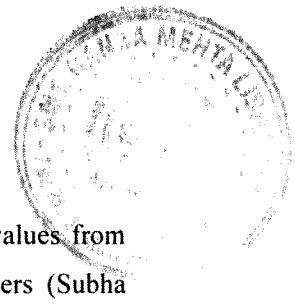
time, to assess the effect of iron enrichment on the N-uptake rates in this part of the world ocean.

2.2 Ocean colour studies

The only possible way to monitor the variation in the biological properties of the ocean on a larger spatial scale is by remote sensing. Ocean colour remote sensing is a method of collecting information about the constituents of water using optical signals in the visible range. It is well established that the concentration of phytoplankton influences the colour of the ocean water. Chlorophyll-a, which is the main photosynthetic constituent in the phytoplankton, absorbs more in blue than in green; as the concentration of phytoplankton increases, the backscattered light progressively shifts towards the green. This property is successfully used to derive the Chl-*a* concentration with the help of a sensor in a satellite. In the tropics, where variation in sunlight is not significant on an interannual scale, variation in chlorophyll concentration can indicate the variation in primary production. Satellite ocean colour data provides the spatial and temporal variations in phytoplankton biomass and hence in the primary production on a larger scale. Since the launch of SeaWiFS (Sea-viewing Wide Field of view Sensor) in August 1997, global ocean colour data are available to the science community on a regular basis.

2.2.1 Analysis of ocean colour data

For present study, monthly composites Level-3 Version 49-km resolution mapped SeaWiFS chlorophyll images were taken. From these images chlorophyll values were obtained using SEADAS software (provided by NASA for ocean colour image processing). SeaWiFS uses OC2 algorithm (O'Reilly et. al., 1998) for deriving Chl-*a* values from the recorded radiance. Sensitivity studies on the algorithm for Chl-a retrieval from measured sensor detected radiances show that the retrieved Chl-a values have the accuracy of ~ 30% (the radiance has an error of ~ 1%). For this analysis, pixel values more then 5 mgm^{-3} were not considered because, OC2 algorithm which is used for deriving Chl-a values from the recorded radiance overestimates Chl-*a* when it is more than 1.5 mg m^{-3}. Apart from that, in our analysis pixels having more then 1.5 mgm^{-3} correspond to coastal waters which are case 2



waters. It is well established that the algorithms used to derive Chl-a values from case 1 waters i.e., open ocean waters, break down in the case 2 waters (Subha Sathyendranath, IOCCG report no 3). Case 2 waters are influenced not only by the phytoplankton but also by the inorganic suspended particles and dissolved organic matter. Presence of these in coastal waters interferes with the phytoplankton signals and algorithm OC2 tends to overestimate the Chl-*a*.

The seasonality of the north-east Arabian Sea SST (Sea Surface Temperature) is also inspected over the same region for the same time period in order to analyse the effect of sea surface cooling due to upwelling on the chlorophyll-*a* concentration. Monthly composite SST data are taken from AVHRR (Advanced Very High Resolution Radiometer) pathfinder version 5 (June-1997 to Dec-2004) and MODIS (Moderate Resolution Imaging Spectro-radiometer) data (Jan-2005 to June-2005). We have also analyzed version-3 QuickScat data in order to monitor change, if any, in wind speed over past 6 years (1999-2005).

2.2.2 Study area

The remote sensing studies were done for the eastern Arabian Sea. We have divided the eastern Arabian Sea into two zones (Fig. 2.1): Zone 1 extends from 20°N to 25°N and 62°E to 75°E and zone 2 extends from 20°N to 10°N and 62°E to 75°E. This division into two different zones is based on the observed physical forcing responsible for the high production in each zone. Our aim, here, is not to subdivide the basin into different hydrographic regimes but to see the change in production in the basin as a whole. The proposed subdivision allows us to monitor any change over this area.

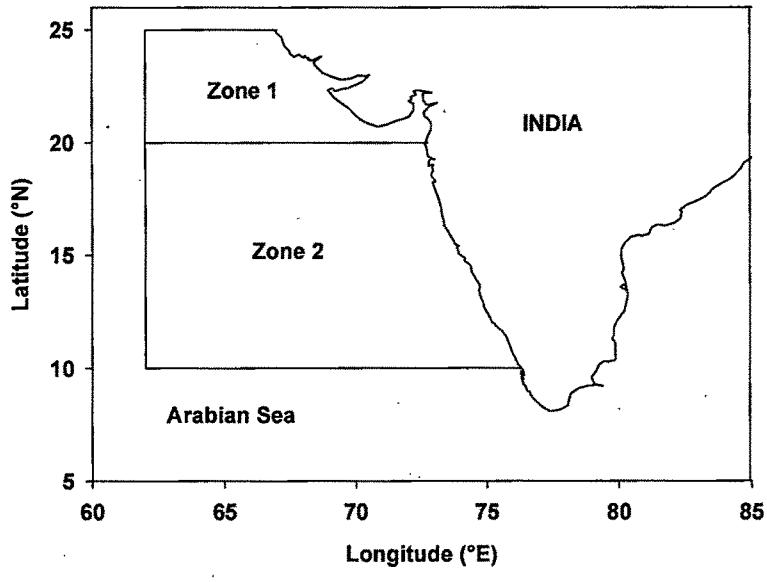


Fig. 2.1 Study Area for Ocean color studies

2.2.3 Limitations of Ocean remote sensing

One of the major limitations of the ocean colour remote sensing is that it is very sensitive to the meteorological conditions. The present sensors used in SeaWiFS and MODIS give abnormal values when cloud cover or some other interference like land etc is present. We could not get enough data for the months of June, July and August because of very dense cloud cover in this area. However, since the zone 2 encompasses a large area we could get some cloud free pixels which certainly could not be neglected. Therefore some chlorophyll values have been obtained in these months.

2.3 Primary Production

Joint Global Ocean Flux Study (JGOFS) protocol (Fig 2.2) was followed for the estimation of total primary production which is the sum of new and regenerated production. For the present study nitrate uptake rate is considered as new production and a sum of ammonium and urea uptake rates as regenerated production. New production is measured as the uptake rate of ^{15}N-labelled nitrate by the phytoplankton during deck incubation and regenerated production as a sum of ^{15}N-

labelled ammonium and urea uptake rates. Though the objectives and the research teams were different during different cruises, the same experimental procedures were followed, which are discussed in detail in the following subsections.

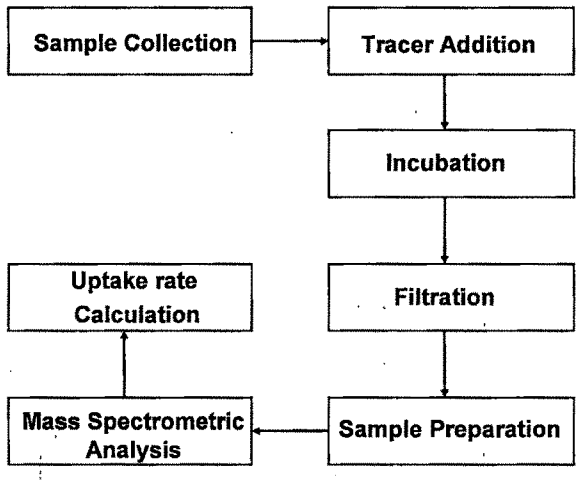


Fig. 2.2 Block diagram showing steps involved in estimation of new and regenerated production

2.3.1 Arabian Sea

India launched its first Ocean Color Sensor called Ocean Color Monitor (OCM) on Indian Remote Sensing Satellite IRS P4 in May 1999. The cruises in the Arabian Sea were a part of satellite ocean-color data validation cruises for OCM and were undertaken in collaboration with Space Application Centre (SAC), Ahmedabad. Cruises were undertaken in two seasons: during the late winter monsoon (Feb.-March 2004) and during the early winter monsoon (Dec-2004). During the late winter monsoon sampling was done in the eastern Arabian Sea onboard *FORV Sagar Sampada* (SS-222). Water samples were collected at 11 different stations, shown in Fig. 2.3. Here PP1 to PP11 denote primary productivity stations 1 to 11. The details of sampling locations along with dates of sampling and sampling depths are listed in Tables 2.2 and 2.3, respectively.

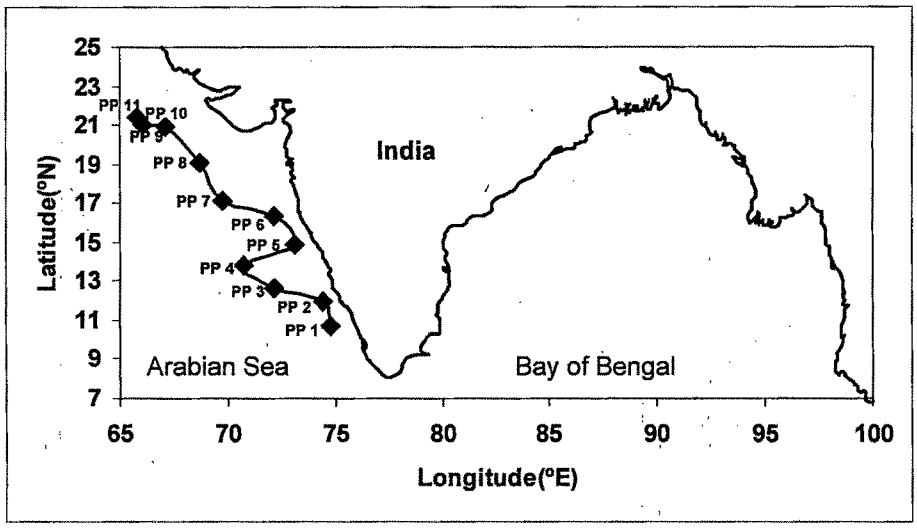


Fig. 2.3 The cruise track along which sampling was done during SS-220. PP denotes primary productivity stations

New Production Stations	Latitude(°N)	Longitude(°E)	Date
PP1	10.72	74.78	22/02/2004
PP2	12.01	74.45	23/02/2004
PP3	12.65	72.16	24/02/2004
PP4	13.81	70.73	25/02/2004
PP5	14.84	73.11	26/02/2004
PP6	16.38	72.16	27/02/2004
PP7	17.10	69.79	28/02/2004
PP8	19.08	68.71	29/02/2004
PP9	20.91	67.08	01/03/2004
PP10	21	66.08	02/03/2004
PP11	21.41	65.82	03/03/2004

Table 2.2 Sampling location along with dates of sampling during the Arabian Sea cruise in late winter (Feb-March 2004)

%Light	Sampling Depths (m)										
	PP 1	PP 2	PP 3	PP 4	PP 5	PP 6	PP 7	PP 8	PP 9	PP10	PP11
100	0	0	0	0	0	0	0	----	----	----	----
80	4	4	2	1.5	2	2	1.5	0	0	0	0
64	7	7	4	3.5	5.5	5	4.5	2.5	3	3	2.5
20	30	17	27	19	30	11	18	15	11	11	9
5	57	45	51	51	55	---	40	35	26	25	16
1	95	83	100	81	115	---	130	67	55	55	25

Table 2.3 Sampling depths during Feb-March 2004

During the early winter monsoon (Dec-2004) sampling was again done at 11 stations, again in the eastern Arabian Sea onboard *ORV Sagar Kanya* (SK-214) but this time the sampling locations (Fig. 2.4), though in the same region, were different from the earlier ones. The details of the sampling locations for this cruise, with sampling dates are given in Table 2.4 and sampling depths are given in Table 2.5.

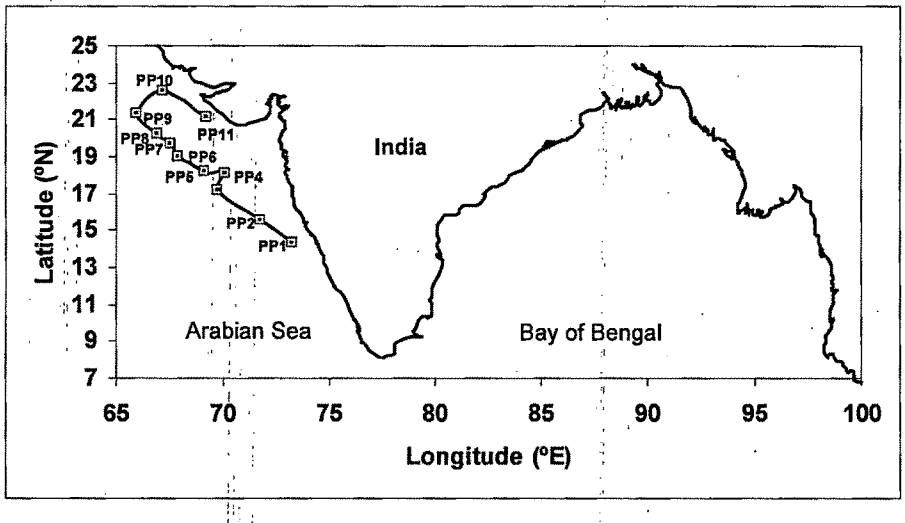


Fig. 2.4 The cruise track along which sampling was done during SK-214. PP denotes primary productivity stations

New Production Stations	Latitude(°N)	Longitude(°E)	Date
PP1	14.33	73.28	05/12/2004
PP2	15.58	71.73	06/12/2004
PP3	17.20	69.72	07/12/2004
PP4	18.10	70.08	08/12/2004
PP5	18.17	69.14	09/12/2004
PP6	19.00	67.92	10/12/2004
PP7	19.74	67.54	11/12/2004
PP8	20.22	66.96	12/12/2004
PP9	21.30	65.94	13/12/2004
PP10	22.59	67.22	14/12/2004
PP11	21.17	69.18	15/12/2004

Table 2.4 Sampling location along with dates of sampling during the Arabian Sea cruise in early winter (Dec-2004)

%Light	Sampling Depths (m)										
	PP 1	PP 2	PP 3	PP 4	PP 5	PP 6	PP 7	PP 8	PP 9	PP10	PP11
100	0	0	0	0	0	0	0	0	0	0	0
80	3	3	2.5	3	3	4	3	3	3	2	2.5
64	6	6	5	6	6	7	6	6	6	4	5
20	21	21	18	21	21	25	21	22	22	14	19
5	40	40	33	40	40	46	40	42	42	26	35
1	60	60	51	60	60	70	60	64	64	40	54

Table 2.5 Sampling depths during Dec-2004

2.3.2 Equatorial Indian Ocean

This cruise was aimed at studying equatorial Indian Ocean processes, in which a thorough measurement of physical, chemical and biological properties were carried out jointly by scientists from the Physical Research Laboratory (PRL), Ahmedabad and the National Institute of Oceanography (NIO), Goa, India.

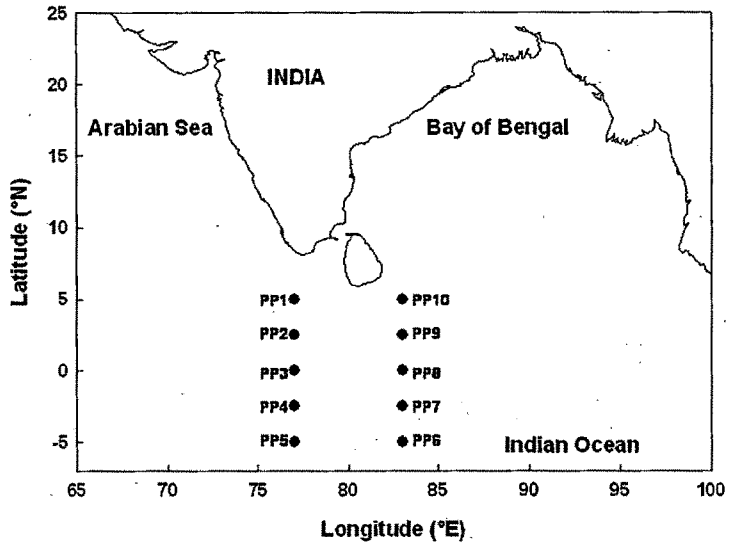


Fig. 2.5 The cruise track along which the sampling was done during SK-220.

In the equatorial Indian Ocean measurements were made in the pre-monsoon season (May-June 2005) on board *ORV Sagar Kanya* (SK-220). Here we collected samples from 10 stations along two transects, 77°E and 83°E (Fig. 2.5). Sampling was done at every 2.5° starting from 5° N to 5° S, with 5 stations along each transect. The details of the sampling locations for this cruise, with sampling dates are given in Table 2.6 and sampling depths are given in Table 2.7.

New Production Stations	Latitude	Longitude	Date
PP1	5 °N	77 °E	12/05/2004
PP2	2.5 °N	77 °E	14/05/2004
PP3	0 °N	77 °E	16/05/2004
PP4	2.5 °N	77 °E	19/05/2004
PP5	5 °N	77 °E	22/05/2004
PP6	5 °N	83 °E	25/05/2004
PP7	2.5 °N	83 °E	27/05/2004
PP8	0 °N	83 °E	31/05/2004
PP9	3 °N	83 °E	03/06/2004
PP10	5 °N	83 °E	04/06/2004

Table 2.6 Sampling location along with dates of sampling during the equatorial Indian Ocean cruise in pre-monsoon season (May-June 2005)

%Light	Sampling Depths (m)									
	PP 1	PP 2	PP 3	PP 4	PP 5	PP 6	PP 7	PP 8	PP 9	PP10
100	0	0	0	0	0	0	0	0	0	0
80	6	5	6	7	5	8	6	6	5	6
64	12	10	12	15	11	15	12.5	12	11	12
20	43	36	43	53	39	55	45	43	39	41
5	80	66	80	99	72	102	84	80	72	76
1	124	101	124	152	111	157	129	124	111	117

Table 2.7 Sampling depths during SK-220 (May-June 2005)

2.3.3 Southern Indian Ocean

The Southern Ocean cruise was a part of second expedition to the Southern Ocean and Larsemann Hills, Antarctica. The main task was to make a bathymetric survey of the approach channel to the proposed new station of India at the Antarctica. We carried out our experiments at a few selected sites on our way to Antarctica. Sampling was done in the late austral summer (Feb-March 2006), on board *RV Akedemik Boris Petrov* (ABP-15), at eight different stations (details of sampling locations and with dates are given in Table 2.8) covering a large area from the equator (0°) to the Antarctic coast (69°S) along the track shown in the Fig 2.6. Details of the sampling depths are listed in Table 2.9.

New Production Stations	Latitude(°S)	Longitude(°E)	Date
PP 1	69	76	24/02/2006
PP 2	65	56.3	03/03/2006
PP 3	58	50	06/03/2006
PP 4	43	48	13/03/2006
PP 5	40	48	15/03/2006
PP 6	35	48	17/03/2006
PP 7	7.5	61	02/02/2006
PP 8	0°	64	30/01/2006

Table 2.8 Sampling Location along with dates of sampling during the Southern Indian Ocean cruise (Jan-April 2006)

%Light	Sampling Depths (in Meter)							
	PP 1	PP 2	PP 3	PP 4	PP 5	PP 6	PP 7	PP 8
100	0	0	0.5	1	1	0	1	1
80	6	6	1.5	5	5	5	5	6
64	12	12	3	10	10	10	10	11
20	43	43	10	35	30	35	35	41
5	80	80	19	65	60	65	70	75
1	124	124	29	100	90	100	100	115

Table 2.9 Sampling depths during the Southern Indian Ocean cruise (Jan-April 2006)

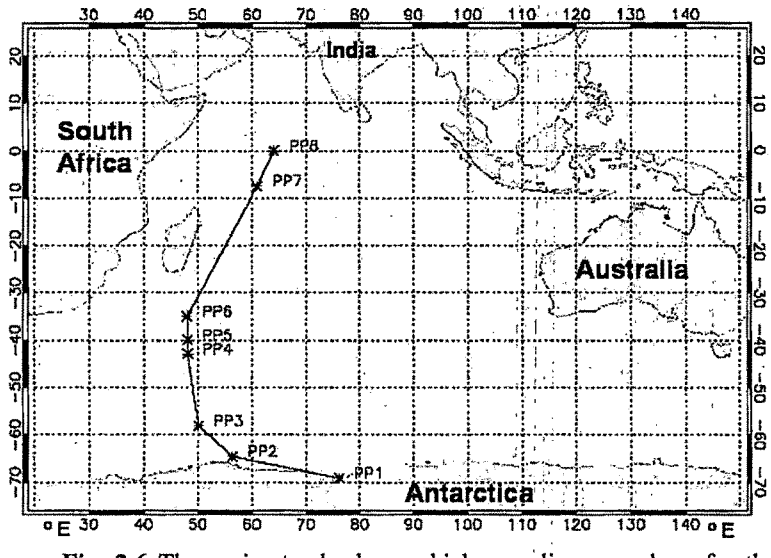


Fig. 2.6 The cruise track along which sampling was done for the present study in the Southern Ocean (ABP15).

2.4 Experimental Procedures

2.4.1 Sample collection

Common sampling procedure was followed during all the four cruises. Water samples were collected from six different depths to cover the entire photic zone. The depth of the photic zone i.e., the depth at which light falls to 1% of the surface level, was estimated using an underwater radiometer (Satlantic Inc.). Based on these light measurements six different depths were chosen for collecting water samples. The corresponding light levels are 100%, 80%, 64%, 20%, 5% and 1% of the surface

value. During all the cruises water samples were collected using clean Go-Flo bottles (General Oceanic, Miami, Florida, USA) attached to a CTD rosette (Fig. 2.7).

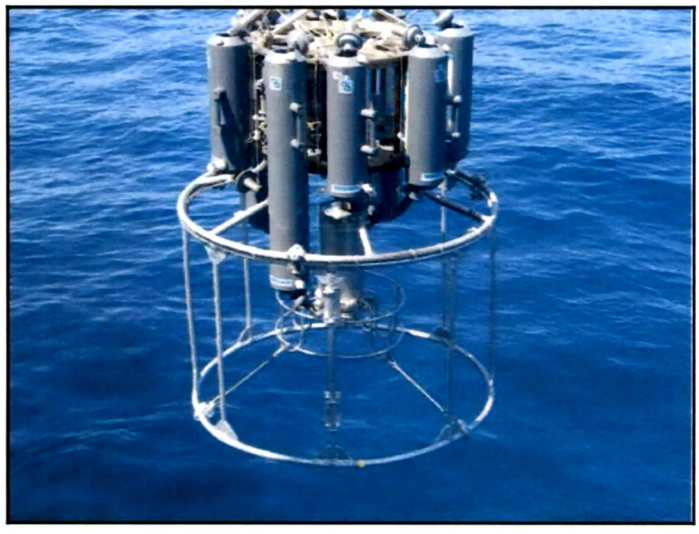


Fig. 2.7 Go-Flo bottles attached to a CTD rosette used to collect sea-water samples from different depths

An electronic CTD (Sea-Bird) was used to obtain conductivity-temperature-depth profiles. The temperature and pressure (*i.e.*, depth) sensors were calibrated before the cruise. The SEASOFT software package was used to process the raw CTD data. The CTD attached rosette was first lowered to the lowest desired depth and water samples were collected while hauling up by closing the bottles one by one at the desired depths. To ensure that the samples are collected from the desired depths, the CTD rosette was allowed to stabilize at that particular depth for some time, generally for one minute. Once the rosette was hauled back to the deck, the samples were immediately transferred to pre-washed polycarbonate Nalgene bottles of two (or one) liter capacity. Individual water samples were taken for nitrate (2L volume), ammonium (2L) and urea (1L) enrichment experiments. This was followed by the addition of ^{15}N (99 atom %) enriched tracer followed by incubation. Details of preparation of tracer solution, addition of tracer and incubation are discussed in following subsections.

2.4.2 Tracer preparation

^{15}N enriched nitrate, ammonium and urea tracers were made by dissolving ^{15}N-labelled (99 atom% enriched) dry nitrate, ammonium and urea salts, procured from Sigma-Aldrich (USA). First of all, stock solution of higher concentration for each tracer was made. 43.5 mg of nitrate (molecular weight ~ 85.98 g), 27.3 mg of ammonium (molecular weight ~ 54.48 g) and 16.8 mg of urea (molecular weight ~ 62.04 g) salt were dissolved separately in 250 ml of double distilled water in three different, well cleaned flasks, to make a stock solution of concentration ~2 mM. This stock solution was diluted to make working solutions of lower concentrations. To make a working solution of concentration 0.01 µmole/ml, 5 ml of stock solutions of each compound was added to 995 ml of double distilled water. One ml of this solution, when added to one liter of sample, raised the concentration of respective tracers in the sample by 0.01 µM. Another working solution of nitrate of concentration 0.1 µmole/ml was made by adding 12.5 ml of the stock solution in 237.5 ml of double distilled water. 1 ml of the resultant solution contains 0.1 µmole of nitrate and when added to a litre of sample, increased the concentration of nitrate in that sample to 0.1 µM. Each stock and working solution was then kept in separate 250 ml Nalgene bottles. The weighing of the salts was done on Sartarious microbalance (Model No. MC-5; Germany) using Thomas Scientific weighing paper.

2.4.3 Ambient Nutrient Measurement

Nitrate, ammonium and urea were the nutrients of interest for the present study. During the Arabian Sea and Southern Ocean cruises, water samples (~100 ml) from each depth were taken in separate bottles and preserved in deep freezer to carry out the nitrate measurements at Dr. S. W. A. Naqvi's lab at NIO, Goa at the end of the cruise using an autoanalyzer. During the equatorial Indian Ocean cruise, nitrate was measured by onboard by Dr. Sugandhini Sardesai of NIO, Goa and her colleagues using an autoanalyzer. Ambient ammonium and urea could not be measured because of logistic reason but was calculated indirectly using regeneration rates for ammonium and urea by the mesozooplankton given by Mullin et al. (1973). Mesozooplankton was converted into dry weight using equation of Weibe et al. (1975). Using average excretion rates of 0.59 and 0.32 mg at-N (g dry wt.)$^{-1}$ d^{-1}, the

release rates were calculated for 12 hrs residence time of mesozooplankton in the mixed layer. According to this calculation the average ammonium and urea concentration in the upper layer were 0.017 μM and 0.009 μM respectively. These values could be at the detection limit (~0.01 μM) if we take the uncertainties involved in the calculation into consideration and therefore, for the present study it was assumed that the tracer added was the only source for the planktons. Hence the uptake rates calculated here for ammonium and urea are conservative estimates.

2.4.4 Addition of tracers

Tracers were added to the samples just before the start of the incubation. For the Arabian Sea and Southern Ocean cruises, since the ambient nutrient measurement was not done onboard, a prefixed amount of nitrate was added. The nitrate concentration for these regions is well documented, so an attempt was made to add less than 10% of the possible ambient value. The actual nitrate measurements, which were done after the end of cruise, were used to calculate the uptake rates. During the equatorial Indian Ocean cruise, although nutrient measurements were done onboard, due to some logistics problems ambient nitrate value was not available on the day of sampling but was available only towards the end of the cruise. So here also prefixed concentrations of nitrate were added. Ambient ammonium and urea were not measured, so constant amount of ammonium and urea tracers were added; 0.01 μM during the Arabian Sea and equatorial Ocean cruises and 1 μM during southern ocean cruises (except at PP8 and PP9 which were in the equatorial region and hence 0.01 μM of each was added). During the Southern ocean cruise 1 μM ammonium and urea were added following Reay et al., (2001). Addition of high concentration of ammonium, here, may lead to the overestimation of ammonium uptake and hence the underestimation of the *f-ratio* (Binachi et al., 1997; Oslon et al., 1980)

2.4.5 Incubation

Addition of tracers was followed by incubation. Soon after the addition of tracers, appropriate neutral density filters were put on the bottles. This was done in order to simulate the in-situ light condition. The neutral density filter was chosen in

such a way that the samples receive the same amount of sunlight that was available at the depth from which samples were taken. The filters were well calibrated using lux-meter in both dry and wet conditions. Once the filters were put on, the samples were left for incubation on the deck for four hours, symmetric to the local noon i.e., from 10.00 Hrs to 14.00 Hrs. A similar procedure was followed during the Arabian Sea and the equatorial Ocean cruises. For the Southern Ocean stations incubation was done for 6 hrs, except at two equatorial stations (PP7 and PP8). Since the quantum efficiency (production per unit chlorophyll per unit time) is very low in the case of Southern Ocean phytoplankton because of low sea surface temperature (~1°C), six hours incubation was done to ensure that the phytoplankton get enough time for photosynthetic production.

Flowing sea water from 5 m depth was continuously maintained into the incubation crates during the whole incubation period to regulate the temperature. After the incubation i.e., exactly after 4 hrs (6 hrs in case of Southern Ocean stations), samples were transferred to the shipboard laboratory for filtration and were kept wrapped in a thick black blanket in a dark cabin and were kept in dark till the filtrations were over. Filtrations were finished within 2 hours after the incubation.

2.4.6 Filtration

All samples are filtered subsequently through 47 mm diameter and 0.7 μm pore size Whatman GF/F filters. These filters were calcinated for 4 hours at 400°C. Before calcination, filters were wrapped in an aluminium foil; one set of foil containing six filters. Well cleaned forceps were used to handle these filters as a precautionary measure to avoid any contamination. Samples were filtered under low vacuum (<100 mm Hg) using a manifold unit procured from Millipore, USA. Samples treated with nitrate, ammonium and urea tracers were filtered using separate glass cups. After filtration, filter papers were "washed" using filtered sea-water to remove any left over ^{15}N. Filter papers were taken out carefully, after the filtration, with the help of forceps. Separate forceps were used for nitrate, ammonium and urea treated plankton samples. Filter papers were then kept in pestrislide boxes (Millipore make, procured from Millipore, USA). This was followed by keeping filters in an oven, at 50°C for 12 hrs, for drying and bringing to shore for isotopic analysis.

Fig. 2.8 Author filtering samples in dark on board R/V *Akademik Boris Petrov*

2.5 Instruments and analysis

In the recent years, improvement in mass spectrometry has revolutionized the application of different stable isotopes, especially the use of ^{15}N as a tracer to understand the oceanic processes and their role in the global carbon cycle. ^{15}N, a stable isotope of nitrogen, has an important application in the estimation of oceanic export production but low concentrations of nitrogen in waters had limited its use in the marine applications. Methods such as wet chemical sample preparation and isotope ratio mass spectrometry requires large amount of nitrogen (~100 μmole) and is mainly used in agricultural research. Another method, emission spectroscopy, is suitable for measuring low amounts of nitrogen (<1 μmole) and is suitable for marine applications but the precision is low.

With improved electronics, vacuum system and ion optics, even low concentrations of nitrogen (<1 μmole) can be measured with sufficiently high precision using an isotope ratio mass spectrometer. For the present study, all samples were analysed using a *CarloErba* elemental analyser interfaced via conflo III to a *Finnigan Delta Plus* mass spectrometer, using a technique for sub-microgram level ^{15}N determination (Owens and Rees, 1989).

2.5.1 Instrumentation

As discussed earlier, samples were analysed using an elemental analyser interfaced with a mass spectrometer. Elemental analyser is based on the Dumas principle of high temperature flash combustion. It consists of two reactors: a combustion or oxidation chamber and a reduction chamber. Both, the combustion and reduction chambers, are prepared in quartz tubes (length 41 cm); combustion chamber is prepared by filling silvered cobaltous oxide and chromium oxide, separated by quartz wool. Reduction chamber is filled with reduced copper with quartz wool at the top and bottom. Quartz wool was also used at the top and bottom of the chamber. Quartz wool separates different chemicals used and second, being porous it allows free movement of gas. The chemicals required for combustion and reduction chambers were procured from Courtage Analyses Services (France).

Before analysis, the filter containing the sample is packed into a pellet using a silver foil. Well cleaned forceps are used for packing the samples which were then loaded in a turret on the top of the oxidation chamber. At a time a maximum of 50 samples could be loaded. There is a well between the turret and the oxidation chamber where the pellet falls. There the sample is purged with pure He gas (grade 5, 99.999%, procured from Hydragas, Bangalore). This is followed by allowing the sample to fall into the oxidation chamber. As soon as the sample falls, a one-second pulse of oxygen is given at a flow rate of 175 ml/sec. The temperature of the oxidation chamber is maintained at 1060°C but as soon as the sample falls and oxygen pulse is given, this temperature increases to 1800°C for a moment, which leads to the combustion of the sample with a flash. Combustion in the presence of a large quantity of oxygen results into production of oxides of nitrogen, CO_2 and H_2O. Helium acts as a carrier gas and carries these gases to the reduction chamber. The reduction chamber contains reduced copper at 680°C. Here different oxides of nitrogen are reduced to N_2 gas. The gases, which now contain N_2, CO_2 and H_2O, are then passed through magnesium perchlorate, which absorbs moisture and water vapor present in the gas mixture. The remaining gases are then carried to gas chromatographic column (maintained at 60°C) which contains a molecular sieve. The sample gas, here a mixture of N_2 and CO_2, passes in a gas stream (called as the *mobile phase*) at different rates in the gas chromatographic column depending on

their various chemical and physical properties and their interaction with a specific column filling, called the *stationary phase* (here the molecular sieve). The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time, called the retention time. The retention time of N_2 is less than CO_2, as a result of which N_2 moves faster than CO_2 and comes out earlier from the chromatographic column. Once the N_2 exits from the column, it is injected into the mass spectrometer through the Con-Flo.

2.5.2 Con-Flo

Con-Flo is an interface between the elemental analyzer and the mass spectrometer. N_2 released from the chromatographic column is introduced into the mass spectrometer through Con-Flo. It contains two inlets and one outlet capillary tube; one inlet each for the reference and the sample gases and one common oulet. Thus the Con-Flo transfers the reference or the sample gas into the mass spectrometer.

2.5.3 Mass Spectrometer

Analysis was done using a Finnigan Delta Plus stable isotope ratio mass spectrometer. This has an impact ionization source where ions are generated in high vacuum by electron impact. The energy of the ionizing electrons is 80eV. The ions, once produced, are accelerated towards the magnetic sector by a 3 KV potential. The ion beam exits the ion source through a slit with a width of 0.3 mm and then enters the magnetic sector where the magnetic field strength is 0.75 Tesla. The direction of the magnetic field is perpendicular to the direction of the moving ions. The geometry of the magnetic sector is such that the ion beam enters the magnetic sector at an angle of 26.5° and also exits the magnetic sector at the same angle. This is done in order to maintain the radius of curvature of flight at 9 cm. This mass spectrometer has a resolution ($m/\Delta m$) of 95 for C, N and O. The collector system consists of three Faraday cups connected to amplifiers.

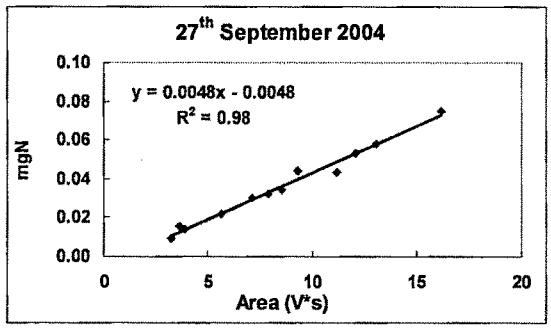
The elemental analyzer and mass spectrometer are fully automated and are controlled by the Finnigan MAT software ISODAT. Important instructions such as the opening and closing of sample and reference valves, their timings, time of helium

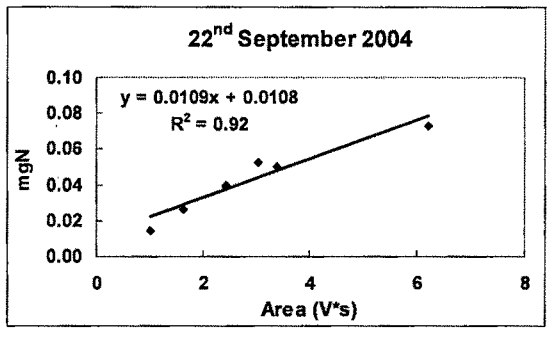
dilution and oxygen pulse rate etc. are controlled using this software. The typical data acquisition time for a sample is 450 seconds. Reference gases are introduced into the mass spec after the 37^{th} and 87^{th} seconds respectively. Sample gas is introduced at the ~150^{th} second and analysis is continued till the ~225^{th} second. Nitrogen peaks appear at the 187^{th} second. The rest of the time is used for He flushing to minimize the memory effect. Ratio of 29/28 and 30/28 are used to calculate the ^{15}N atom% and the total area under peaks 28, 29 and 30 is used to calculate the nitrogen content.

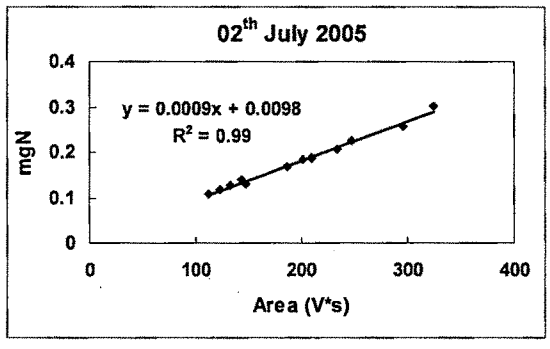
2.5.4 Calibration of the mass spectrometer

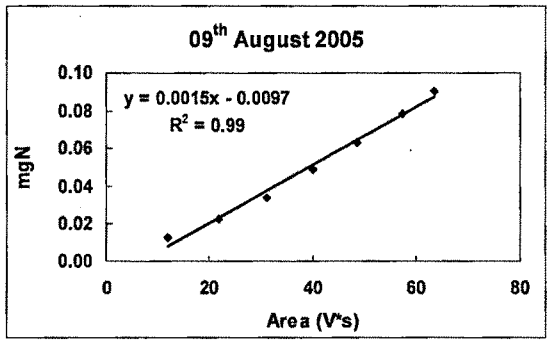
The aim is to measure the total organic nitrogen content of natural and enriched samples and the atom% ^{15}N. To this end, it is important to check the stability and working of the mass spec. For this, the mass spec is calibrated using some standard material, inorganic as well as organic, of known nitrogen content. Main standards used are: Potassium nitrate, ammonium sulphate, acetp[illegible] and BSA (an organic compound) which contain 13.8, 21.2, 10.3 and 17.8% nitrogen respectively. Since the typical concentration of particulate organic nitrogen is very less (typically 1 μmole), weight of the standards is taken in such a range that it contains similar concentrations of nitrogen (generally 0.10 to 0.50 mg). For each such standard, the total area (a sum of area under the 28, 29 and 30 peaks in Vs) is plotted against the nitrogen content and a regression equation is derived. Some typical examples of such plots are given below (Fig. 2.9a):

a.


27th September 2004
y = 0.0048x - 0.0048
R² = 0.98
mgN
0.10
0.08
0.06
0.04
0.02
0.00
0
5
10
15
20
Area (V*s)


22nd September 2004
y = 0.0109x + 0.0108
R² = 0.92
mgN
0.10
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
Area (V*s)


02th July 2005
y = 0.0009x + 0.0098
R² = 0.99
mgN
0.4
0.3
0.2
0.1
0
0
100
200
300
400
Area (V*s)


09th August 2005
y = 0.0015x - 0.0097
R² = 0.99
mgN
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
Area (V*s)

b.

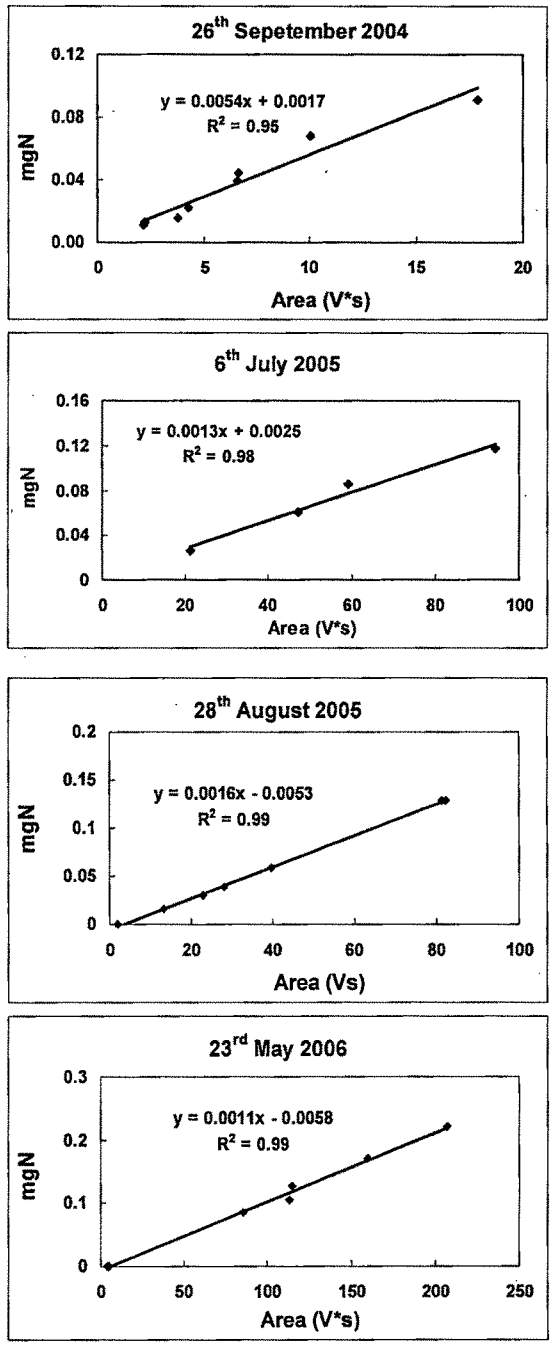


Fig. 2.9 Some typical examples of (**a.**) calibration curve obtained immediately after putting fresh chemicals in the oxidation/reduction chambers and (**b.**) examples of calibration curve used for the estimation of particulate organic nitrogen in the post incubation sample.

Only when the stability of the mass spec is confirmed, the sample analysis is started. In a set of 50 samples loaded in the turret for analysis, eight standards (8 standards + 42 samples) are also analyzed to check for possible stability change, if any. Overall precision on the basis of the standards measured during the analysis of the samples are shown in Table 2.10. These calibrations are used to calculate the organic nitrogen content of the sample. Some examples of such plots and equations are shown in Fig. 2.9b.

Standards Used	Quoted isotopic ratio	Values obtained during the present analysis
$(NH_4)_2SO_4$ (IAEA-N-2)	0.3753	0.3751 ± 0.0009 (n=22)
KNO_3 (USGS 32)	0.4340	0.4329 ± 0.0012 (n=16)
KNO_3 (IAEA-NO-3)	0.3695	0.3689 ± 0.0004 (n=11)

Table 2.10 Table showing overall precision, based on the standard measurements, during the analysis of the samples

During the Arabian Sea and equatorial Indian Ocean cruises only nitrate assimilation experiments was done in duplicates but during the Southern Indian Ocean cruises all the three i.e., nitrate, ammonium and urea, were done in duplicates. Table 2.11 shows the upper limit of the error in the PON estimation and atom % for the primary and duplicates.

Cruises	**PON**	**Atom %**
SS 222	< 35 %	< 0.5%
SK 214	< 9 %	< 5.5 %
SK 220	<7%	< 2 %
ABP 15	< 0.3%	< 3%

Table. 2.11 Difference between duplicates

2.5.5 Estimation of uptake rate

Nitrogen uptake by the phytoplankton is estimated from ^{15}N incorporation in the particulate organic matter. Several equations have been given in the past (Nees et al., 1962; Dugdale and Goering, 1967; Eppley et al., 1977) to estimate the uptake rate using ^{15}N labeled techniques but all equations are based on some primary assumptions which fail under some conditions. The general equation for the

estimation of nitrogen uptake rate is derived from the concept of isotopic mass balance at the end of the incubation and was first shown by Collos and Slawyk (1985). According to the isotopic balance the number of ^{15}N atoms in the final particulate organic nitrogen is equal to the sum of the number of ^{15}N atoms in the initial particulate matter and the number of ^{15}N atoms taken up.

If C_p: ^{15}N atom % in the particulate phase after incubation

C_d: ^{15}N atom % in the dissolved phase before incubation

C_o: ^{15}N atom % in the particulate phase before incubation

N_o: Concentration of particulate nitrogen before incubation

N_f: Concentration of particulate nitrogen after incubation

And ΔN: Nitrogen taken up during incubation

Then isotope mass balance equation can be written as:

$$C_p*N_f = C_o*N_o + C_d*\Delta N \quad \text{.....(1)}$$

Since $N_f = N_o + \Delta N$

Equation (1) can be rewritten as

$$C_p*N_f = C_o*(N_f - \Delta N) + C_d*\Delta N \quad \text{.....(2)}$$

Or $C_p*N_f = C_o*N_f - C_o*\Delta N + C_d*\Delta N$

Or $\Delta N*(C_o - C_d) = N_f*(C_o - C_p)$

Or $\Delta N = N_f*(C_o - C_p) / (C_o - C_d)$

Or $\Delta N = N_f*(C_p - C_o) / (C_d - C_o)$

If Δt is the time of incubation, then the uptake rate is given as:

$$\Delta N / \Delta t = N_f*(C_p - C_o) / \Delta t*(C_d - C_o) \quad \text{.....(3)}$$

2.5.6 Equation used for the estimation of uptake rates

During the present study uptake rates were calculated using the equation of Dugdale and Wilkerson (1986):

Uptake rate $= [PON * {}^{15}N_{xs}] / [{}^{15}N_{enrch} * t]$

Where PON = particulate organic nitrogen content of the sample in unit of µmolN/L

$^{15}N_{xs}$ = Excess ^{15}N in post-incubation samples. This is calculated as difference in atom % between measured ^{15}N in the post-incubation sample and ^{15}N natural abundance (0.36781 atom %).

$^{15}N_{enrch}$ = ^{15}N enrichment in the dissolved fraction relative to the ambient

$^{15}N_{enrch}$ is calculated as:

$^{15}N_{enrch}$ = [{(99 * tracer conc.) + ($^{15}N_{natural}$ * ambient conc.)} / (tracer conc. + ambient conc.)] - $^{15}N_{natural}$

And t = time of incubation

The above formula takes two parameters for the estimation of uptake rates: Particulate nitrogen and ^{15}N atom % in the post-incubation samples. These two parameters are measured on the same sample during mass spectrometer analysis and therefore this method gives the most accurate estimation of uptake rates. The hourly uptake rate is converted into daily uptake rate by multiplying it by day length *i.e.*, 12 hours, for nitrate and urea, as no nitrogen fixation takes place during night from nitrate and urea. For ammonium, the daily uptake rate is calculated by multiplying it by 18 hrs (Dugdale and Wilkerson, 1986). The nitrogen uptake rate is converted into carbon uptake rate using the Redfield ratio (C/N = 6.62), ignoring small variations in this ratio. Finally the *f*-ratio is calculated as:

f-ratio = nitrate uptake/ total N-uptake

where the total uptake rate is the sum of nitrate, ammonium and urea uptake rates.

2.6 Iron Experiment

In the Southern Ocean, apart from ^{15}N experiments, bottle scale iron enrichment experiments were also done. The productivity of the southern Ocean is believed to be limited by the presence of the micro nutrient "Iron" (Martin and Fitzwater 1988, Martin et al., 1990). Iron helps in plant metabolism (Geider and La Roche 1994). and lack of iron may cause a decline in the photosynthetic electron transfer (Geider and La Roche 1994: Hutchins 1995) and this may decrease the photosynthetic efficiency of phytoplankton present there. Martin and his colleagues (Martin and Fitzwater, 1988) measured the concentration of iron in the waters of Gerlache strait (Antarctic coastal water) and Drake passage (off shore) and concluded that off shore location is less productive because it is not having enough iron to facilitate the consumption of nitrate present in the water whereas the coastal stations receive iron from continental margins, which are rapidly consumed in the surrounding water which is very productive and this iron is not getting transported to the open ocean (Martin et al., 1990). The idea of iron limitation got momentum when

it was shown, through bottle scale Fe enrichment experiments carried out for the first time at station PAPA (50°N, 145°W) by Martin and colleagues, that there is rapid increase in chlorophyll concentration and nitrate was totally consumed after 4 days since iron enrichment (Martin and Fitzwater 1988). This was followed by a number of iron enrichment experiments both in equatorial Pacific (IronEx I and IronEx II) and Southern ocean (EisenEx, SOIREE, SOFeX, EIFEX). No iron enrichment experiment has been done so far in the Indian sector of the Southern Ocean. We, for the first time, did bottle scale iron enrichment experiment in the Southern Indian Ocean at two different stations, the locations of which are shown in Table 2.12.

Station Name	Geographical Location		Date of sampling
IEE 1	43°S	48°E	13/03/2006
IEE 2	35°S	48°E	17/03/2006

Table 2.12 The locations of the stations for Iron enrichment experiments along with the date of sampling. IEE stands for Iron Enrichment Experiment

2.6.1 Experimental Procedure

Sampling was done in late austral summer (Feb-March 2006), on board *Akedemik Boris Petrov* (ABP-15). Water samples from a fixed depth (in this case depth corresponding to deep chlorophyll maxima) were collected, using pre-cleaned Go-Flo bottles attached to a CTD rosette, to carry out the iron enrichment experiment. Samples were transferred directly into 1L Nalgene bottles to avoid any trace metal contamination. Samples were collected in eighteen 1L Nalgene bottles. These bottles were divided into two sets (set 1 and set 2) of 9 bottles each (Fig. 2.10). Further, each set of 9 bottles were subdivided into subsets of three bottles each (subset 1N, 2N and 3N) one bottle each for nitrate, ammonium and urea uptake rates (henceforth referred as 'control experiment'). This was followed by addition of respective nutrients tracers. The second set was also treated similarly but along with nutrient tracers, iron solution was also added in each bottle (henceforth referred as 'Fe experiment'). The purpose was: samples where only ^{15}N labelled tracers were added help calculate the total uptake during the whole incubation period (the control experiment), whereas the samples where iron tracer was also added help monitor the

change in production, if any, because of the iron addition. 1 μM of ^{15}N enriched (99%) nitrate, ammonium and urea tracers were added to the respective water samples. The nitrate tracer added corresponded to ~6% and ~18% of the ambient concentration at station IEE1 and 2 respectively. Ambient ammonium and urea were not measured because of logistic reasons and it was assumed, for the calculation of uptake rates, that the tracer added was the only source available to planktons. In the surface waters of the open ocean, which is well oxygenated, the ambient concentrations of ammonium (~0.2 μM; Binachi et al., 1997) and urea are low but still a high concentration of ^{15}N enriched tracer of ammonium and urea were added. This was done in order to artificially simulate the ammonium and urea uptake rates.

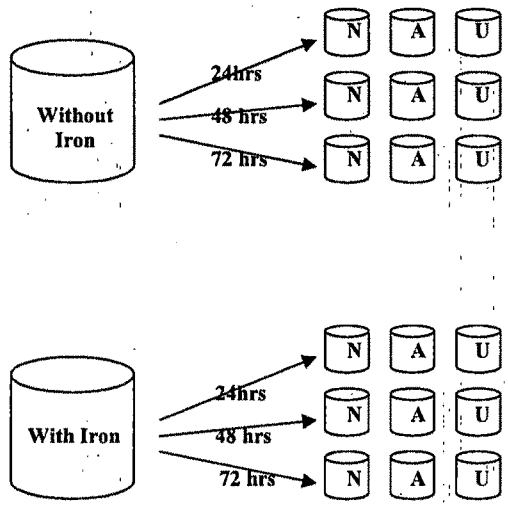


Fig 2.10 Schematic representation of the sampling adopted in the present study for iron enrichment experiment. (N = Nitrate tracer; A = Ammonium tracer; U = Urea tracer. All tracers were 99% enriched in ^{15}N)

The above methodology allowed us to monitor the effect of iron enrichment on nitrate, ammonium and urea uptake rates as well as on the *f*-ratio. This also helped to determine the preferred nutrient taken up during the iron enrichment experiment. It is very well established, now, through a number of iron enrichment experiments that there is some time-lag between the addition of iron and the consequent increase in production. To establish the role of the time of incubation on the iron and nitrogen

uptake kinetics, the three sets were incubated for three different time periods; subsets 1, 2 and 3 were incubated for 24, 48 hours and 72 hours respectively.

2.6.2 Iron tracer preparation

Iron exists mainly in two oxidation states: Fe (II) and Fe (III) but only Fe (II) is soluble in seawater and thus is bio-available. Even though Fe (II) is soluble in water, it precipitates at the present day sea water pH, *i.e.*, ~8. As a result of which its concentration in surface water decreases and becomes insufficient to meet the demand of phytoplankton to sustain biological production. To dissolve Fe in adequate concentration the pH of sea water must be lower. For tracer preparation 2L of surface water sample (pH 7.76) was taken from a station in the Southern Ocean and its pH was reduced to 1.91. This was followed by addition of 0.5 g of Iron (II) sulphate 7-hydrate ($FeSO_4.7H_2O$, molecular weight 278.02, procured from VWR International Limited, UK). The concentration of the resulting solution was 0.93 mM. 1ml of this solution (i.e., 930 nmol of Fe) was added to 1 L of the sea water sample for the iron enrichment experiment. This has already been shown that addition of iron increases productivity. Thus our aim of the present study was not to test whether addition of Fe increases the productivity but to check what happens to the new and regenerated productions because of the Fe addition. According to Michelis-Menten kinetics, the effect of iron will saturate for large concentrations of Fe, which we have added. In addition, some experiments have shown that the effect of Fe addition is not immediate; rather it takes a few days. To see the effect of iron enrichment within a day or two on potential uptakes of nitrate, ammonium and urea, we increased the Fe concentration.

2.6.3 Chlorophyll measurement

In situ chlorophyll measurements were made by scientists from NCAOR (National Centre for Antarctic and Ocean Research), Goa, using a submersible fluorescence probe (*FluoroProbe, bbe-Moldaenke,* Kiel, Germany) for the determination of chlorophyll-a. This probe contains five light emitting diodes (450, 525, 570, 590, and 610 nm) for the excitation of pigments present in the phytoplankton. Chlorophyll fluorescence was measured at 685 nm. The excitation

spectrum obtained was compared to normal curves stored in the probe and the amount of chlorophyll was then calculated.

2.7 Quality Control

Special care was taken during experiments to avoid any contamination. For collection of samples Teflon coated Go-Flo bottles were used to avoid any trace metal contamination. These bottles were rinsed thoroughly before using them for sample collection. Samples were directly transferred from Go-Flo bottles to polycarbonate Nalgene bottles. No other plastic cans or pipes were used as a precautionary measure to avoid contamination. Always new and separate pipette tips were used for different tracers. Running seawater was used during incubation to maintain the temperature. Samples were immediately transferred to the ship-board laboratory after incubation and kept covered under dark cloths till the filtration was over. Filtration cups were thoroughly washed with milliQ water before the filtration and were rinsed properly once the filtration of each sample was over. This was done to restrict cross contamination. Filter papers were handled using well cleaned and separate forceps for each tracer. After filtration each bottles were washed thoroughly using 10% HCl, followed by fresh water for the next experiment. For the mass spectrometric analysis samples were packed in clean silver foils. Blanks with only silver foils were run in the mass spectrometer during every batch.