

# Effect of high level iron enrichment on potential nitrogen uptake by marine plankton in the Southern Ocean

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**Iron fertilization of the Southern Ocean is believed to counter the increasing CO<sub>2</sub> concentration in the atmosphere and the consequent global warming. Though a number of large scale iron enrichment experiments have been done in the recent past in different parts of the world ocean, little effort has been made to understand the effect of iron enrichment on nitrogen uptake rates and *f*-ratios. Here we assess the effect of iron addition on N-uptake rates and *f*-ratio in the Indian sector of the Southern Ocean. This study shows, in contrast to the earlier belief, that iron addition enhances not only nitrate uptake (~3×) but it causes a significant increase in ammonium (~2×) and urea (~3×) uptakes as well. Also, since iron enrichment caused significant increase in the uptake of all N-substrates, its effect on *f*-ratio was insignificant.**

**Keywords:** Iron enrichment, marine plankton, nitrogen uptake, Southern Ocean.

THE oceans play an important role in controlling the atmospheric CO<sub>2</sub> concentration on glacial–interglacial time scales<sup>1</sup>, thereby affecting global climate. This is mainly achieved by single-celled microscopic plants (phytoplankton) which can convert inorganic CO<sub>2</sub> into organic carbon through photosynthesis in the sunlit upper layers of the ocean. The organic matter synthesized is transported to the deep; this is known as the ‘biological pump’. This strips the nutrients, such as nitrate, silicate and phosphate out of the surface layers of the ocean<sup>2,3</sup>. Some of the areas of the world’s ocean such as the Southern Ocean, the equatorial and the north Pacific Ocean contain excess amounts of these unused macro-nutrients in their surface waters. Despite this, the productivity in these areas is low (column integrated primary productivity varies between 130 and 220 mgC m<sup>-2</sup> d<sup>-1</sup>) (ref. 4). Because of this unique property these areas are described as High Nutrient Low Chlorophyll or HNLC regions<sup>5</sup>.

Persistence of large concentrations of nutrients in the surface ocean suggests the retardation of productivity due to some reason. The insufficient availability of micronutrients such as iron (Fe) (<10<sup>-3</sup> nM in the open ocean waters) appears to be the main cause for the observed low productivity<sup>6</sup>. The role of iron in limiting productivity in the open ocean and, consequently the climate is called the ‘iron hypothesis’.

Martin and Fitzwater<sup>7</sup> were the first to measure the concentration of iron in the waters of the Gerlache strait (Antarctic coast) and the Drake Passage (offshore). They concluded that the offshore locations were less productive (~100 mgC m<sup>-2</sup> d<sup>-1</sup>) due to lack of iron (<0.16 nM). They also proposed that the coastal stations received iron (~7.4 nM) from the continental margins and so the productivity was high (3 gC m<sup>-2</sup> d<sup>-1</sup>) and therefore the supplied iron was consumed and could not be transported to the open ocean<sup>8</sup>. Iron can limit productivity because (i) it is required for the synthesis of chlorophyll and it helps in plant metabolism<sup>9</sup> and (ii) lack of iron may also cause decline in the photosynthetic electron transfer<sup>9,10</sup> which in turn may lead to low photosynthetic efficiency (i.e. carbon fixation per unit chlorophyll). The idea of iron limitation got momentum when it was shown, through bottle scale experiments at station PAPA (50°N, 145°W) in the sub-arctic north Pacific, that there is a rapid increase in the chlorophyll concentration and nitrate was totally consumed after four days since the iron enrichment<sup>7</sup>. This was followed by a number of large-scale Fe-enrichment experiments carried out to test the hypothesis of Fe-limitation in different HNLC regions<sup>10</sup>.

The Southern Ocean, also an HNLC area, is now delimited as the world’s fifth ocean. It comprises the southern portions of the Pacific, Atlantic and the Indian Oceans of which the Indian sector constitutes ~39% (13.1 × 10<sup>6</sup> sq. km) by area<sup>11</sup>. In the Southern Ocean four large scale iron enrichment experiments have been done, two each in the Pacific sector (Southern Ocean Iron Enrichment Experiment (SOIREE) and Southern Ocean Iron Experiment (SOFeX)) and the Atlantic (‘Iron’ Experi-

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ment (EisenEx) and the European Iron Fertilization Experiment (EiFex)); the Indian sector of the Southern Ocean still remains unexplored. For details reference is made to Singh *et al.*<sup>12</sup>.

All these experiments have proved that the Southern Ocean is iron limited and addition of iron enhances productivity. However, the effect of iron on uptake of different N-substrates is still poorly known. A few studies<sup>13,14</sup> determined the effect of iron enrichment on the uptake of different substrates such as nitrate and ammonium. The present work is the first study in the Indian sector of the Southern Ocean to measure urea uptake as well. We carried out bottle scale <sup>15</sup>N tracer-iron enrichment experiments in the Indian sector of the Southern Ocean at two different stations to get a preliminary estimate of the role of iron on productivity and on the individual nutrient uptake rates and *f*-ratios. The locations of the stations are shown in Table 1.

Iron, in the ocean water, 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 (oxic) sea water pH, i.e. ~8. Consequently its concentration in surface water decreases and becomes insufficient to meet the demand of phytoplankton to sustain production. To dissolve Fe in adequate concentration the pH of the sea water must be low (pH ~2). For tracer preparation, 2 l of surface water sample (pH 7.76) was taken from the Southern Ocean waters (56°S 50°E) and its pH was reduced to 1.91. This was followed by addition of 0.515 g of iron (II) sulphate 7-hydrate (FeSO<sub>4</sub>·7H<sub>2</sub>O, molecular weight 278.02, procured from VWR International Limited, UK). The concentration of the resulting solution was 0.93 mM. 1 ml of this solution (i.e. 930 nmol of Fe) was added to 1 l of the sea water sample for the iron enrichment experiment (the natural concentration of Fe was ~7.5 µg/l). As it has already been shown earlier that addition of iron increases productivity, our aim was not to test the validity of 'iron hypothesis' but to estimate the effect of iron enrichment on the uptake rates of different N-substrates. As most of the experiments done earlier have shown that the effect of Fe addition is not immediate, rather it takes a few days, we added a large concentration of iron to detect any immediate effect in our samples. Also, according to the Michaelis–Menten kinetics, addition of large concentration of iron is unlikely to simulate higher uptake rates than saturation values.

**Table 1.** Sampling locations in the Southern Indian Ocean along with the date of sampling

Station	Geographical location		Date of sampling
IEE1	43°S	48°E	13 March 2006
IEE2	35°S	48°E	17 March 2006

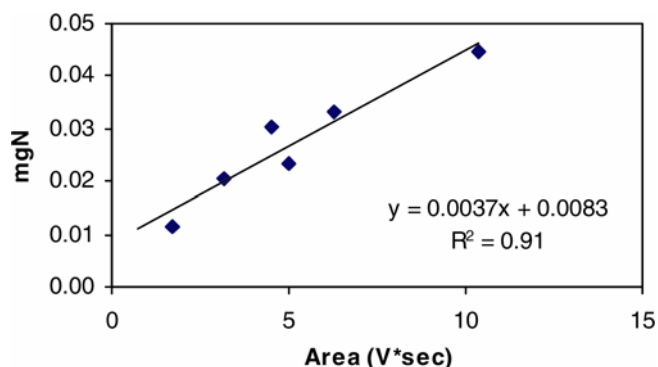
IEE, iron enrichment experiment.

Sampling was done in late austral summer (March 2006), onboard *Akademik Boris Petrov* (ABP-15). Water samples were collected from the depths of deep chlorophyll maxima (60 m at IEE1 and 80 m at IEE2), using pre-cleaned Go-Flo bottles attached to a CTD rosette. Samples were collected in eighteen 1 l Nalgene bottles and were transferred directly from Go-Flo bottles to Nalgene bottles to avoid any trace metal contamination. These bottles were divided into two sets (set 1 and set 2) of nine bottles each. Further, each set of nine bottles was 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 set'). This was followed by the addition of respective nutrient tracers. Nutrient tracers were prepared using <sup>15</sup>N enriched (99 atom% <sup>15</sup>N) nitrate (Na<sup>15</sup>NO<sub>3</sub>), ammonium (<sup>15</sup>NH<sub>4</sub>Cl) and urea (CO(<sup>15</sup>NH<sub>2</sub>)<sub>2</sub>). 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 <sup>15</sup>N labelled tracers were added help calculate the total uptake for the whole incubation period (the control experiment), whereas the samples with added iron would help monitor the change in production, if any. 1 µM of <sup>15</sup>N enriched (99%) nitrate, ammonium and urea tracers were added to the respective water samples. The nitrate tracer added corresponded to ~6% and ~12% of the ambient concentration at station IEE1 and IEE2 respectively; ammonium and urea were added following Reay *et al.*<sup>15</sup>. 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 plankton. Addition of high concentration of ammonium, here, may lead to the overestimation of ammonium uptake and hence to underestimation of the *f*-ratio<sup>16,17</sup>. 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 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 uptakes. To establish the role of the time of incubation on nitrogen uptakes due to iron enrichment, the three sets were incubated for three different time periods; subsets 1, 2 and 3 were incubated for 24, 48 and 72 h respectively. Flowing seawater from a depth of 4 m was continuously maintained at the local SST during the whole incubation period.

All samples were filtered subsequently through pre-combusted (4 h at 400°C) 47 mm diameter and 0.7 µm pore size Whatman GF/F filters, then dried in oven at 60°C overnight and finally brought to the shore for mass spectrometric analysis. Samples were analysed using a CarloErba elemental analyser interfaced via conflo III to a Finnigan Delta Plus mass spectrometer, using a

**Table 2.** Values and precision of the standards analysed during the sample analysis

Standards used	Quoted isotopic ratio	Values obtained
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (IAEA-N-2)	0.3753	0.3751 ± 0.0009 (n = 22)
KNO <sub>3</sub> (USGS 32)	0.4340	0.4329 ± 0.0012 (n = 16)
KNO <sub>3</sub> (IAEA-NO-3)	0.3695	0.3689 ± 0.0004 (n = 11)

**Figure 1.** Calibration equation used for estimation of particulate organic nitrogen in the sample.

technique for sub-microgram level <sup>15</sup>N determination. The aim of the mass-spectrometric analysis was to measure the particulate organic nitrogen (PON) content and atom% <sup>15</sup>N simultaneously in the sample<sup>18</sup>. For this, the mass spectrometer was first calibrated by combusting known amounts of organic and inorganic standards of known nitrogen content. Only when the stability of the mass spec was ascertained, sample analysis was started. During the analysis of samples some standards were also analysed to check the possible stability change, if any. The overall precision on the basis of the standards measured in between the analysis of the samples is shown in Table 2. 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. The equation derived gives a relationship between the nitrogen content (mgN) and the area under peak (of masses 28, 29 and 30 in units of Vs). A typical calibration plot is shown in Figure 1. Using such a regression equation the nitrogen content (PON) in the sample was calculated. The uptake rates for nitrate, ammonium and urea were calculated using the equation of Dugdale and Wilkerson<sup>19</sup>. The PON and atom% <sup>15</sup>N were measured within an overall precision of less than 5% and <3% in PON and <sup>15</sup>N atom% respectively, based on duplicate analyses and hence the maximum uncertainty in the uptake rate is <10%. The *f*-ratio was calculated as a ratio of nitrate uptake to total N-uptake<sup>20</sup>.

*In situ* chl *a* measurements were carried out using a submersible fluorescence probe (FluoroProbe, bbe-Moldaenke, Kiel, Germany). 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 estimated.

Station IEE1 lay in typical Southern Ocean waters, i.e. south of sub-tropical front (STF), and IEE2 lie north of STF. The 40°–41°S latitude marks the presence of STF in the Indian Ocean which defines the northern boundary of the Antarctic Circumpolar Current (ACC) and it separates the subtropical warm waters from sub-Antarctic cold waters<sup>21</sup>. This is marked by the sudden change of surface temperature (up to 9°C) and salinity<sup>22</sup>. Upwelling of the Antarctic Intermediate Water (AAIW) takes place at STF which triggers high carbon uptake. The upwelled water is carried northward as surface advection<sup>3</sup>. During the present study, STF was located at 41°S and was marked by large temperature gradient<sup>23</sup>. The same is reflected in SST at IEE1 and IEE2 as well; SST at IEE1 and IEE2 were 11.3°C and 20.3°C respectively. Euphotic zone depths were 100 m and 115 m and temperature-based mixed layer depths were 45 m and 39 m at IEE1 and IEE2, respectively. The nitrate concentrations were 18.1 and 8.6 μM, and silicate concentrations were 1.66 μM and 0.3 μM respectively at both the stations. The concentration of nitrate and silicate both decreased significantly at IEE2 compared to IEE1. Both these stations lay in a low silicate zone<sup>24</sup> where silica concentration in the surface waters is not enough to support a large population of diatoms. This was reflected in surface chlorophyll and species composition as well: at IEE1 surface chlorophyll was ~1 μg l<sup>-1</sup> where green algae were the dominant species contributing more than 65% of the total chl *a*. Euphotic zone integrated chl *a* was significantly high ~134 mg m<sup>-2</sup>. At IEE2 surface chlorophyll was significantly less (0.1 μg l<sup>-1</sup>) and euphotic zone integrated chlorophyll was ~49 mg m<sup>-2</sup>, significantly less compared to IEE1. Diatoms were absent at this station and only green algae contributed to the total chlorophyll.

The results of nitrate, ammonium and urea uptake and the *f*-ratios, from both the stations, under controlled and enriched iron conditions are shown in Tables 3 and 4, respectively. At IEE1 the total N-uptake did not show any significant change (within the uncertainty limit) in the first 24 h. Since the replicate measurements were not possible, from the indicative results it can be suggested that even sudden supply of a large amount of dissolved iron was not able to simulate nitrogen uptake immediately but phytoplankton responded to the same as the time pro-

## INDIAN CONTRIBUTION IN SOUTHERN OCEAN

**Table 3.** Nitrate, ammonium and urea uptake and the  $f$ -ratios at station IEE1 under controlled and enriched iron conditions

Duration of incubation (h)	Nitrate uptake (nM N)		Ammonium uptake (nM N)		Urea uptake (nM N)		Total N-uptake (nM N)		$f$ -ratio	
	No iron	With iron	No iron	With iron	No iron	With iron	No iron	With iron	No iron	With iron
24	29	19	11	6	17	17	58	42	0.50	0.44
48	24	73	12	21	14	41	50	134	0.47	0.54
72	12	36	10	24	11	47	33	107	0.38	0.34

**Table 4.** Nitrate, ammonium and urea uptake and the  $f$ -ratios at station IEE2 under controlled and enriched iron conditions

Duration of incubation (h)	Nitrate uptake (nM N)		Ammonium uptake (nM N)		Urea uptake (nM N)		Total N-uptake (nM N)		$f$ -ratio	
	No iron	With iron	No iron	With iron	No iron	With iron	No iron	With iron	No iron	With iron
24	28	30	39	74	36	31	103	136	0.27	0.22
48	31	31	48	53	72	52	151	135	0.20	0.23
72	37	23	70	5	71	63	178	91	0.21	0.26

gressed. This is why N-uptake increased almost thrice under Fe enrichment compared to the control case at the end of 48 h; nitrate and urea uptake increased more than three-fold and ammonium uptake increased two-fold. An increase in the absolute nitrate uptake by a factor of 15 in another silicon-less zone in the Pacific sector of the Southern Ocean has been reported earlier<sup>24</sup>. In HLNC area of the equatorial Pacific Ocean iron enrichment caused 14-fold increase in the nitrate uptake on 6th day<sup>13</sup>; it increased from  $<10 \text{ nM h}^{-1}$  to  $133 \text{ nM h}^{-1}$ ; the effect of iron enrichment on ammonium and urea uptake was not studied earlier. An increase in specific nitrate uptake rate, under iron enriched condition, has also been reported from the Southern Ocean sector of the Atlantic Ocean<sup>14</sup>. The same effect of iron enrichment could not be seen at IEE2 on the total N-uptake; within the limit of uncertainty, it remained almost the same in 'control' and 'enriched' conditions. This may be because the water here is not Fe-starved. Station IEE2 lay north of STF, in a zone where upwelled AAIW gets advected. This water may be transporting macro-nutrients and iron. As planktons of this station are not Fe-starved, they are not responding to iron enrichment.

The  $f$ -ratio did not show any significant change due to iron enrichment at IEE1 during the 1st and the 2nd day but it reduced slightly on the 3rd day in both the 'control' as well as 'enriched' sets. The  $f$ -ratio has reported to increase from 0.1–0.2 to 0.3–0.4 on Fe-enrichment in silica-depleted zone of the Southern Pacific<sup>24</sup>. This result was based on nitrate uptake and carbon uptake measurements; ammonium and urea uptake rates were not measured. During the present study Fe-enrichment not only enhanced nitrate uptake but increased ammonium and urea uptake as well and this is the reason their net effect on  $f$ -ratio was not significant at IEE1. The  $f$ -ratio showed considerable variation; it varied from 0.68 to 0.85 in

spring and from 0.17 to 0.63 in summer in Indian sector Southern Ocean waters near Kerguelen Island<sup>25</sup>. A mean  $f$ -ratio of 0.42 in coastal zone and 0.68 in the open ocean zone has also been reported for the Prydz Bay area<sup>26</sup>. The  $f$ -ratio from the western Pacific sector of the Southern Ocean along 170°W has been reported to vary from 0.04 to 0.5; high  $f$ -ratios were measured at the ice edge during spring and lower  $f$ -ratios were measured during summer<sup>27</sup>. At IEE2 also no significant change was observed in the  $f$ -ratio because of iron enrichment; it remained almost the same in both 'control' and 'enriched' conditions.

In summary, preliminary results of <sup>15</sup>N tracer-Fe enrichment experiment from IEE1 suggest that addition of iron does not enhance primary productivity during the initial stage of the enrichment but takes some time to increase the uptake. The reason for this remains unclear. The availability of iron increases uptake of all substrates of nitrogen, i.e. nitrate, ammonium and urea. This was clearly reflected in enhancement in uptake rates of all substrates at station IEE1 under 'enriched condition'. This is in contrast to the earlier belief that availability of iron enhances uptake of nitrates only and not of ammonium and urea. As it enhanced uptake of all forms of nitrogen at IEE1, the  $f$ -ratio remained almost the same under both 'control' and 'enriched' conditions.

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