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**Natural isotopic composition of nitrogen**

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# Natural isotopic composition of nitrogen in suspended particulate matter in the Bay of Bengal

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## Abstract

We present the first measurement of nitrogen isotopic composition ( $\delta^{15}\text{N}$ ) in suspended particulate matter (SPM) of the surface Bay of Bengal (BOB) at 24 different locations during pre- (April–May 2003) and post- (September–October 2002) monsoon seasons. The  $\delta^{15}\text{N}$  of particulate organic nitrogen (PON) in surface suspended matter of coastal as well as northern open BOB shows signatures of a two end-member mixing between continental inputs and marine sources. Dilution by the organic and detrital continental material brought in by rivers leads to consistently lower  $\delta^{15}\text{N}$ , evident from the relationship between surface salinity and  $\delta^{15}\text{N}$ .  $\delta^{15}\text{N}$  of surface PON of open ocean locations during both seasons, and also at coastal locations during pre-monsoon suggest the nitrate from deeper waters as a predominant source of nutrient for planktons. The depth profiles of  $\delta^{15}\text{N}$  of SPM during pre-monsoon season at nine different locations are also presented. These indicate an increase in  $\delta^{15}\text{N}$  by a maximum of 2.8‰ between euphotic depth and 300 m, which is lower than that observed in the eastern Indian Ocean, indicating the role of higher sinking rates of particles ballasted by aggregates of organic and mineral matter in BOB.

## 1. Introduction

Particulate organic matter (POM) is known to play an important role in marine nitrogen and carbon cycles (Saino and Hattori, 1980). The study of the nitrogen isotope ratio  $^{15}\text{N}/^{14}\text{N}$  ( $\delta^{15}\text{N}$ , expressed as deviation in per mil from that of atmospheric  $\text{N}_2$ ) of POM provides an insight into the availability and utilization of nutrients and the transformation processes it undergoes during its transportation to greater depths. Several such studies have been done in different parts of the world ocean (Wada and Hattori, 1976; Saino and Hattori, 1980; Altabet, 1996). Similar studies in the ocean sediments have been used for the reconstruction of past changes in surface ocean nutrient utilization (e.g. Altabet and Francois, 1994; Farrell et al., 1995).

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The  $\delta^{15}\text{N}$  of the marine particulate organic nitrogen (PON) has a strong dependence on the  $\delta^{15}\text{N}$  of source material (atmospheric  $\text{N}_2$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ).  $\delta^{15}\text{N}$  of marine PON shows a wide variation (Saino and Hattori, 1980; Altabet, 1988; Rau et al., 1998), due to the changes in  $\delta^{15}\text{N}$  of source, caused by fractionation of isotopes during various biogeochemical/biological processes involved during its formation. These processes are  $\text{N}_2$  fixation, denitrification, nitrification, and nitrate assimilation. Denitrification leads to  $^{15}\text{N}$  enrichment of the remaining nitrate while nitrification causes enrichment of  $^{15}\text{N}$  in the ammonium pool (Mariotti et al., 1984). Enrichment of up to 20‰ in the remaining pool has been found due to denitrification (Miyake and Wada, 1971; Cline and Kaplan, 1975), nitrification (Miyake and Wada, 1971) and nitrate assimilation (Wada et al., 1971). During the uptake of dissolved nitrogen in eutrophic waters, phytoplankton prefers  $^{14}\text{N}$  over  $^{15}\text{N}$  (Wada and Hattori, 1978). Fixation of atmospheric nitrogen is known to lower the  $\delta^{15}\text{N}$  of PON (−2 to 0‰, Minagawa and Wada, 1986) because of the depleted source (atmospheric  $\text{N}_2$ , 0‰). Therefore, the areas with lower  $\delta^{15}\text{N}$  may involve  $\text{N}_2$  fixation. Furthermore, the  $\delta^{15}\text{N}$  of PON in marine organic matter also depends on phytoplankton species (composition), physiology and the rate and phase of growth of planktons (Montoya and McCarthy, 1995).

This study reports the first detailed observations of  $\delta^{15}\text{N}$  of suspended PON in the Bay of Bengal (BOB), the eastern counterpart of Arabian Sea in the northern Indian Ocean, where data on  $\delta^{15}\text{N}$  of suspended matter is scarce. We examine the observed variation in  $^{15}\text{N}$  and PON content in two different seasons i.e. between post- (September–October 2002) and pre- (April–May 2003) monsoon periods. The vertical profiles of  $\delta^{15}\text{N}$  in suspended matter at different locations, and the depth related changes during the pre-monsoon season have also been discussed.

## 2. Material and methods

Sampling was performed along the cruise track shown in Fig. 1, onboard ORV Sagar Kanya (SK-182 and SK-191), as a part of Bay of Bengal process study (BOBPS). Surface sea water samples were collected using a clean plastic bucket and thirty litre Go Flo bottles attached to a CTD rosette were used to collect sea water samples from various depths (up to 500 m). Immediately after collection, four to six litres of sea water were filtered through a precombusted (400°C for 4 h) Whatman GF/F glass fibre filter (47 mm diameter, 0.7 µm pore size). After the filtration the samples were dried at 60°C and stored at room temperature for isotopic analysis in the shore laboratory.

Measurements of nitrogen isotope ratio and PON were carried out using a Carlo Erba elemental analyser interfaced via Confloll to a Finnigan Delta Plus mass spectrometer. Due to the small amount of nitrogen gas recovered from the samples (typically <1 µMN), for precise analysis, the method of Owens and Rees (1989) with a modification in oxygen injection time was used. Integration of ion beam areas (m/z 28+29+30) after the calibration against standard material (IAEA-NO-3, KNO<sub>3</sub>) provided the measurement of PON. The advantage of this technique lies in the simultaneous measurement of isotope ratio and PON in the same sample. PON was measured to a precision of less than 10%, while that for δ<sup>15</sup>N was 0.3‰. δ<sup>15</sup>N measurement of the standard (IAEA-NO-3, KNO<sub>3</sub>, δ<sup>15</sup>N=4.7‰) yielded a value of 4.9±0.3‰ (n=19).

## 3. Study area: hydrography and nutrients

BOB is a semi-enclosed tropical basin in the northern Indian Ocean. It experiences seasonal changes in circulation and climatic conditions due to monsoons. BOB receives excess precipitation and large quantities of freshwater influx (1.6\*10<sup>12</sup> m<sup>3</sup> yr<sup>-1</sup> compared to 0.3\*10<sup>12</sup> m<sup>3</sup> yr<sup>-1</sup> of Arabian Sea; Subramanian, 1993) from the major rivers draining the Indian subcontinent. This riverine freshwater input results in considerable salinity variations during and post-monsoon over the whole basin, thereby

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inducing stratification of the upper 100 m of the water column (Prasanna Kumar et al., 2002). The surface salinity of the open ocean stations during post-monsoon decreased from south to north (34 psu at 7° N to 32 psu at 16° N) and dropped by 3 psu at 17° N. The coastal stations showed similar distribution pattern of salinity but the drop was more pronounced from 16° N to 17° N (34 psu to 21 psu). During pre-monsoon the overall variation in salinity was between 32 to 34 psu. Sea surface temperature (SST) during post-monsoon along open BOB varied marginally from 28.2 to 29°C from south to north, while along coastal transect it did not show any trend and the average was around 30°C. During pre-monsoon SST varied from 29 to 31.4°C in the open ocean and showed a decrease of ~2°C from 14° N to 15° N. For the coastal locations, it varied from 29.1°C to 30.4°C. The riverine inputs are a major potential source of nutrients such as nitrate, phosphate and silica to the Bay. Also, one of the major suppliers of the nutrients to the surface Bay is mixing due to cyclones, frequent in the BOB during post monsoon season. The formation of localized intense blooms and also the intensification of bloom generated by anticyclonic gyre are known due to injection of nutrients by cyclonic activity (Vinaychandran and Mathew, 2003). During the pre-monsoon period there is a poleward East India Coastal Current (EICC) active at north of 10° N which brings cooler, more saline water with nutrients to the surface (Shetye et al., 1993). Upwelling has also been observed along the western boundary of basin in a 40 km wide band due to local longshore wind stress (Shetye et al., 1991). The measured nitrate concentrations in the surface Bay is presented in Fig. 2; in general, the surface nitrate during post monsoon was very low (mostly below detection limit, <0.1 μM) and for pre-monsoon it was mostly around 0.2 μM. However, it increased sharply between 40 and 60 m (maximum ~15 μM and average 7 μM during pre-monsoon).

#### 4. Results

Stations studied are divided into two transects: one along the 88° E longitude (Stn. 1–Stn. 13), defined here as open ocean stations (transect) and the other parallel to the

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Indian coast (Stn. 14 to Stn. 24) as the coastal stations (transect).

#### 4.1. Surface suspended matter

Overall, it has been observed that the average surface PON concentration during post-monsoon season ( $1.4 \mu\text{MN}$ ) is nearly twice that of pre-monsoon ( $0.7 \mu\text{MN}$ ). During the post-monsoon the difference in average coastal ( $1.3 \mu\text{MN}$ ) and open ocean ( $1.4 \mu\text{MN}$ ) surface PON is insignificant. The same is true for the pre-monsoon season, where it averages  $0.7 \mu\text{MN}$  for coastal and  $0.6 \mu\text{MN}$  for open ocean locations. During the whole period of study, the maximum surface PON of  $2.5 \mu\text{MN}$  has been observed at Stn. 15, which is the nearest to the coast with the shallowest water column depth of 620 m. The  $\delta^{15}\text{N}$  of surface PON for both pre- and post-monsoon seasons ranges from 2 to 7.6‰ and falls in the general range of known oceanic PON  $\delta^{15}\text{N}$ . The overall  $\delta^{15}\text{N}$  of surface PON averages around 4.1‰ for post-monsoon and 3.8‰ for pre-monsoon season, which agree within analytical error. There is a significant difference of 1.5 between average  $\delta^{15}\text{N}$  of open ocean (4.8‰) and coastal (3.3‰) stations during post-monsoon. However, no such difference has been observed for the samples collected during pre-monsoon where it averages around 4‰ for both open and coastal stations. There is no significant latitudinal variation in  $\delta^{15}\text{N}$  during both the seasons. There exists a positive linear correlation between PON and  $\delta^{15}\text{N}$  (Fig. 3). This relationship is more significant during post-monsoon ( $R^2=0.42$ ,  $n=24$ ) than pre-monsoon ( $R^2=0.21$ ,  $n=22$ ). For post-monsoon season the coastal stations show less variability and data points lie in the lower regime i.e. low PON- low  $\delta^{15}\text{N}$  zone. On the other hand, the open ocean stations show a bimodal distribution, one with low PON-low  $\delta^{15}\text{N}$  which averages around 3.0‰ and other with high PON-high  $\delta^{15}\text{N}$  with an average of 5.8‰. During pre-monsoon no clear cut distinction exists between  $\delta^{15}\text{N}$  of PON in coastal and open ocean transects.

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## 4.2. Depth profile of suspended matter

Figure 4 presents the vertical profile of  $\delta^{15}\text{N}$  and PON for pre-monsoon season at different locations upto 300 m or more except Stn. 3, where it is only up to 100 m. Average  $\delta^{15}\text{N}$  of PON in the euphotic zone ( $\sim 60$  m) varies between 1.9 to 4.9‰ for different stations with an average of 4.2‰. Below the euphotic zone,  $\delta^{15}\text{N}$  increases with depth and reaches an average value of 5.9‰ at 300 m. For the open ocean stations the average euphotic zone  $\delta^{15}\text{N}$  shows a decreasing trend from south to north with a maximum of 4.5‰ for southernmost station (Stn. 23) and minimum of 2.9‰ for northern station (Stn. 12).  $\delta^{15}\text{N}$  also shows subsurface minimum between 10 to 60 m varying with location. PON, in general, decreases with depth showing subsurface maxima within the euphotic zone. Euphotic zone average of PON is  $0.7 \mu\text{MN}$  which decreases to  $0.3 \mu\text{MN}$  at 300 m.

## 5. Discussion

In oceanic environments, PON is mainly derived from phytoplankton, microzooplankton, bacteria and detritus. The nitrogen isotopic signature of PON in suspended matter depends on the isotopic fractionation associated with its formation, and in turn upon the isotopic composition of inorganic form of dissolved nitrogenous sources (such as  $\text{NO}_3^-$ , 3–7‰;  $\text{NH}_4^+$ , 6–8‰; and atmospheric  $\text{N}_2$ , 0‰; Miyake and Wada, 1967) available for the utilization by phytoplankton. The variation in  $\delta^{15}\text{N}$  of PON reveals the utilization of different nitrogen sources by planktons as these sources have distinct isotopic compositions. Our  $\delta^{15}\text{N}$  data precludes the possibility of  $\text{N}_2$  fixation in BOB. Cyanobacteria “Trichodesmium”, a well known  $\text{N}_2$  fixer exhibits low  $\delta^{15}\text{N}$  values around  $-2$  to  $0$ ‰ (Minagawa and Wada, 1986). All our  $\delta^{15}\text{N}$  data are above the required value for an area dominated by  $\text{N}_2$  fixers.

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## 5.1. Surface suspended matter

For the purpose of discussion, stations in BOB may be classified into two based on the surface salinity of the stations. The first includes the stations with salinity less than 32 psu (the six coastal stations and three open ocean stations during post monsoon; Fig. 5) and second includes the stations with surface salinity more than 32 psu (all the rest). The former are influenced by the riverine discharge whereas the latter are not.

The salinity and  $\delta^{15}\text{N}$  of suspended PON (Fig. 5) for the two seasons indicate that when salinity is low (<32 psu), as in the case of six coastal locations and three open ocean stations, due to riverine discharge during post-monsoon, the  $\delta^{15}\text{N}$  is consistently on lower side (2–3‰) (except one location each in coastal and open ocean which have  $\delta^{15}\text{N}$  values of 4.9 and 4.6‰, respectively). The rivers draining the BOB bring lot of terrestrial organic as well as detrital material (Unger et al., 2003). The consistent low  $\delta^{15}\text{N}$  suggests that isotopic signature of PON at these locations have been influenced by terrestrial inputs. Terrestrial particulate matter, brought by major rivers, might have diluted the overall  $\delta^{15}\text{N}$  signal of PON, although there exists no literature regarding the  $\delta^{15}\text{N}$  of such particulate matter. But, the naturally occurring land derived materials are known to have low  $\delta^{15}\text{N}$  (mean of 2.5‰ for terrestrial organic matter, Sweeney et al., 1978; and 1.5‰ for terrestrial detrital component, Mariotti et al., 1984).

The stations which are not influenced by riverine discharge show a wide isotopic variability (2–7.6‰). However, high average  $\delta^{15}\text{N}$  of surface suspended matter (5.3‰ for open ocean stations during post-monsoon and 4‰ for both open and coastal stations during pre-monsoon) have been observed for these stations. Since these locations are unaffected by the terrestrial influence the variability observed may be attributed to the two possible reasons: first, uptake of regenerated ammonium (Wada and Hattori, 1976); and second, supply of nitrate from deeper waters due to the presence of shallow nitracline, which is between 50–100 m (Prasanna Kumar et al., 2002). In the former case, regenerated ammonium produced by excretion of zooplankton and heterotrophs in the surface layer has been considered as a source. In most oceanic regions, ammo-

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5 nium is the preferred substrate and normally does not accumulate in the surface layer (Mino et al., 2002). Soon after regeneration of ammonium, it is rapidly taken up by the algae; there is little time for isotopic fractionation and the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  is imprinted in PON without much modification. Unfortunately,  $\delta^{15}\text{N}$  of ammonium in the BOB has not been measured to directly assess the role of ammonium on  $\delta^{15}\text{N}$  of PON. Values in the range of 6–8‰ have been reported for ammonium in other oceans (Miyake and Wada, 1967). However, indirect estimation of degree of contribution of ammonium in  $\delta^{15}\text{N}$  of PON may be obtained from new production measurement (Dugdale and Goring, 1967) in the region. If the new production is less, there could be a prominent effect of the regenerated ammonium on the  $\delta^{15}\text{N}$  of PON. But in BOB, in general, high new production has been observed by us during both post- (4 mmol N m<sup>-2</sup> d<sup>-1</sup>) and pre- (6.6 mmol N m<sup>-2</sup> d<sup>-1</sup>) monsoon during the two cruises. Therefore, regenerated ammonium is likely to have played a limited role in observed  $\delta^{15}\text{N}$  of PON. However, significant ammonium contributions cannot be ruled out for three locations in the open ocean during post-monsoon and at one location during pre-monsoon, where values higher than 6‰ have been observed.

20 The nitrate from deeper water is a known source of nutrients in the Indian Ocean for planktons (Vinaychandran and Mathew, 2003); however, its possible imprint on  $\delta^{15}\text{N}$  of PON and related fractionation mechanism could only be estimated if the nitrate  $\delta^{15}\text{N}$  is known. But, as in the case of ammonium, nitrate  $\delta^{15}\text{N}$  has also not been measured in the Bay.  $\delta^{15}\text{N}$  values of 3–7‰ have been reported for nitrate in deeper waters lacking significant column denitrification as in BOB (Miyake and Wada, 1967; Cline and Kaplan, 1975). The average value reported here for  $\delta^{15}\text{N}$  of PON in open ocean during both post- and pre-monsoon seasons (5.3 and 4‰) are found to be of similar magnitude. However, the observed variability can be explained in two different ways: first, the rapid uptake of the nitrate without fractionation (with changing source isotopic composition) and second, the fractionation of nitrate during uptake by the phytoplankton. In the first scenario, the consumption of nitrate has to be fast enough for little or no isotopic fractionation and the original  $\delta^{15}\text{N}$  of nitrate would be reflected in the  $\delta^{15}\text{N}$  of

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PON (Altabet and McCarthy, 1985; Wada and Hattori, 1991). In this case, complete consumption of nitrate from the surface would be expected. The open ocean stations during post monsoon shows virtually absence of nitrate (below the level of detection; Fig. 2) from the surface in most open ocean stations during post-monsoon, implying its complete consumption.  $\delta^{15}\text{N}$  of PON at these locations shows a general increase in  $\delta^{15}\text{N}$  of from south to north, indicating the existence of deeper nitrate with higher  $\delta^{15}\text{N}$  at southern locations.

The  $\delta^{15}\text{N}$  of pre-monsoon locations vary from 2.7 to 7‰ with relatively higher surface nitrate concentration (0.2 to 1.1  $\mu\text{M}$ ). The availability of nitrate pool in the surface water suggests the luxury of phytoplankton to discriminate and hence the fractionation during uptake of nitrate by the planktons during pre-monsoon. The exact mechanism by which these nutrients reach the surface in the open Bay during pre-monsoon is a subject of speculation. However, nitrate for coastal locations during pre-monsoon might have been supplied by the EICC acting north of about 10°N. The EICC is best developed during March–April and decays only by June (Shetye et al., 1993).

Overall, the  $\delta^{15}\text{N}$  values of PON in the surface waters observed in the Bay may be explained in terms of a two end-member mixing: first, the terrestrial particulate matter with low  $\delta^{15}\text{N}$  which has mostly influenced the six coastal locations and three open ocean locations during post-monsoon, and the other, marine phytoplankton, which has mainly inherited the higher  $\delta^{15}\text{N}$  of nitrate from deeper waters.

### 5.2. Depth profile of $\delta^{15}\text{N}$ suspended matter

The depth related distribution of  $\delta^{15}\text{N}$  in suspended matter is in agreement with the general pattern observed in the world ocean i.e. it increases with depth (Saino and Hattori, 1980). At most locations there is an increase in  $\delta^{15}\text{N}$  below 60 m. This increase is 0.21 to 2.8‰ between 60 and 300 m. High  $\delta^{15}\text{N}$  below euphotic depth has been observed mainly due to two reasons: (1) degradation of suspended matter itself causing the preferential release of  $^{14}\text{N}$  leaving the remaining PON enriched in  $^{15}\text{N}$ . (2) The PON below euphotic depth is produced due to fragmentation of sinking particles

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(Bacon et al., 1985). These sinking particles are enriched in  $\delta^{15}\text{N}$  by 3–4‰ relative to suspended particle in euphotic zone because these particles are formed as a by-product of zooplankton feeding, causing an increase in  $\delta^{15}\text{N}$  with each trophic step (DeNiro and Epstein, 1981). There is no data regarding  $\delta^{15}\text{N}$  of sinking particles for BOB for 300 or 500 m depths. However, the sediment traps placed at around 2000 m show the  $\delta^{15}\text{N}$  variation in the range of 2.2–6.2‰ (Schafer and Ittekkot, 1995). But  $\delta^{15}\text{N}$  of deeper PON (~2000 m) is known to be less (Saino and Hattori, 1987) and starts decreasing below 500 m. Based on this argument the  $\delta^{15}\text{N}$  of sinking particles around 300 m during present study should be more than 6‰. Saino and Hattori (1980) have found the  $\delta^{15}\text{N}$  is high as ~12‰ at 300 m depth in the far eastern Indian Ocean. However our data suggest the average value of ~6‰ for BOB at the same depth. This may be due to the high sinking rate of particles in the BOB allowing it lesser time for degradation. Here, the particle removal to the deep sea occurs in the form of large aggregates formed by the interaction of organic and mineral matter introduced from external sources like rivers and wind. This increases the density and consequently the settling rate in water column (Ittekkot, 1991). Minima in the  $\delta^{15}\text{N}$  of PON within the surface layer as reported by Saino and Hattori (1980), have been observed during present study too, possibly due to the isotopic fractionation during nitrate uptake in light limited conditions.

## 6. Conclusions

First systematic measurements of  $\delta^{15}\text{N}$  of surface PON in BOB shows it to be a two end-member mixing between continental input and marine phytoplankton.  $\delta^{15}\text{N}$  values in surface PON of open ocean locations during both pre- and post-monsoon seasons and coastal location during pre-monsoon season is primarily supported by nutrients of marine origin. However, during post-monsoon coastal locations as well as northern open ocean stations appear to be influenced by the continental run off as is evident by the salinity and  $\delta^{15}\text{N}$  dilution at these locations. The depth dependent increase in

$\delta^{15}\text{N}$  of suspended PON appears to be a general feature. This increase to a maximum of 2.8‰ is lower than that observed for the eastern Indian Ocean (Saino and Hattori, 1980) possibly due to the high settling rates of sinking particles in BOB due to formation of organic and mineral aggregates.

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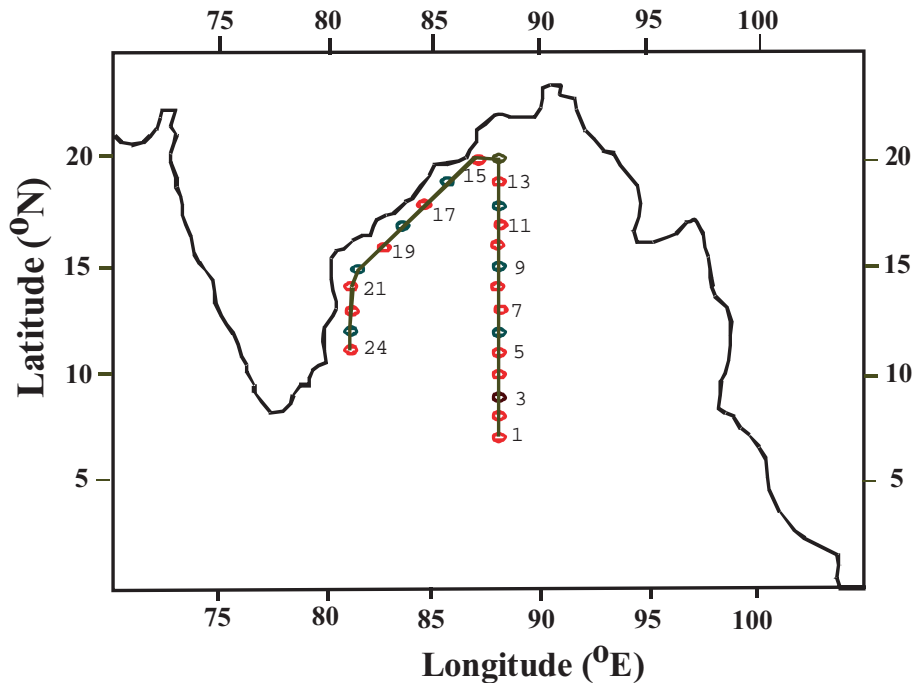
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**Fig. 1.** The cruise track along which the study was performed, during both cruises.

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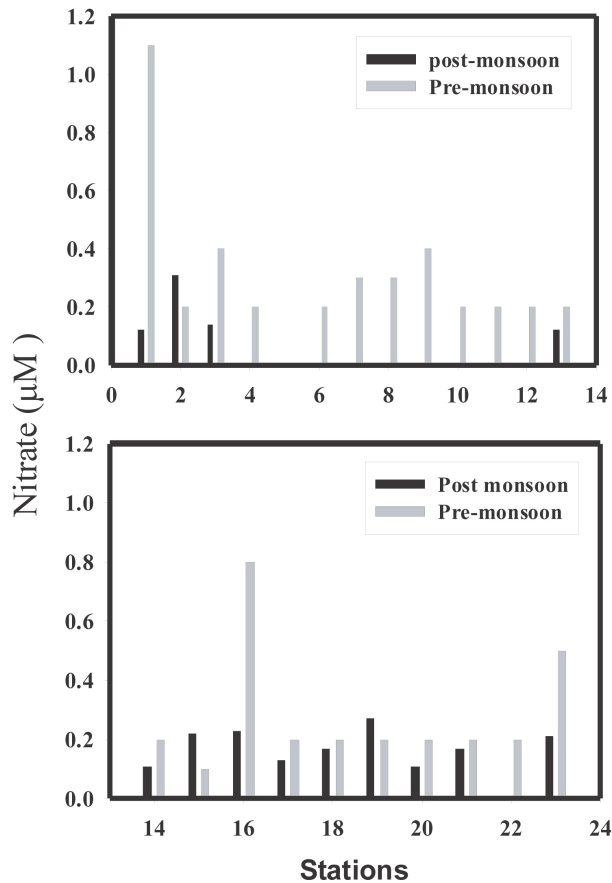
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**Fig. 2.** The surface nitrate concentrations at different stations during post- (dark bars) and pre- (light bars) monsoon. The absence of bars indicate the values below detection limit ( $<0.1 \mu\text{M}$ ).

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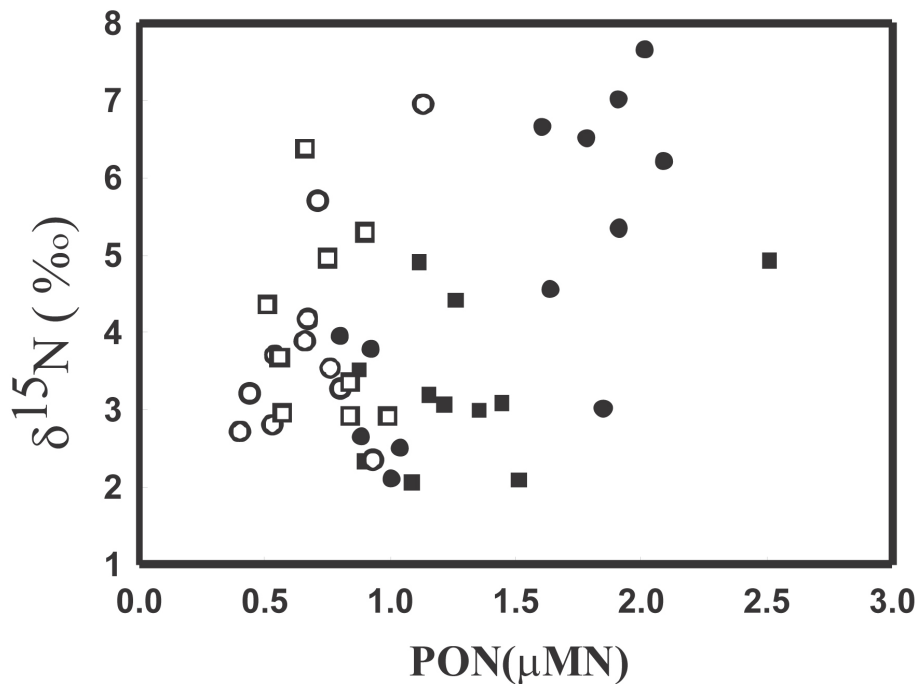


Fig. 3. The relationship between  $\delta^{15}\text{N}$  and PON during post- (filled circles and squares represent open ocean and coastal stations) and pre-monsoon (open circle and squares represent open ocean and coastal stations).

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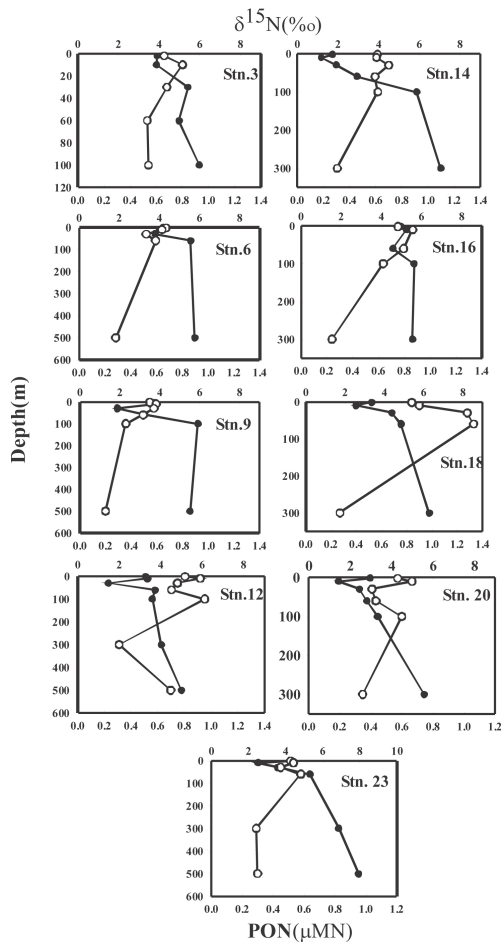
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**Fig. 4.** The depth profiles of  $\delta^{15}\text{N}$  and PON for pre-monsoon season at different locations. The filled and unfilled circles indicate  $\delta^{15}\text{N}$  and PON, respectively.

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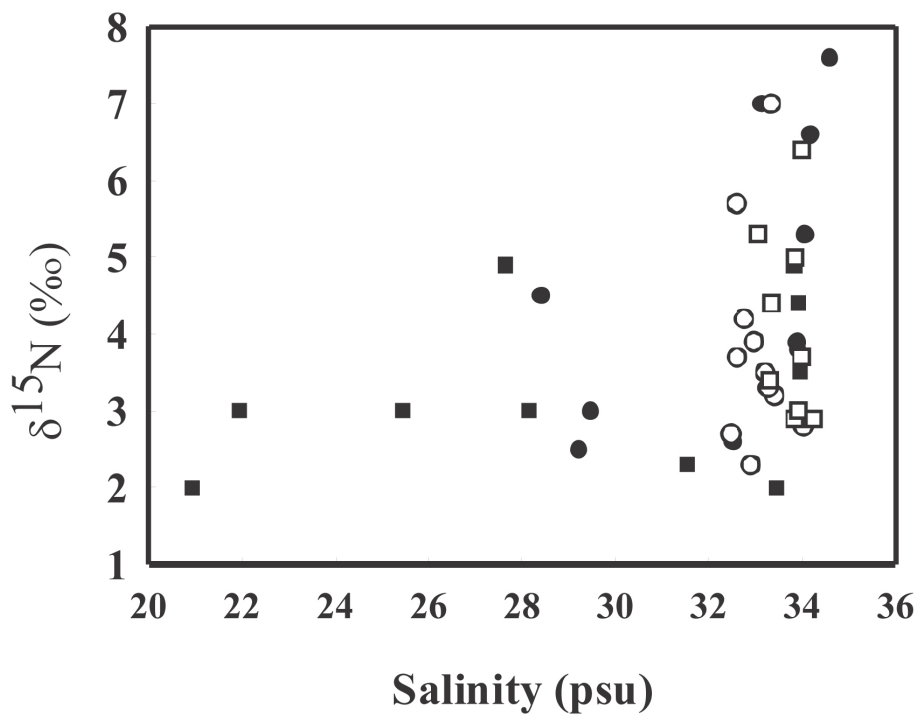
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**Fig. 5.** The relationship between salinity and  $\delta^{15}\text{N}$  for pre- and post-monsoon seasons. The annotations are same as Fig. 2.

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