

# Nitrogen isotopic studies in the suboxic Arabian Sea

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Measurements of  $^{15}\text{N}/^{14}\text{N}$  in dissolved molecular nitrogen ( $\text{N}_2$ ), nitrate ( $\text{NO}_3^-$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) and  $^{18}\text{O}/^{16}\text{O}$  in  $\text{N}_2\text{O}$  [expressed as  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , relative to atmospheric  $\text{N}_2$  and oxygen ( $\text{O}_2$ ), respectively] have been made in water column at several locations in the Arabian Sea, a region with one of the thickest and most intense  $\text{O}_2$  minima observed in the open ocean. Microbially-mediated reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  (denitrification) in the oxygen minimum zone (OMZ) appears to greatly affect the natural isotopic abundances. The  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  increases from 6‰ in deep waters (2500 m) to 15‰ within the core of the denitrifying layer (250–350 m); the  $\delta^{15}\text{N}$  of  $\text{N}_2$  concurrently decreases from 0.6‰ to 0.20‰. Values of the isotopic fractionation factor ( $\epsilon$ ) during denitrification estimated using simple advection-reaction and diffusion-reaction models are 22‰ and 25‰, respectively. A strong decrease in  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  is observed from ~200 m (> 11‰) to 80 m (~6‰); this is attributed to the input of isotopically light nitrogen through nitrogen fixation. Isotopic analysis of  $\text{N}_2\text{O}$  reveals extremely large enrichments of both  $^{15}\text{N}$  and  $^{18}\text{O}$  within the OMZ, presumably due to the preferential reduction of lighter  $\text{N}_2\text{O}$  to  $\text{N}_2$ . However, isotopically light  $\text{N}_2\text{O}$  is observed to accumulate in high concentrations above the OMZ indicating that the  $\text{N}_2\text{O}$  emitted to the atmosphere from this region cannot be very heavy. The isotope data from the intense upwelling zone off the southwest coast of India, where some of the highest concentrations of  $\text{N}_2\text{O}$  ever found at the sea surface are observed, show moderate depletion of  $^{15}\text{N}$ , but slight enrichment of  $^{18}\text{O}$  relative to air. These results suggest that the ocean-atmosphere exchange cannot counter inputs of heavier isotopes (particularly  $^{18}\text{O}$ ) associated with the stratospheric back flux, as proposed by previous workers. This calls for additional sources and/or sinks of  $\text{N}_2\text{O}$  in the atmosphere. Also, the  $\text{N}_2\text{O}$  isotope data cannot be explained by production through either nitrification or denitrification, suggesting a possible coupling between the two processes as an important mechanism of  $\text{N}_2\text{O}$  production.

## 1. Introduction

In spite of the great abundance of nitrogen in the earth's surface environment, photosynthetic production in the oceans is generally nitrogen limited

(Codispoti 1989). This is because molecular nitrogen ( $\text{N}_2$ ), the dominant gaseous constituent of the atmosphere, has low chemical reactivity, and as such only a few organisms of the sea have the capability to utilize or "fix" it (Galloway *et al* 1995). Most organisms can

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only make use of nitrogen in the combined forms, such as nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ), which occur in much lower concentrations than  $\text{N}_2$ , but can be assimilated with greater ease. Nitrogen in these forms occurs in a number of oxidation states ranging from  $-3$  to  $+5$ . The speciation is strongly dependent on the redox potential of seawater determined mainly by the ambient oxygen ( $\text{O}_2$ ) levels. The bulk of the oceanic water column is well-oxygenated, making  $\text{NO}_3^-$  the predominant species of combined nitrogen in the sea.

The inventory of combined nitrogen in the oceans is regulated by several processes. Inputs of nitrogen to the oceans occur mainly through river runoff, atmospheric deposition and nitrogen fixation, while its loss is principally through conversion of combined nitrogen into  $\text{N}_2$  (Codispoti and Christensen 1985). This process, known as denitrification, takes place when the  $\text{O}_2$  concentrations are close to zero and bacteria utilise  $\text{NO}_3^-$  instead of  $\text{O}_2$  as an oxidant for decomposing organic matter (Richards 1965). Conditions conducive for denitrification commonly develop within the coastal sediments. In the water column, however, the oceanic currents supply enough  $\text{O}_2$  at all depths to prevent the development of anoxia in most oceanic areas. However, there are three regions where some unusual oceanographic processes cause the  $\text{O}_2$  demand to exceed its supply leading to an almost complete  $\text{O}_2$  depletion (suboxia) at mid-depths (Deuser 1975). Two of these sites are located in the eastern tropical north and south Pacific, while the third is found in the Arabian Sea. Although these regions account for only  $\sim 2\%$  of the total oceanic area, they are extremely important from the biogeochemical and climatic points of view. This is because the high and temporally variable rate of denitrification in these regions has the potential to greatly alter the oceanic combined nitrogen inventory. This in turn may cause variations in the rate of photosynthetic carbon fixation and thereby in the rate at which carbon dioxide ( $\text{CO}_2$ ) is sequestered from the atmosphere. Thus the oceanic  $\text{O}_2$ -deficient zones probably contribute very significantly to global climatic changes (McElroy 1983; Codispoti et al 1996a). Another important way in which these environments affect the atmospheric composition is through production of trace gases, particularly nitrous oxide ( $\text{N}_2\text{O}$ ). The importance of  $\text{N}_2\text{O}$  owes to its capacity to strongly absorb the back radiation emitted by the earth in the infrared range and to the role it plays in the chemistry of stratospheric ozone depletion. Recent studies show that the oceans may account for about half of the total inputs of  $\text{N}_2\text{O}$  to the atmosphere from all natural sources, and that a large fraction of the oceanic efflux comes from the  $\text{O}_2$ -deficient zones particularly the Arabian Sea (Codispoti and Christensen 1985; Law and Owens 1990; Naqvi and Noronha 1991; Bange et al 1996).

A considerable amount of work on nitrogen cycling in suboxic waters of the Arabian Sea, carried out over the past two decades, has established it to be globally significant (Naqvi 1994). However, these studies largely involved chemical and, to a lesser extent, biochemical measurements. Investigations on the natural abundance of stable isotopes in various dissolved nitrogen species have been initiated only recently (Yoshinari et al 1997; Naqvi et al 1998; Brandes et al 1998). This approach is based on the fact that the biogeochemical processes involve characteristic mass-dependent discriminations between various isotopes, and so the isotopic data can provide useful insights into the pathways of nitrogen transformations (Wada and Hattori 1991). A brief account of the progress made in this area is given here.

## 2. Material and methods

Isotopic measurements on three dissolved nitrogen species ( $\text{N}_2$ ,  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$ ) were made during four cruises of FORV *Sagar Sampada* (SS106, November 1992; SS119, April 1994; SS128, January 1995; and SS141, May 1996) and two cruises of ORV *Sagar Kanya* (SK87, September 1993; and SK103, June-July 1995) at the stations shown in figure 1. However, concurrent measurements on all these species were made only at one station (3204/SS119). While the

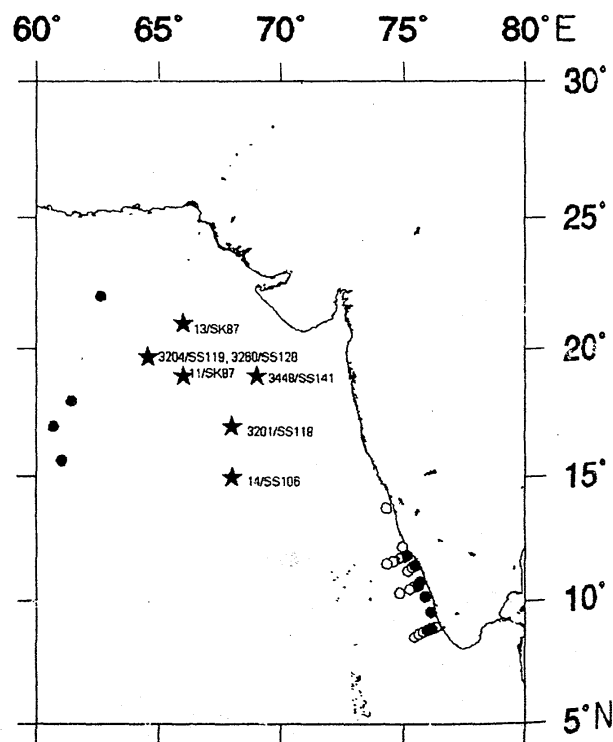


Figure 1. Station locations (open circles: surface  $\text{N}_2\text{O}$  concentration only; filled circles: surface  $\text{N}_2\text{O}$  concentration and isotopes; stars: vertical profiles).

$^{15}\text{N}/^{14}\text{N}$  ratio was measured in  $\text{N}_2$  and  $\text{NO}_3^-$ , both  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$  were determined in  $\text{N}_2\text{O}$ . In addition, routine hydrographic (temperature and salinity) and hydrochemical (dissolved  $\text{O}_2$  and nutrients) data were collected during all the cruises.

Water samples for  $^{15}\text{N}/^{14}\text{N}$  analysis in  $\text{N}_2$  and/or  $\text{NO}_3^-$  were taken during SK87, SS119, SS128 and SS141 from 1.8-litre Niskin samplers mounted on the CTD (conductivity-temperature-depth) rosette. Utmost care was taken to avoid atmospheric contamination while sampling for  $\text{N}_2$ . Sub-samples (200–250 ml) were drawn immediately after recovery into evacuated,  $\text{HgCl}_2$ -poisoned 500-ml glass bottles. The  $\text{N}_2$  extraction and analysis was done at the University of Washington using a Finnigan MAT 251 mass spectrometer (Brandes *et al* 1998). Reproducibility was within  $\pm 0.05\%$ . Subsamples (1 litre) for  $\text{NO}_3^-$  were stored after poisoning with 1 ml saturated  $\text{HgCl}_2$  for analysis at the University of Washington following the method described by Brandes and Devol (1997). Briefly, it involved reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  using Devardas alloy. The  $\text{NH}_4^+$  was distilled and sorbed into an ion sieve. The sorbed material was then filtered onto quartz QM-A filters and sealed into evacuated quartz combustion tubes containing Cu and  $\text{CuO}$ . Ampoule contents were converted into  $\text{N}_2$  with a micro-Dumas combustion method, extracted into liquid  $\text{N}_2$ -cooled cold fingers containing molecular sieve and analysed for  $^{15}\text{N}/^{14}\text{N}$  using a Finnigan MAT 251 mass spectrometer. The results, reproducible to  $\pm 0.2\%$ , were corrected for an isotopic shift of  $-0.9\%$  to account for the reagent blank.

Samples for the  $\text{N}_2\text{O}$  isotope analysis were collected using specially modified 20-litre Go-flo samplers mounted on a hydrowire (during SS106 and SK87) or a CTD rosette sampler (during SS119 and SK103). The modified samplers enabled extraction of  $\text{N}_2\text{O}$ , without transferring the water to another vessel, with high-purity argon that was recirculated after removal of moisture and  $\text{CO}_2$  and adsorption of  $\text{N}_2\text{O}$  on a molecular sieve (MS 5A) packed in stainless steel columns. Extraction was carried out for 90 minutes at an Ar flow rate of  $\sim 1.5 \text{ l min}^{-1}$  (Yoshinari *et al* manuscript in preparation). After thermal desorption in the shore laboratory,  $\text{N}_2\text{O}$  was purified by gas chromatography and its isotopic composition ( $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$ ) measured at Woods Hole Oceanographic Institution by continuous-flow isotope-ratio monitoring using a Finnigan MAT 251 mass spectrometer (Yoshinari *et al* 1997). Reproducibility was better than  $\pm 0.3\%$  for both isotopes.

Dissolved  $\text{O}_2$  measurements were made with the Winkler titration technique during all cruises while nutrients were analysed using a Technicon Autoanalyser II on board *Sagar Sampada* and a Skalar 6-channel Analyser 5100/1 on board *Sagar Kanya* following standard methods (Grasshoff *et al* 1983).  $\text{N}_2\text{O}$  concentration was determined on board ships by head-

space extraction with helium followed by injection into a Perkin-Elmer 3920B gas chromatograph equipped with an electron capture detector (precision  $\sim 4\%$ ).

### 3. Results and discussion

#### 3.1 Nitrogen isotopic fractionation during denitrification

The reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  by denitrifying bacteria is believed to occur through the pathway (Payne 1973):



Nitrite ( $\text{NO}_2^-$ ), the first intermediate of this reduction sequence, accumulates in the oceanic suboxic zones (Richards 1965). In the Arabian Sea, as in the eastern tropical Pacific Ocean (Cline and Richards 1972), the secondary nitrite maximum (SNM), located in the upper portion of the oxygen minimum zone (OMZ), is invariably associated with  $\text{O}_2 < 0.1 \text{ ml l}^{-1}$ , in contrast to the primary  $\text{NO}_2^-$  maximum commonly found at the base of the euphotic zone. Precise  $\text{O}_2$  measurements carried out during a U.S. JGOFS cruise in the Arabian Sea revealed that the threshold  $\text{O}_2$  levels required for the  $\text{NO}_2^-$  build-up lie between 0.01 and  $0.02 \text{ ml l}^{-1}$  (S W A Naqvi and L A Codispoti, unpublished data). Dramatic changes in water chemistry are seen once this threshold is crossed. An apparent spurt in denitrification activity causes a minimum in the vertical profile of  $\text{NO}_3^-$  (figure 2). Interestingly, while  $\text{NO}_2^-$  accumulates under these conditions,  $\text{N}_2\text{O}$ , the other major intermediate, shows an opposite trend (e.g., figures 2 and 3), indicating that the reduction of  $\text{NO}_2^-$  may be the rate limiting step. These microbially-mediated transformations leave a strong imprint on the isotopic composition of dissolved nitrogen species. This is because, of the two nitrogen isotopes with mass numbers of 14 and 15 ( $^{14}\text{N}$  and  $^{15}\text{N}$ ),  $\text{NO}_3^-$  ions containing the more abundant  $^{14}\text{N}$  are preferentially

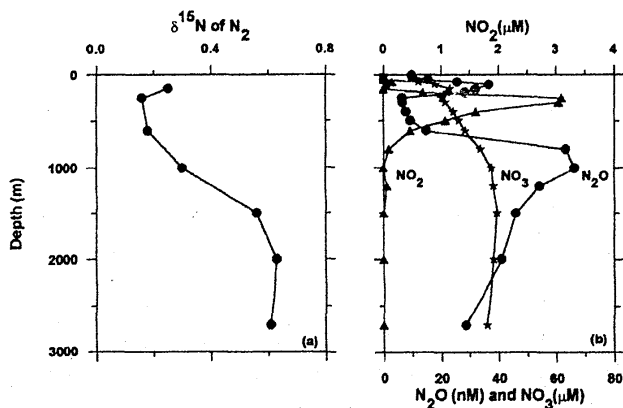


Figure 2. Vertical profiles of (a)  $\delta^{15}\text{N}$  of  $\text{N}_2$  (‰ relative to air) and (b) concentrations of  $\text{NO}_3^-$  ( $\mu\text{M}$ ),  $\text{NO}_2^-$  ( $\mu\text{M}$ ) and  $\text{N}_2\text{O}$  (nM) at Sta. 3448/SS141.

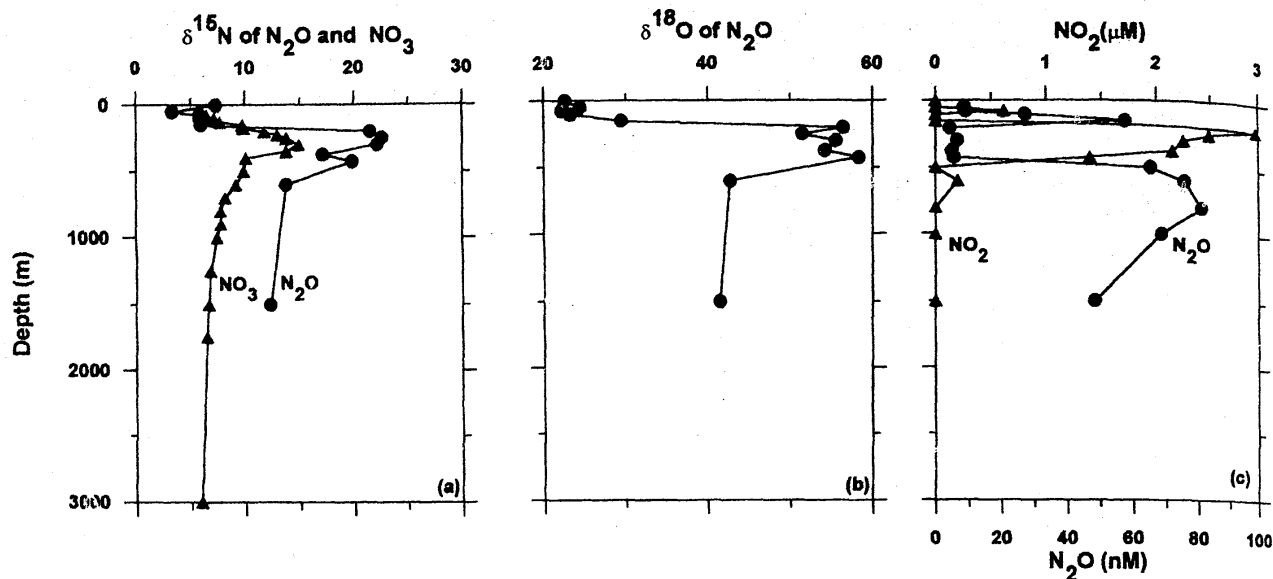


Figure 3. Vertical profiles of (a)  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$  (‰ relative to air), (b)  $\delta^{18}\text{O}$  of  $\text{N}_2\text{O}$  (‰ relative to air) and (c) concentrations of  $\text{NO}_2^-$  ( $\mu\text{M}$ , triangles) and  $\text{N}_2\text{O}$  (nM, circles) at Sta. 3201/SS119 (modified from Naqvi et al 1998).

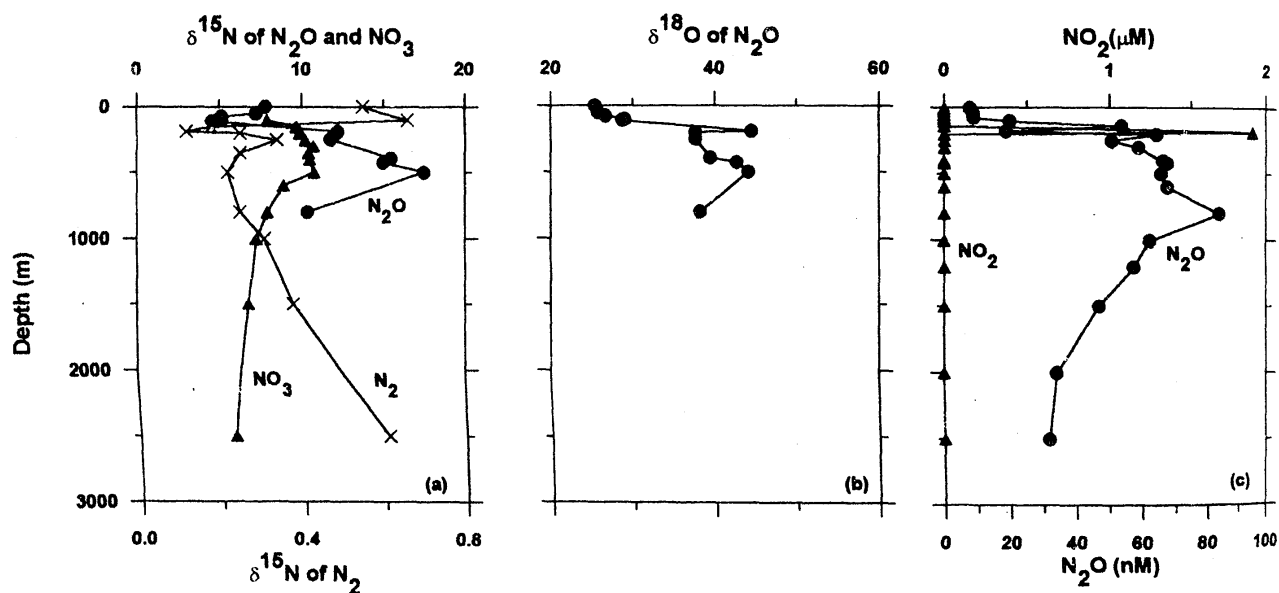


Figure 4. Vertical profiles of (a)  $\delta^{15}\text{N}$  of  $\text{N}_2$ ,  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$ , (b)  $\delta^{18}\text{O}$  of  $\text{N}_2\text{O}$  and (c) concentrations of  $\text{NO}_2^-$  ( $\mu\text{M}$ , triangles) and  $\text{N}_2\text{O}$  (nM, circles) at Sta. 3204/SS119 (the  $\delta^{15}\text{N}$ - $\text{NO}_3^-$  profile at this site was obtained during SS128) (modified from Brandes et al 1998; Naqvi et al 1998).

reduced during denitrification, leaving the residual  $\text{NO}_3^-$  enriched with  $^{15}\text{N}$ . On the other hand  $\text{N}_2$ , the end product of denitrification, gets greatly enriched with  $^{14}\text{N}$ . This is strikingly demonstrated by our results. The vertical profile of  $\delta^{15}\text{N}$  of  $\text{N}_2$  at the strongly nitrate reducing Sta. 3448/SS141, located in the northeastern Arabian Sea, exhibits a pronounced minimum coinciding with the SNM with values dipping below 0.2‰ (figure 2) (the  $\delta^{15}\text{N}$  of  $\text{N}_2$  in surface waters in this region, not determined at this station, is generally greater than 0.5‰). Interestingly at 150 m depth, where the  $\text{NO}_3^-$  concentration was

high ( $23\mu\text{M}$ ) and  $\text{NO}_2^-$  was not detectable, the  $\delta^{15}\text{N}$  was still quite low (0.25‰) i.e., only marginally higher than the minimal value but less than half of the values observed in waters outside the  $\text{O}_2$ -deficient zone. Even at 1000 m depth the  $\delta^{15}\text{N}$  was only 0.3‰. This indicates that the denitrification signature extends well outside the SNM. A similar pattern is also seen at Sta. 3204/SS119 (figure 4). This station had a thin, weakly-developed SNM associated with a minimum in  $\delta^{15}\text{N}$  of  $\text{N}_2$  (0.18‰) at 150-190 m. This was underlain by a layer with relatively high  $\delta^{15}\text{N}$  of  $\text{N}_2$  (maximum 0.39‰) which, going by its high salinity,

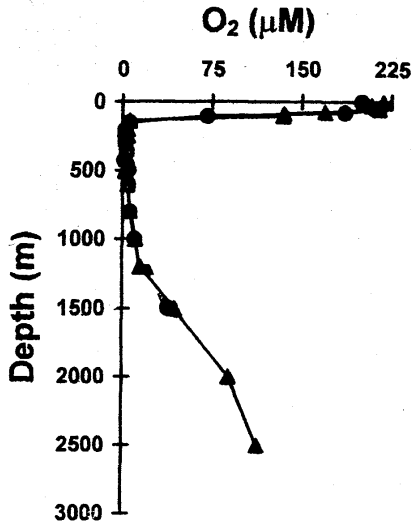


Figure 5. A comparison of vertical profiles of  $O_2$  ( $\mu M$ ) at Stas. 3201/SS119 (circles joined by dotted lines) and 3204/SS119 (triangles, joined by continuous lines) (modified from Naqvi *et al* 1998).

evidently originated in the Persian Gulf. The  $O_2$  concentrations, as determined by the Winkler method, were not appreciably different from those at Sta. 3201/SS119 (figure 5). The association of a maximum in  $\delta^{15}N$  of  $N_2$  with the Persian Gulf salinity maximum layer shows that the former is a sensitive tracer of advection. Again the vertical extent of the  $\delta^{15}N-N_2$  minimum is much larger than the SNM. This would mean that substantial  $N_2$  may be produced without the accumulation of  $NO_2^-$  or/and the rapid mixing results in the export of lighter  $N_2$  out of the SNM.

The distribution of  $\delta^{15}N$  of  $NO_3^-$  shows a good correspondence to that of secondary  $NO_2^-$  (figures 3, 4 and 6). Sharp increases in  $\delta^{15}N$  of  $NO_3^-$  (up to 15‰) were observed within the SNM at Stas. 3201/SS119 (figure 3) and 13/SK87 (figure 6) with both maxima being thicker at the latter site. In contrast, at Sta. 3204/SS119 where the SNM was weakly developed, the extent of  $^{15}N$  enrichment in  $NO_3^-$  was considerably smaller (figure 4). At all the three stations, significant  $^{15}N$  enrichment in  $NO_3^-$  [relative to the average value of 5.7‰ for the oceanic subsurface waters (Altabet *et al* 1995)] persisted down to at least 1,500 m, consistent with the observed trends in  $\delta^{15}N$  of  $N_2$ .

These data on  $\delta^{15}N$  of  $N_2$  and  $NO_3^-$  can be used to compute the fractionation factor,  $\epsilon_{denit} [= 10^3(1 - \alpha)]$ , where  $\alpha$  is the ratio between the rates of utilisation of  $^{15}NO_3^-$  and  $^{14}NO_3^-$ , during denitrification using simple advection-reaction and diffusion-reaction models (Brandes *et al* 1998). In the first case, we ignore diffusion and the water mass is assumed to advect into the OMZ without undergoing any mixing in the vertical and horizontal directions. The isotopic distribution can then be modelled with a simple Rayleigh equation (Bender 1990):

$$\delta^{15}N-NO_3 = 10^3(\alpha - 1) \ln f_{NO_3} + (\delta^{15}N-NO_3)_{init} \quad (1)$$

where  $f_{NO_3}$  is the ratio between the observed and expected  $NO_3^-$  concentrations and  $(\delta^{15}N-NO_3)_{init}$  gives the isotopic composition of the initial (unaltered) material. Values of  $f_{NO_3}$  were computed using the relationships between a nitrate tracer ( $NO_3^-$ ) and potential temperature and the observed  $NO_3^-$  and  $NO_2^-$  concentrations (Naqvi *et al* 1990).

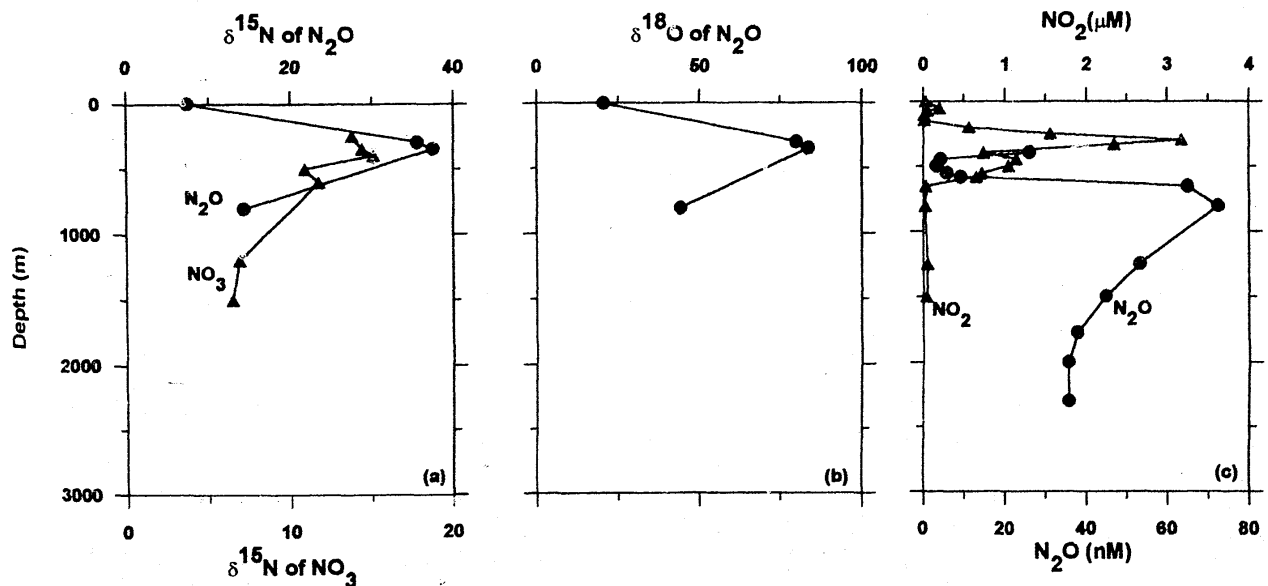


Figure 6. Vertical profiles of (a)  $\delta^{15}N$  of  $NO_3^-$  and  $N_2O$ , (b)  $\delta^{18}O$  of  $N_2O$  and (c) concentrations of  $NO_2^-$  ( $\mu M$ , triangles) and  $N_2O$  (nM, circles) at Sta. 13/SK87 (modified from Brandes *et al* 1998; Yoshinari *et al* 1997).

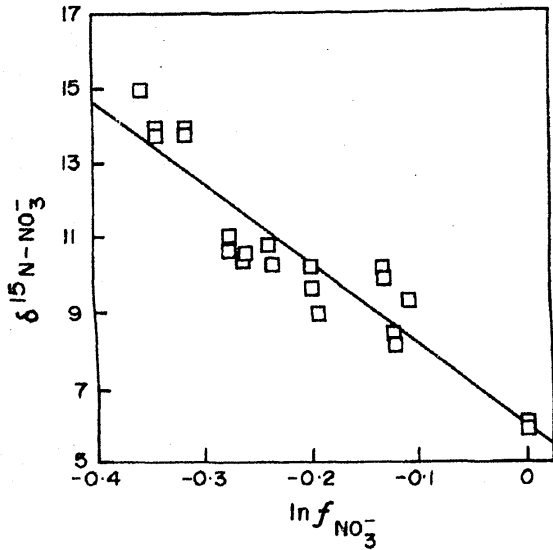


Figure 7. The  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  (% relative to air) vs. the natural log of  $f$ , the ratio of measured to expected nitrate concentrations (pooled data from three stations). The line gives the least squares fit to the data (modified from Brandes *et al* 1998).

A plot of  $\delta^{15}\text{N}\text{-NO}_2$  versus  $\ln f_{\text{NO}_3}$  for the depth range 200–600 m (figure 7) shows a good linear relationship between the two ( $r^2 = 0.9$ ). The slope of the regression line yields a value of 22‰ for  $\epsilon_{\text{denit}}$ , not too different from a similarly computed value (25‰) for the eastern tropical north Pacific (ETNP) (Brandes *et al* 1998).

The opposite extreme to the advection-reaction model is the reaction-diffusion model having no advective component. Such a model is expected to yield a larger fractionation factor than the advective model because the system being “open” allows the addition of new, unaltered material into the reaction region and the removal of old, altered material.

The reaction-diffusion equation is:

$$\partial[^{14}\text{NO}_3]/\partial t = J_{14\text{NO}_3} + K_d \partial^2[^{14}\text{NO}_3]/\partial x^2 \quad (2)$$

where  $J$  is the denitrification rate and  $K_d$  the horizontal eddy diffusivity along the 300 m isopycnal which was chosen because it lies close to the core of the denitrifying layer (Naqvi 1987);  $x$  increases from the edge to the centre of the denitrifying zone. For  $^{15}\text{NO}_3^-$  the equation is:

$$\partial[^{15}\text{NO}_3]/\partial t = \alpha([^{15}\text{NO}_3]/[^{14}\text{NO}_3])J_{14\text{NO}_3} + K_d \partial^2[^{15}\text{NO}_3]/\partial x^2. \quad (3)$$

Because there is no exact analytical solution for these equations, the values of both  $^{14}\text{N}$  and  $^{15}\text{N}$  were calculated numerically using a tridiagonal matrix solution method (Press *et al* 1992). The boundary conditions were that the  $\text{NO}_3^-$  and  $\delta^{15}\text{N}\text{-NO}_3$  compositions at  $x = 0$  were 28  $\mu\text{M}$  ( $\text{NO}_3^-$  deficit = 0) and 6‰, respectively, and that there was no  $\text{NO}_3^-$  or isotopic flux out of the centre of the OMZ. Alternative

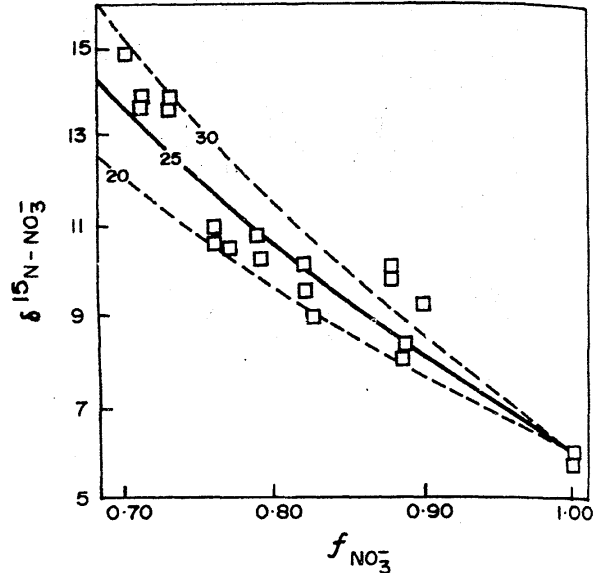


Figure 8. The  $\delta^{15}\text{N}$  of  $\text{NO}_3$  (% relative to air) vs.  $f$ , the ratio of measured to expected nitrate concentrations. Also plotted are the diffusion-reaction model results with the numbers denoting fractionation factors (modified from Brandes *et al* 1998).

boundary conditions, such as an interior concentration boundary condition, would change  $\epsilon$  by no more than 2 (Brandes *et al* 1998).

The value of  $J$  was set at  $1 \times 10^{-7} \mu\text{moles N l}^{-1} \text{sec}^{-1}$ . Although this value is only 1/2 of the rate estimated by Naqvi and Shailaja (1993) from the ETS data, it gives realistic nitrate deficit given a  $x_E$  of 500 km, which is the minimum distance between the southern boundary of the suboxic zone at 12°N and the southernmost station located at 17°N. The value of  $K_d$  was taken as  $1800 \text{ m}^2 \text{sec}^{-1}$  (Jenkins 1990). Using these parameters,  $\text{NO}_3^-$  concentrations dropped to  $\sim 70\%$  of the initial values in the interior of the denitrification zone, in agreement with the observations (Naqvi 1987). With these values of  $J$  and  $K_d$  the best-fit to the data was obtained with a fractionation factor of 25‰ (figure 8), only slightly lower than a similarly calculated value of 30‰ for the ETNP (Brandes *et al* 1998). These values of  $\epsilon$  are higher than those (15–20‰) derived from laboratory experiments of denitrification (Delwiche and Steyn 1970; Mariotti *et al* 1981), but lower than the estimate (30–60‰) of Cline and Kaplan (1975) derived from one-dimensional model fits to their ETNP data. However, the same data when fitted to the advection-reaction model described above yielded a value of  $\epsilon$  (25‰) which compares very well with our results.

Although, the above models ignore either diffusion or advection, and a more realistic approach should simultaneously consider both of these, the results of the two models are not too divergent. More significantly, as the estimates from the Arabian Sea and ETNP are not very different, it would appear that the

fractionation factor is not strongly dependent on either the denitrification rate or the local hydrography of the region.

### 3.2 Nitrogen fixation

A very important aspect of the data is the strong decrease in  $\delta^{15}\text{N-NO}_3$  in near-surface waters (figures 3 and 4). At 200 m, for example, the value is  $> 10\text{‰}$  but it declines to  $\sim 6\text{‰}$  (close to the oceanic background) at 80 m. This trend is exactly opposite to that seen outside the OMZ (Sigman *et al* 1997). The observed decrease can only result from an input of light nitrogen to the surface waters. The two possible sources are unaltered  $\text{NO}_3^-$  horizontally advected from outside the central Arabian Sea, and nitrogen fixation. The known pattern of water circulation in the Arabian Sea (Naqvi 1987; Olson *et al* 1993) makes the first mechanism less likely, and so the most plausible mode of input of isotopically light  $\text{NO}_3^-$  to the surface layer appears to be nitrogen fixation.

If  $\text{NO}_3^-$  from 200 m at  $11\text{‰}$  is being upwelled to the surface, and all nitrogen is removed by primary producers at 70–80 m, one can estimate the amount of nitrogen reaching the surface ocean that originally came from nitrogen fixation (Brandes *et al* 1998). Assuming that the changes in isotopic composition below 80 m are due to dilution by remineralised nitrogen fixed at  $0\text{‰}$ , about 40% of the  $\text{NO}_3^-$  present at 80 m should have been derived from nitrogen fixation. Using the data on primary productivity in this region this translates into a nitrogen fixation rate of up to  $6 \text{ Tg N y}^{-1}$  ( $1 \text{ Tg} = 10^{12} \text{ g}$ ) or about 25% of the denitrification rate. Thus, in spite of a high rate of nitrogen fixation, the Arabian Sea still serves as a net sink of combined nitrogen. This is consistent with the data on N:P ratios in this region (Codispoti 1997).

### 3.3 Dual isotopic composition of nitrous oxide

As stated earlier, the eastern boundary upwelling environments that contain  $\text{O}_2$ -depleted waters are important for global  $\text{N}_2\text{O}$  cycling. This is because of the extreme sensitivity of the processes involved in  $\text{N}_2\text{O}$  production and consumption to minor changes in ambient  $\text{O}_2$  levels in the low range ( $< \sim 0.5 \text{ ml l}^{-1}$ ; Codispoti and Christensen 1985). An intense  $\text{N}_2\text{O}$  accumulation is found to occur as the  $\text{O}_2$  concentrations approach, but do not reach, suboxia. However, under strongly nitrate reducing conditions, such as those found within the SNM,  $\text{N}_2\text{O}$  concentrations fall rapidly below saturation, evidently as a result of its reduction to  $\text{N}_2$  (e.g., figures 2 and 3). Thus, the Arabian Sea contains sites, located in close proximity of each other, which act either as strong source or strong sink of  $\text{N}_2\text{O}$  (Naqvi and Noronha 1991). Why does a strong net accumulation of  $\text{N}_2\text{O}$  occur in the low- $\text{O}_2$  zones is an issue that has not been fully

resolved so far. This is because  $\text{N}_2\text{O}$  is an intermediate of both the oxidative and reductive sequences of the nitrogen cycle and can be produced both during nitrification and denitrification. The two pathways leave very different isotopic signatures:  $\text{N}_2\text{O}$  produced during denitrification is enriched with  $^{15}\text{N}$  and  $^{18}\text{O}$  while that formed during nitrification is depleted in these isotopes (e.g., Yoshida 1988; Wada and Hattori 1991; Yoshinari *et al* 1997). Hence measurements of the  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$  ratios in  $\text{N}_2\text{O}$  dissolved in seawater are expected to provide insights into the mechanisms of its transformations. However, while substantial data have now been generated on the dual isotopic composition of  $\text{N}_2\text{O}$  in seawater, their interpretation has not been straightforward, and indeed often contradictory (Yoshida *et al* 1989; Kim and Craig 1990). The Arabian Sea contains several diverse biogeochemical environments and offers the most extreme conditions for  $\text{N}_2\text{O}$  cycling (Burkill *et al* 1993). The isotopic composition of  $\text{N}_2\text{O}$  in this region may therefore be helpful in improving the understanding of its transformations.

Data on the isotopic composition of  $\text{N}_2\text{O}$  at five stations (3201/SS119, 3204/SS119, 13/SK87, 11/SK87 and 14/SS106) are shown in figures 3, 4, 6, 9 and 10, respectively. The most prominent feature of the isotope profiles is the large enrichment of both  $^{15}\text{N}$  and  $^{18}\text{O}$  at all the stations, although the degree of enrichment varies considerably. As stated earlier, Stas. 14/SS106 and 3204/SS119 experienced mildly reducing conditions (thin SNM) while the other three stations were strongly nitrate reducing. These differences in redox conditions are reflected in the isotope data. The greatest enrichment is seen at Sta. 13/SK87; peak  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values at this site ( $37.5$  and  $83.6\text{‰}$ , respectively) are the highest reported yet from a natural environment (Yoshinari *et al* 1997). In contrast, the most southern Sta. 14/SS106 exhibits the least enrichment\*. Preferential loss of lighter  $\text{N}_2\text{O}$  to  $\text{N}_2$  within the SNM evidently leads to the observed enrichments of  $^{15}\text{N}$  and  $^{18}\text{O}$  in residual  $\text{N}_2\text{O}$ . The  $\text{N}_2\text{O}$  concentration minimum is embedded between two maxima, but the isotopic compositions of these  $\text{N}_2\text{O}$ -rich layers are very different. Going upward from the OMZ, the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values fall sharply across the oxic-suboxic boundary with the former generally lower than the tropospheric value ( $7\text{‰}$  – Kim and Craig 1990) in the upper 150 metres. In contrast, although  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  also decrease below SNM, their levels remain much higher than the tropospheric values ( $20.7\text{‰}$  for  $\delta^{18}\text{O}$  – Kim and Craig 1990) in the

\* Samples from Sta. 14/SS106 were analysed following an older method [combustion of  $\text{N}_2\text{O}$  to  $\text{N}_2$  and  $\text{CO}_2$  (Yoshinari and Koike 1994)]. It is possible that the oxygen isotope data at this station suffer from some contamination in  $\text{CO}_2$  with traces of water leading to somewhat lower  $\delta^{18}\text{O}$  values; this would, however, not affect the nitrogen isotope data.

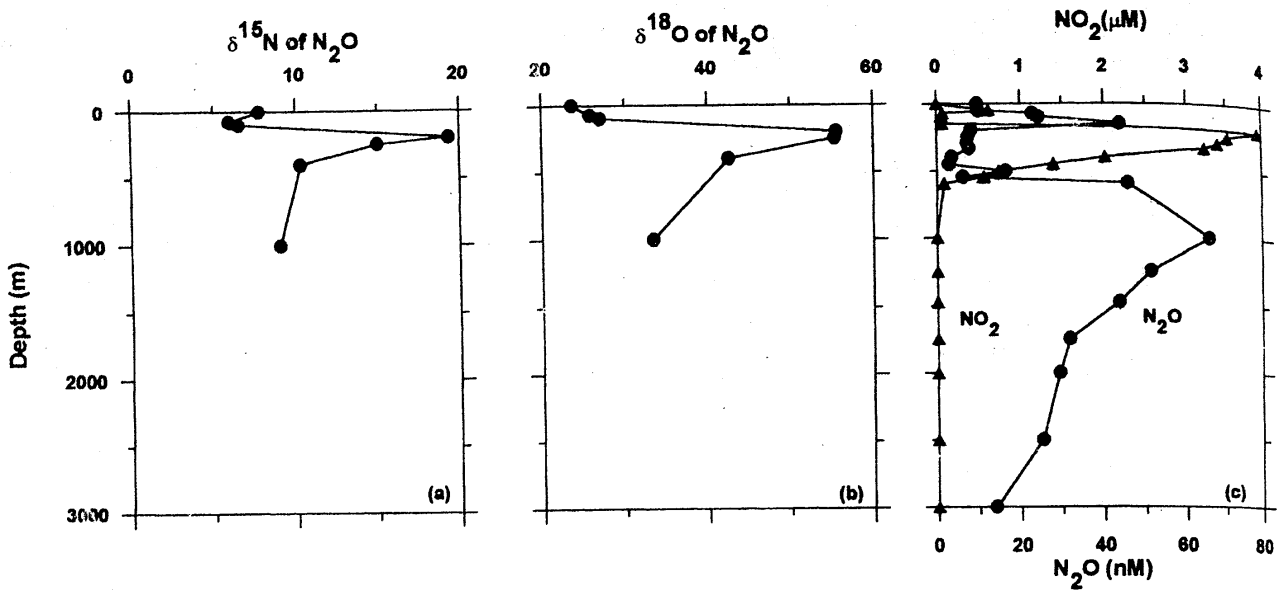


Figure 9. Vertical profiles of (a)  $\delta^{15}\text{N}$  and (b)  $\delta^{18}\text{O}$  of  $\text{N}_2\text{O}$ , and (c) concentrations of  $\text{NO}_2^-$  ( $\mu\text{M}$ , triangles) and  $\text{N}_2\text{O}$  (nM, circles) at Sta. 11/SK87 (modified from Yoshinari *et al* 1997).

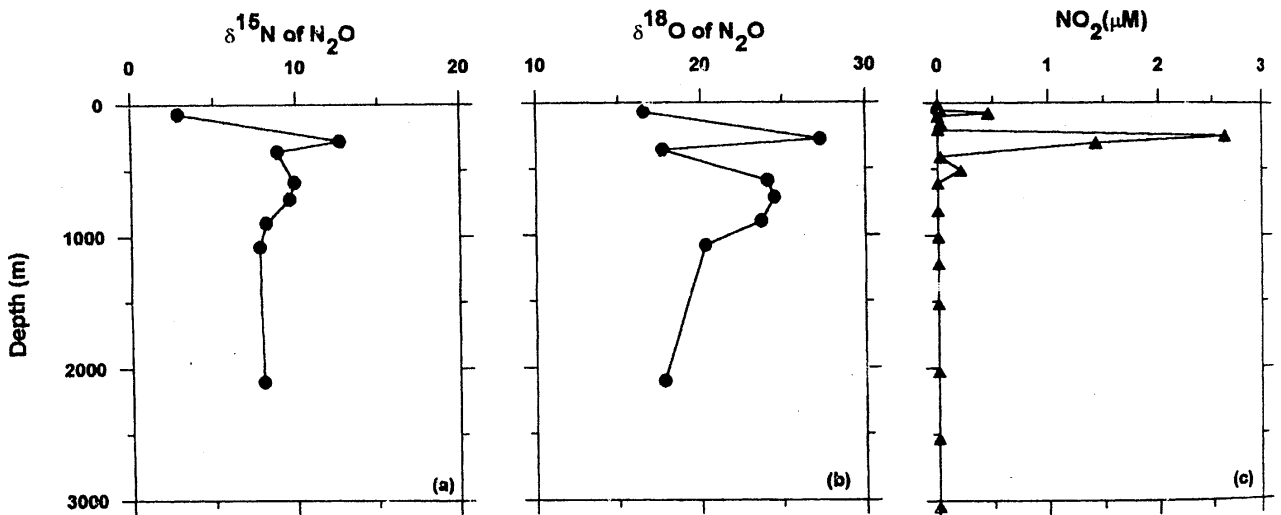


Figure 10. Vertical profiles of (a)  $\delta^{15}\text{N}$  and (b)  $\delta^{18}\text{O}$  of  $\text{N}_2\text{O}$ , and (c) concentration of  $\text{NO}_2^-$  ( $\mu\text{M}$ ) at Sta. 14/SS106.

deep waters. Since the suboxic waters with huge enrichments of heavier isotopes are capped by a layer which has high concentrations of light  $\text{N}_2\text{O}$ , the  $\text{N}_2\text{O}$  escaping to the atmosphere in the region cannot be heavy. Thus the near-tropospheric isotopic values at the sea surface at the open ocean sites (mean  $\pm$ s.d =  $7.65 \pm 0.47\%$  for  $\delta^{15}\text{N}$  and  $23.45 \pm 1.35\%$  for  $\delta^{18}\text{O}$ ;  $n = 11$ ) probably reflect an active air-sea exchange. The fate of heavy  $\text{N}_2\text{O}$  is not known, but it is expected to be advected out of the region and contribute to the elevated  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{N}_2\text{O}$  observed outside the suboxic zones (Kim and Craig 1990).

An important aspect of the data is that the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  is consistently lower than that of  $\text{N}_2\text{O}$  at depths exceeding  $\sim 200$  m, and higher at shallower depths

(figures 3 and 4). This indicates that the intense  $\text{N}_2\text{O}$  accumulation in the upper and lower parts of the OMZ may be through different mechanisms. Greater enrichment of  $\delta^{15}\text{N}$  in  $\text{N}_2\text{O}$  relative to  $\text{NO}_3^-$  appears to be characteristic of denitrification as revealed by recent culture experiments (Altabet M A, unpublished data). It may arise from a combination of much lower  $\text{N}_2\text{O}$  concentration than the amount of denitrified nitrogen and large kinetic isotope fractionation associated with the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Yoshida *et al* 1984). This pattern is the most pronounced within  $\text{N}_2\text{O}$ -depleted SNM at 3201/SS119. Its persistence even below SNM at 3201/SS119 and at depths exceeding  $\sim 200$  m at 3204/SS119 suggests significant  $\text{N}_2\text{O}$  production through denitrification. This obser-



vation is consistent with the view that denitrification may lead to a net  $N_2O$  accumulation under certain conditions (Betlach and Tiedje 1981; Capone 1996). However, we also note that heavy isotope enrichment has also been observed, albeit to a lesser degree, in the more oxygenated deep waters of the Pacific Ocean by Kim and Craig (1990). It was attributed to partitioning of isotopes during nitrification by which the precursor species of  $N_2O$  [such as hydroxylamine ( $NH_2OH$ )] is enriched with  $^{15}N$  and  $^{18}O$ . But the problem with this mechanism is that it cannot explain the observed large increases in  $N_2O$  at  $O_2$  levels approaching suboxia (Law and Owens 1990; Naqvi and Noronha 1991) over concentrations expected from linear relationships between  $N_2O$  production and  $O_2$  utilisation commonly observed in the oceans (Yoshinari 1976; Elkins *et al* 1978). This obviously requires a reductive step in  $N_2O$  production. An alternative mechanism of the observed heavy isotope enrichment could be the consumption of  $N_2O$  within sediments (and in water in areas such as the Arabian Sea and eastern tropical Pacific) and subsequent transport of heavy  $N_2O$  to the ocean interior (Zafriou 1990). Lastly, coupling of nitrification and denitrification through common intermediates/by-products may also influence the isotopic composition of  $N_2O$  (Zafriou 1990; Naqvi and Noronha 1991; Naqvi 1991). Such a coupling should be especially important near the oxic-suboxic interface, and this could possibly account for the enigmatic isotopic trend seen in the upper layer of the Arabian Sea which defies conventional explanation. That is, while the lighter  $N_2O$  at depths less than ~200m is apparently not produced by denitrification, the classical nitrification pathway ( $NH_4^+ \rightarrow NO_2^- \rightarrow N_2O$ ) (Yoshida 1988) also cannot fully explain the observed data for two reasons. First, as stated earlier, the  $\delta^{15}N$  values are much higher than that reported ( $< -60\text{‰}$ ) by Yoshida (1988) for or the production of  $N_2O$  from  $NH_4^+$  via  $NO_2^-$  by *Nitrosomonas europaea*<sup>1</sup>. Secondly, what is more significant is that the observed  $\delta^{18}O$  is much higher than the value for nitrification expected from  $\delta^{18}O$  of  $H_2O$  and  $O_2$  [assuming that one oxygen atom in  $NO_2^-$  is derived from  $H_2O$  and the other from dissolved  $O_2$  (Yoshinari *et al* 1997) and using the data from GEOSECS station 416 (Ostlund *et al* 1987), the  $\delta^{18}O$  of nitrification-derived  $N_2O$  should have been  $< -3\text{‰}$ ]. Given the very abrupt decrease in  $O_2$  below the surface mixed layer and the expected abundance of large particles at this level, it seems very plausible (Naqvi and Noronha 1991; Naqvi 1991) that an intermediate or by-product of nitrification such as NO enters the denitrification sequence and gets reduced to  $N_2O$ . As the oxygen atom of

$NH_2OH$ , the probable precursor of NO (Ward and Zafriou 1988), is derived from  $O_2$  (Dua *et al* 1979) which is characterised by high  $\delta^{18}O$  (Ostlund *et al* 1987),  $N_2O$  produced through this pathway is expected to be enriched in  $^{18}O$ . Moreover, unlike  $N_2O$ , production of NO by nitrifying bacteria involves modest depletion of  $^{15}N$  (Yoshida 1988). Thus,  $N_2O$  produced through this pathway is expected to be moderately depleted in  $^{15}N$ , but enriched in  $^{18}O$ , as observed.

Therefore, it may be concluded that, in conformity with previous suggestions (Zafriou 1990; Naqvi 1991), there are probably several mechanisms of  $N_2O$  production in the ocean involving different isotopic fractionations. The relative contribution from various pathways is probably determined by the  $O_2$  distribution and organic carbon availability, both of which vary in space and time. This not only brings about large spatial changes in  $N_2O$  production and consumption but, in response to temporal changes in the geographical extent and intensity of the  $O_2$  minimum zones (Altabet *et al* 1995; Ganeshram *et al* 1995) the oceanic  $N_2O$  source strength may also vary greatly with time contributing to fluctuations in the atmospheric  $N_2O$  content as recorded in ice cores (Leuenberger and Siegenthaler 1992).

As stated earlier, the Arabian Sea is a region of high  $N_2O$  efflux to the atmosphere; the emission rate is particularly high in the three upwelling centres located off Somalia, Arabia and Southwest India (Bange *et al* 1996; de Wilde and Helder 1997; Naqvi *et al* 1998). Observations made off the southwest coast of India during the summer monsoon of 1995 yielded some of the highest  $N_2O$  levels reported from the sea. At twenty-three stations sampled during SK103 (figure 1) the surface concentrations and saturations were 11.2–62.5  $\mu M$  (mean  $\pm$  s.d. = 28.5  $\pm$  14.7 nM) and 193–953% (458  $\pm$  223%), respectively. These values were associated with intense upwelling as manifested by low sea surface temperatures (minimum 22.8°C) and high  $NO_3^-$  (maximum 16  $\mu M$ ) which are comparable to those observed in the upwelling zones of the western Arabian Sea (Smith and Codispoti 1980; Codispoti *et al* 1996b). The  $^{15}N/^{14}N$  and  $^{18}O/^{16}O$  ratios in surface-water  $N_2O$  measured at eight of these stations together with those from eight open ocean stations are plotted versus percentage saturation in figure 11. The  $\delta^{15}N$  and percentage saturation appear to be negatively correlated, with  $N_2O$  in upwelling waters of the southeastern Arabian Sea being the most depleted in  $^{15}N$ . A few measurements (not included in figure 11) were also made in subsurface waters. The lowest  $\delta^{15}N$  (0.8‰) was recorded at 26 m at 11°51'N, 75°09'E; the corresponding  $N_2O$  concentration and saturation were 115.6 nM and 1709%, respectively. Since this is the lowest  $\delta^{15}N$  ever recorded from the oceanic water column, and because at such high concentrations the signatures of background  $N_2O$  would be almost

<sup>1</sup> One may question the applicability of these experimental results, conducted with a high ammonium concentration, to the substrate-limited natural environments, but it may be noted that  $N_2O$  emitted from the soils is quite depleted in both  $^{15}N$  and  $^{18}O$  (see figure 12).

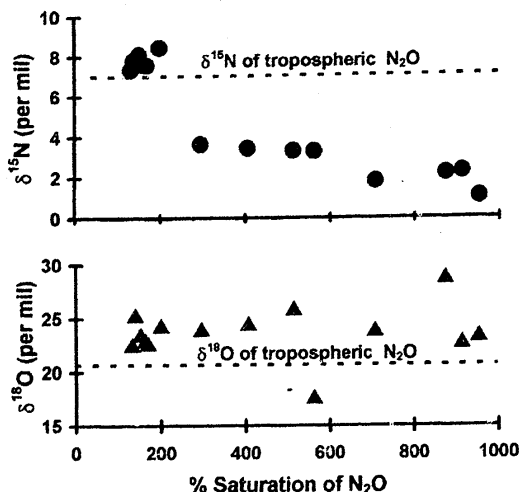


Figure 11. The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{N}_2\text{O}$  as a function of  $\text{N}_2\text{O}$  saturation at sea surface (depth < 5 m). Horizontal dashed lines indicate isotopic composition of  $\text{N}_2\text{O}$  in marine air (Kim and Craig 1990). Values with moderate saturation and  $\delta^{15}\text{N}$  close to the atmospheric value are from the northern Arabian Sea; all others are from the coastal upwelling zone off SW India (modified from Naqvi et al 1998).

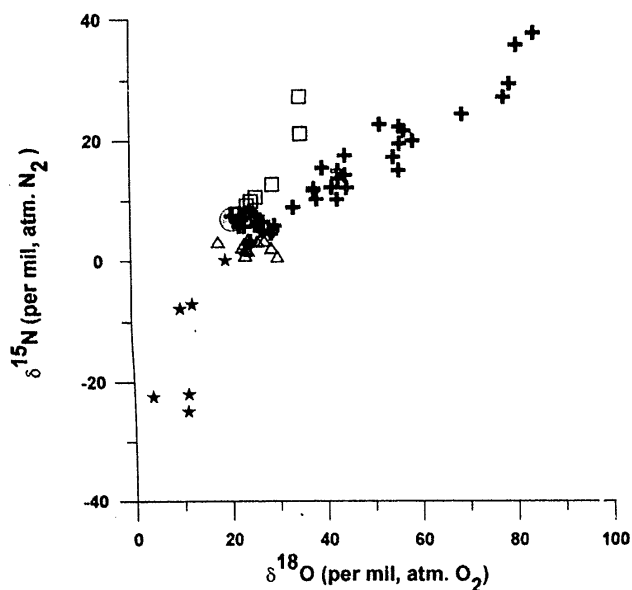


Figure 12. Comparison of isotope data from the Arabian Sea with representative data from other environments (Kim and Craig 1990; Rahn and Wahlen 1997) (stars: soil gas; triangles: upwelling zone off SW India; crosses: northern Arabian Sea; squares: stratosphere; circle: troposphere). Note that the tropospheric value does not fall on vectors joining the other end members (modified from Yoshinari et al 1997; Naqvi et al 1998).

completely erased, it can be taken to represent the end-member composition of  $\text{N}_2\text{O}$  produced in seawater. It is substantially lower than the  $\delta^{15}\text{N}$  of tropospheric  $\text{N}_2\text{O}$ , but much higher than the expected  $\delta^{15}\text{N}$  of  $\text{N}_2\text{O}$  produced through nitrification (Yoshida 1988). In contrast to  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  did not exhibit a

discernible relationship with percentage saturation, and with the exception of one sample, all  $\delta^{18}\text{O}$  values were consistently higher than the  $\delta^{18}\text{O}$  of tropospheric  $\text{N}_2\text{O}$ .

The above observations have an important implication for the atmospheric  $\text{N}_2\text{O}$  budget. Isotopic analysis of stratospheric  $\text{N}_2\text{O}$  at  $68^\circ\text{N}$  has shown large enrichments of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  ( $\sim 21$  and  $35\%$ , respectively) relative to the troposphere (Kim and Craig 1993). This trend has been confirmed by more recent observations at  $33\text{--}48^\circ\text{N}$ , albeit with somewhat smaller enrichments in the lower stratosphere, but the causative mechanism is still not fully understood (Rahn and Wahlen 1997; Cliff and Thiemens 1997; Yung and Miller 1997). However, the magnitude of  $^{15}\text{N}$  and  $^{18}\text{O}$  fluxes ( $\sim 400$  and  $500 \text{ TgN}\% \text{ y}^{-1}$ , respectively, obtained from the older data and about half as much from recent results) are such that these cannot be balanced by air-sea exchange, as proposed (Kim and Craig 1993; McElroy and Jones 1996). Even if, as an extreme case, the lowest observed  $\delta^{15}\text{N}$  value from the ocean ( $\sim 1\%$ ) is combined with the largest estimate of oceanic  $\text{N}_2\text{O}$  production ( $11 \text{ TgN}\% \text{ y}^{-1}$ ) (Capone 1996), the air-sea exchange can counter no more than  $66 \text{ TgN}\% \text{ y}^{-1}$  of the stratospheric  $^{15}\text{N}$  flux. The reported  $^{18}\text{O}$  fluxes are even more problematic since the observed opposite trends of  $^{15}\text{N}$  and  $^{18}\text{O}$  imply that the air-sea exchange will lead to an increase in the tropospheric  $^{18}\text{O}$  inventory. The gravity of this issue is evident from the fact that on a  $\delta^{15}\text{N}$  vs.  $\delta^{18}\text{O}$  diagram constructed with the published data representing various planetary reservoirs of  $\text{N}_2\text{O}$ , the stratospheric composition conspicuously forms a "corner" (figure 12). Thus the tropospheric value, which does not fall on vectors joining the other end-members, cannot be explained by simple mixing between various reservoirs (Kim and Craig 1993). In view of these observations, it is imperative that more measurements be made on  $\text{N}_2\text{O}$  in the stratosphere and that emitted from different terrestrial reservoirs. If the published data are representative, however, then there ought to exist some hitherto poorly known sources and/or sink of  $\text{N}_2\text{O}$  that may be vital for tropospheric isotopic balance (Prasad 1994; McElroy and Jones 1996; Cliff and Thiemens 1997).

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