

# **REPORT ON 209<sup>TH</sup> OCEANOGRAPHIC CRUISE OF O.R.V. SAGAR KANYA**

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## **1. Cruise Summary**

The Arabian Sea is an area where the physical and biological processes are linked on the basin scale such that biogeochemical processes along the Indian coast are affected to very large extent by those in the economic zones of other countries especially Oman and Somalia. An international oceanographic expedition was planned and undertaken by India for the first time involving five scientists from USA and four scientists from the Sultanate of Oman. This enabled the first ever observations within Oman's EEZ by an Indian group. In addition to intensive sampling at several stations located over the Omani shelf, a coast-perpendicular section extending all across the Arabian Sea from the Omani coast towards the Indian coast was worked out for the first time. Such sampling had not been achieved earlier in the Arabian Sea and, along with the data taken on the preceding cruise (SK208), it will allow, for the first time, a comparison of upwelling conditions prevailing along the Omani and Indian coasts during the same southwest monsoon. The specialized investigations undertaken on the cruise at four stations located outside the national exclusive economic zones included innovative experiments to understand the role of trace metals (iron and copper) in redox transformations of nitrogen (especially denitrification), distribution and speciation of these metals, measurement of the rate of denitrification including the anaerobic ammonia oxidation, and characterization of the microbes involved in the denitrification process using advanced molecular techniques.

It is hoped that the expedition will provide an impetus for active collaboration between India and Oman in ocean sciences. It generated enormous interest in Oman and was covered by most of the print media.

## **2. Participants**

### **2.1. Scientific Compliment**

#### *National Institute of Oceanography, Goa*

Dr. S.W.A. Naqvi, Chief Scientist

Dr. P.V. Narvekar

Dr. V.P.C. Rao

Dr. Sugandha Sardesai

Dr. M.S. Shailaja

Dr. D.M. Shenoy

Dr. Hema Naik

Dr. Mangesh Gauns

Mr. H.S. Dalvi

Dr. P.V. Bhaskar

Shri A.K. Pratihary

Shri B.R. Thorat

***National Centre for Antarctic and Ocean Research, Goa***

Shri G. Chandvale  
Dr. Ravi Mishra  
Dr. M.V. Ramesh  
Shri K. Verma  
Shri. D.R. Singh  
Shri V.S.K. Rao

***Princeton University, Princeton, USA***

Dr. D.A. Jayakumar  
Dr. J.J. Rich  
Dr. C.B. Tuit

***Woods Hole Oceanographic Institution, Woods Hole, USA***

Dr. J.W. Moffett  
Mr. T.J. Goepfert

***University of Washington, Seattle, USA***

Ms. Bonnie Chang

***Sultan Qaboos University, Al Khod, Oman***

Dr. Adnan Al-Azri  
Mr. Harib Al-Habsi  
Mr. Salim Al-Khusaibi

***Marine Science and Fisheries Centre, Muscat, Oman***

Mr. Salim Al-Hajri

***NORINCO***

Mr. Biju Nair  
Mr. R.M. Ismail  
Mr. P Boopathy  
Mr. P.J. George

**2.2. Ship's Compliment**

Capt. M.D. Sanap, Master  
Shri K. Pandian, Chief Officer  
Shri B.C. Kalita, Second Officer  
Shri P.P. Rawat, Third Officer

Shri James Jose, Medical Officer  
Shri R.M. Horwood, Radio Officer  
Shri M. Ali, Purser  
Shri R.I. Chougule, Chief Engineer  
Shri V. Muraleedharan, Second Engineer  
Shri Varun Raina, Third Engineer  
Shri T.N. Ulde, Fourth Engineer  
Shri A.K. Singh, Fifth Engineer  
Shri P.K. Patunnayil, Electrical Officer  
Shri A.K. Chenal, Electrical Officer  
Shri A.A. D'Silva, Catering Officer  
Shri A.A. Silveira, Assistant Catering Officer

### **3. Cruise Schedule**

The vessel sailed from Goa on 4<sup>th</sup> September with the Indian and US scientists on board. She arrived at Muscat on 12<sup>th</sup> September. The Omani scientists joined the team at Muscat before the vessel sailed on 14<sup>th</sup> September. She reached Goa on 25<sup>th</sup> September, 2004.

### **4. Objectives**

While the central and eastern parts of the Arabian Sea have been fairly well investigated as a result of numerous surveys carried out by the National Institute of Oceanography (NIO), the available information from the western Arabian Sea is rather sparse. The only recent data from this region are those collected during the JGOFS, but since JGOFS largely focused on the open ocean, only a few of the sampling sites were located over the shelf off Oman. The other major deficiency of the existing data sets is that a zonal section extending from coast-to-coast all across the Arabian Sea has not been worked on any of the cruises undertaken so far. Cruise SK209 was planned to remedy these shortcomings by making observations in the upwelling zone off Omani and compare them with the data taken from the Indian EEZ collected during the previous cruise (SK208). The specific questions to be addressed are as follows:

*4.1. Do suboxic conditions develop at shallow depths (over the shelf) seasonally off Oman, and if so how do they affect the cycling of climatically important gases?*

One unusual characteristic of the perennial suboxic zone of the Arabian Sea is that it is geographically separated from the centres of upwelling both along the eastern and western boundaries. However, suboxia over the shelf has been found to occur seasonally off India. It was not known if a similar feature also existed in the western Arabian Sea; its presence was faintly suggested by the limited data generated during the US JGOFS cruises. On one of the cruises of the *Thomas G. Thompson*, water from a mid-depth smelled of hydrogen sulphide. Moreover, episodes of fish kills occur off Oman and could have been caused by the upwelling of oxygen-poor water.

#### *4.2. What is the relationship of Primary Productivity (PP) with Chlorophyll in the Omani Upwelling Zone?*

There are just a few estimates of PP available from the Omani shelf as the JGOFS observations were largely made in the open ocean. These data suggest rather modest PP (generally under  $1.5 \text{ g C m}^{-2} \text{ d}^{-1}$ ) as well as chlorophyll *a* concentrations (rarely exceeding  $1 \text{ mg m}^{-3}$ ). The PP/Chl *a* ratio appears to be higher than other upwelling zones, and it is possible that the grazing pressure keeps the phytoplankton biomass at relatively low levels. However, this interesting conjecture needed to be verified with additional data, particularly from the shelf.

#### *4.3. Has there been a change in upwelling/productivity in the western Arabian Sea in recent years?*

There have been several reports of recent enhancement of biological production in the oceans including the natural upwelling systems. The last major observations in western Arabian Sea were made roughly a decade ago (during the JGOFS, 1995-96). One of the objectives was to look for a shift in the environmental conditions during this period.

#### *4.4. What does the N-isotopic composition of nitrate tell us about the present and past processes?*

As mass-dependent fractionation of isotopes commonly occurs during various biogeochemical transformations, the natural isotope abundance is a very powerful tool to understand these transformations. For example, preferential reduction of  $^{14}\text{N}$  - which is more abundant than  $^{15}\text{N}$ , the other stable isotope of nitrogen - results in marked enrichment of nitrate in the residual pool. Upwelling of this nitrate is expected to produce organic matter enriched with  $^{15}\text{N}$ . Based on this premise, the nitrogen isotopic composition of organic matter in sediments has been used to reconstruct past changes in denitrification in the Arabian Sea. However, there are some problems with this approach: (a) the water upwelling in the western Arabian Sea is derived from outside the upwelling zone. Therefore, the dissolved nitrate may not be very enriched with  $^{15}\text{N}$ ; (b) Fractionation of isotopes in the upwelled nitrate through photosynthesis, as the upwelled water advects offshore, may produce horizontal gradients in  $^{15}\text{N}/^{14}\text{N}$  (a progressive increase offshore until the surface waters become nitrate deficient). Thus the partial utilization would produce the same signature as denitrification; (c) In the central Arabian Sea, that experiences the most intense denitrification, the  $\delta^{15}\text{N}$  values within the core of the oxygen minimum layer (ca. 200-300 m) reach up to 16 per mil. However, the  $\delta^{15}\text{N}$  of nitrate decreases rapidly across the upper boundary of the suboxic-oxic layer to ~6 per mil in near-surface waters. This indicates inputs of lighter nitrogen close to the surface possibly through nitrogen fixation. As this is indistinguishable from the global average, it is hard to explain the high  $\delta^{15}\text{N}$  in surface sediments. Therefore, more data on the isotopic composition of seawater, suspended material and sediments were needed to understand the dynamics of nitrogen isotopes in this region.

5.5. *What is the rate of denitrification in the Arabian Sea and how much does ANAMMOX contribute to the N<sub>2</sub> production?*

The Arabian Sea is one of the three major oceanic sites where an acute oxygen deficiency within a large body of intermediate waters causes large-scale microbially-mediated reduction of nitrate ions to molecular nitrogen (denitrification), a process of vital biogeochemical significance that keeps the atmospheric nitrogen content constant over geological time scales. Recent attempts to budget combined nitrogen in the ocean reveal an imbalance between the input and sink terms. However, all estimates of water column denitrification, including those from the Arabian Sea, used in current budgets are based on indirect measurements. We have been making direct measurements of denitrification rate in the water based on on-deck and in-situ incubation of water samples spiked with <sup>15</sup>N-labelled nitrate and tracking down the increase in <sup>15</sup>N/<sup>14</sup>N ratio in either N<sub>2</sub> (in samples spiked only with <sup>15</sup>NO<sub>3</sub>) with or N<sub>2</sub>O (samples spiked only with <sup>15</sup>NO<sub>3</sub> in the presence of acetylene). The results are generally consistent with earlier estimates, but for an accurate estimation of the rate, more measurements were needed.

The recently discovered process of anaerobic ammonium oxidation (ANAMMOX) is an additional pathway of N<sub>2</sub> production, the role of which in the open ocean suboxic systems is yet to be evaluated. However, our previous measurements of N<sub>2</sub>/Ar suggest a lot more N<sub>2</sub> than what can be accounted for the nitrate losses. Experiments were planned on this cruise to evaluate the quantitative significance of ANAMMOX in the Arabian Sea. Simultaneously large volumes of water within the OMZ was filtered for the analysis of biomarkers of ANAMMOX bacteria.

5.6. *What is the role of copper in the accumulation of denitrification intermediates in the OMZ of the Arabian Sea?*

Culture experiments suggest that the trace metals such as copper play an important role in regulating redox cycling of nitrogen and trace gas production in the ocean. The activity enzyme nitrous oxide reductase appears to be quite sensitive to the availability and probably speciation of copper. It is possible that the observation that N<sub>2</sub>O accumulates in reducing environments under certain conditions and not in others is related to the changes in the availability of copper. This possibility was investigated on this cruise.

5.7. *What are the relative distribution, abundance, expression and diversity of iron utilizing (nirS) and copper utilizing (nirK) denitrifying bacteria within the Arabian Sea OMZ, with respect to the denitrification rates and speciation of iron and copper?*

As stated above, the availability of trace metals in suitable forms may affect the activity and functions of certain key enzymes. Apart from the N<sub>2</sub>O reductase, the enzymes of special interest are nitrite reductase (NIR), one with copper (NirK) in its active centre and the second,

which requires iron (NirS). An important task to be undertaken during the cruise was to investigate the community structure of the bacterial assemblages in the OMZ in order to evaluate the possible relationship between the diversity and biogeochemistry and also the distribution of the copper type organism versus iron type in relation to the speciation of copper and iron. This involved state of the art molecular biological techniques to investigate relative gene abundance and expression and eventually that would be compared to the denitrification rates in this region and the trace metal speciation.

#### *5.8. How does the iron cycling respond to environmental changes?*

The well developed OMZ within the Arabian Sea has been known to affect the cycling of iron, an important micronutrient. The concentrations of dissolved iron have been known to increase within the OMZ due to reductive mobilization of iron (conversion from oxidation state III to II). But the concentrations of Fe(II) had never been measured. The study of iron speciation was an important component of the work planned for the cruise.

### **5. Sampling and On-board Analysis/Processing**

The cruise was undertaken in two legs (See figure). On the first leg (Mormugoa-Muscat, from 4<sup>th</sup> to 12<sup>th</sup> September), one shallow and three deep stations were worked. However, most of the time was spent at Stations 1 and 2 for process studies dealing with the interactions between trace metals and nitrogen cycling. A piston core was taken from the Murray Ridge at Station 3 before the vessel made a port call at Muscat from 12<sup>th</sup> to 14<sup>th</sup> September.

After leaving Muscat on the second leg, 12 stations were occupied in the Omani waters. Most of these were located over the shelf within the zone of active upwelling along the Omani coast extending to the Ghubbat Sawqirah, where the vessel turned offshore retracing the southern line of the US JGOFS up to 15°N latitude, and then due east up to Goa. A total of 17 stations were located on this line; of this one (Station 23) was for the process studies of the kind carried out at Stations 1 and 2. The vessel arrived at Mormugao on 25<sup>th</sup> September. The data collected on this cruise when combined with those from the preceding one (SK208) constitute the first coast-to-coast data set from the Arabian Sea during the most important upwelling period. Geographical locations of these stations and a summary of various observations made/samples collected are given in the figure and tables.

CTD was deployed at all stations. At all stations, chemical (oxygen, nutrients, nitrous oxide, carbon dioxide, pH and dimethyl sulphide) and biological (chlorophyll) measurements were made on a routine basis.

Multiple plankton net (MPN) was operated at several stations for stratified sampling of zooplankton. The HT net was also used for plankton sampling at selected stations.

Primary productivity was measured through in situ incubations of samples spiked with <sup>14</sup>C at

two stations - one off the Ras al-Hadd and the other in the Ghubbat Sawqirah.

Bacterial production was measured at twenty stations following the  $^3\text{H}$ -thymidine method. At three stations an in situ filtration pump was used for large volume (a few hundred litres) filtration of seawater within the secondary nitrite (also particle) maximum. The filters were deep frozen for analysis of ANNAMOX lipid biomarkers as well as qualitative analysis of bacteria.

### *Copper Experiments*

For copper studies, samples were collected from the core of the OMZ using 30 L GoFlo bottles on a Kevlar rope. The water from the GOFLO was sub-sampled in a flow hood into 10L trilaminate bags, using trace metal clean techniques, by forcing the water out by pressurising the top of the GOFLO outlet with helium. To avoid possible contamination, the GoFlos were not completely drained out into the trilaminate bags, 5L or more sample was always left behind. Prior to the collection the trilaminate bags were flushed with helium twice and then left filled up with He, which was emptied only before subsampling.

Cu experiments were done in Station 1, 2, 17 and 23. Subsamples were collected for nutrients,  $\text{N}_2\text{O}$  and cell counts. At the last time point in some experiments, samples for DNA, Cu speciation and nitrite reductase enzymes were obtained. Subsamples from all bags were collected in 150 or 300 ml evacuated gas flasks for  $^{15}\text{N}$ - $\text{N}_2$  analysis, for denitrification rate measurements. For reference ( $T=0$ ), a similar sample was also taken from the GoFlo bottle. The experiments were carried out in duplicate with  $^{15}\text{NO}_3^-$  in all bags, treatment 1 without Cu, treatment 2 with added Cu and treatment 3 with tetra thiomolybdate. At Station 1 the same three treatments were repeated with carbon addition.

In order to study copper complexation by organic ligands in the OMZ and overlying waters, samples were collected from stations 1, 2, 23 and 27 to determine total Cu, free  $\text{Cu}^{2+}$  and the concentration and conditional stability constants of Cu ligands. These numbers were used to determine whether Cu within the OMZ might be too strongly bound to be biologically available to denitrifiers. The results indicated that free Cu was at or below  $10^{-15}$  M throughout the OMZ. Since the above experiments indicated that rates of  $\text{N}_2\text{O}$  reduction were high and not accelerated by Cu additions, it appears that denitrifiers have a way to access organically complexed Cu. Results for total Cu, and ligand binding constants and concentrations are being generated.



### *Reduced Iron in the OMZ*

Samples were collected from stations 1, 2, 23 and 27 to determine total Fe and Fe(II). Fe(II) measurements, made on board the ship, showed that concentrations ranged from 40 to 800 pM and coincided with the shallow nitrite maximum. Total Fe measurements have since been made, yielding concentrations ranging between 1.0 and 1.4 nM at stations 1 and 2. Fe(II) was up to 80% of the total. Samples were also collected along the Omani coast for total Fe determination.

### *ANAMMOX Experiments*

Stations 1, 2 and 23 were sampled at about 250 m (around the secondary nitrite maximum) for ANAMMOX incubations. Water (5 L) was transferred from Niskin bottles directly into 10 L bags and  $^{15}\text{N}$  and  $^{14}\text{N}$  solutions were added in the following combinations:  $^{15}\text{NH}_4^+$ ,  $^{14}\text{NO}_2^-$ , and  $^{14}\text{NH}_4^+$ ,  $^{15}\text{NO}_2^-$ . There was also a control that did not receive N. The headspace of Niskin bottle was flushed with helium during the transfer of seawater to the bags, except for Station 1. The final concentration of added N was about 10  $\mu\text{M}$  at Station 1 and 5  $\mu\text{M}$  at Stations 2 and 23. The bags were incubated for 5 to 7 days at ambient temperature, where upon seawater from the bags was sampled into 150 or 300 ml evacuated gas flasks for  $^{15}\text{N-N}_2$  analysis. An additional gas sample was taken at 48 hours in the case of Station 23. Nutrients ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ) were monitored throughout the incubation. Ammonium increased by about 5% but no change in  $\text{NO}_3^- + \text{NO}_2^-$  was noticed in Station 1 sample after 6 days. The Station 2 incubation showed exponential decrease in  $\text{NO}_3^-$  and an increase in  $\text{NO}_2^-$  after about three days, with a 50% reduction in  $\text{NH}_4^+$ . After 2 days, there was no change in nutrients in the incubated sample from Station 23; this incubation was sampled for nutrients and  $^{15}\text{N-N}_2$  after 5 day of incubation.

### *Seabed Sampling*

Spade corer was operated at one station off the Ras al Hadd. However, the instrument was unfortunately lost at sea, which prevented sampling for sedimentary respiration measurements. Attempts were made to use the grab available on board for bottom sampling, but out of three attempts sufficient undisturbed sample could be recovered only at one station due to sandy bottom. This sample was incubated for the measurement of sedimentary denitrification rate.

Several attempts were made to collect gravity/piston cores at Station 3 located over the Murray Ridge. Only in one case a core measuring 4.18 m in length could be recovered. It was sectioned at 1-cm intervals up to 3.50 m, and at 2-cm intervals below this level. Another gravity core from 470 m yielded some stones and internal moulds (possibly phosphorites). A second gravity core measuring 5.76 m in length was collected from the deep basin (depth 3920 m). It was also sectioned at 1-cm interval up to 3.50 m and 2-cm intervals below this level.

## **6. Significant Findings**

At several stations over the inner shelf along the Omani coast, O<sub>2</sub> concentrations reached suboxia triggering denitrification in near-bottom waters. This is the first observation of the occurrence of the process in the western Arabian Sea. However, complete denitrification was not observed and the N<sub>2</sub>O concentrations were nowhere as high as those observed over the Indian shelf. The hydrographic conditions including nutrient distribution were comparable to those observed during US JGOFS in August-September 1995. The offshore spreading of the upwelled water maintained high nutrient levels several hundred kilometers from the coast leading to extensive plankton blooms (largely of the dinoflagellate *Noctiluca*). Vertical profiles of Fe(II), measured for the first time in the Arabian Sea, indicated pronounced maximum within the denitrifying zone. The first ever coast-to-coast (trans-Arabian Sea) section during the cruise will enable evaluation of (i) the role of coastal upwelling in influencing processes offshore, (ii) differences between the eastern and western Arabian Sea upwelling systems, and (iii) the change, if any, in the biogeochemical cycling since the region was last surveyed (during 1994-95 as a part of JGOFS).

## **7. Performance of Equipment and General Recommendations**

Most of the equipment used worked quite well throughout the cruise, and the occasional problems with the equipment or deck machinery were fixed by the NORINCO personnel and ship's engineers. The CTD temperature sensor, however, did not work well during the first leg, but a replacement was received at Muscat after which the instrument functioned without any problems.

The autoanalyzer is short of spares, especially the filters that should be procured urgently. The deep freezer in the Wet Starboard Lab is not very efficient, and it needs to be replaced. A new deep freezer capable of giving -80°C is strongly recommended for procurement.

Drainage system from the Chemical Lab and Wet Port Lab needs to be fully refurbished (for the cruise the drainage from the Wet Port Lab was temporarily bypassed).

## **8. Losses**

On 15<sup>th</sup> August, at Station 7 located off the Ras al-Hadd, the spade corer was lowered at a water depth of around 300 m. The depth was quite steady. However, when the corer reached close to the seafloor the depth began to fall very rapidly presumably as the vessel drifted over a shallow feature not marked on the hydrographic chart. The instrument was hauled up quickly, but it probably got entangled with a rock on the seafloor and the cable snapped. This unfortunate loss was not due to any human error, and has been logged as required. While sampling at the Murray ridge, the piston corer hit the hull resulting in a slight deformation of

the top circular fender. However, this did not affect the functioning of the equipment as another core was subsequently taken from the deep seafloor at a depth of about 4 km.

### **9. Acknowledgement**

We, the participants of SK 209 express our gratitude to the Government of the Sultanate to allow us to work in its territorial waters. We express our sincere thanks to the staff of the Indian embassy in Muscat especially Shri Manjit Puri, Minister, who contributed enormously in getting the clearances and made excellent logistic arrangements during the port call. Also acknowledged with thanks are the efforts of Dr. Rajendra Prasad from CSIR Headquarters who was responsible for getting permission from the Government of India and Prof. Saiyed Ahmed, Head of the Department of Marine Science & Fisheries, Sultan Qaboos University, and Dr. Ahmed Al-Mazrooei Director, Marine Science and Fisheries Centre, Oman who worked tirelessly for getting the clearance from the Omani government. We are grateful to Dr. S.R. Shetye, Director, NIO, Dr. P.C. Pandey, Director, NCAOR, and Dr. M. Sudhakar, Director, Vessel Management, for their constant encouragement and support. The Department of Ocean Development kindly sanctioned the cruise for which we sincerely thank its Secretary Dr. Harsh K. Gupta. Finally, this cruise would not have been successful but for the excellent support rendered by the Master, officers and crew of ORV Sagar Kanya.

