

# Reduced iron associated with secondary nitrite maxima in the Arabian Sea

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## Abstract

Dissolved iron and Fe(II) were measured in the oxygen minimum zone (OMZ) of the Arabian Sea in September 2004. The OMZ is a well-demarcated feature characterized by high rates of denitrification, and a deep nitrite maximum coinciding with oxygen levels below  $1 \mu\text{mol L}^{-1}$ . This zone is significantly enriched in dissolved Fe relative to overlying and underlying waters and up to 50% of the dissolved Fe is present as Fe(II). The maxima in Fe(II) are at the same depth as the deep nitrite maxima, centered around 200–250 m. They coincide with a local maximum in total dissolved Fe, suggesting that Fe accumulates at this depth because of the greater solubility of Fe(II) over Fe(III). Fe(II) is thermodynamically unstable even at submicromolar oxygen levels, so active biological reduction is the most plausible source. To our knowledge, this is the first report of a potential link between Fe reduction, elevated dissolved Fe concentrations, and nitrite accumulation within an OMZ. Denitrification has a high Fe requirement associated with the metalloenzymes for nitrate and nitrite reduction, so in situ redox cycling of Fe has important implications for the nitrogen cycle.

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## 1. Introduction

Dissolved iron (Fe) concentrations are generally very low in seawater, with primary production limited by Fe in large regions (de Baar and de Jong, 2001). Concentrations are low because of high biological demand and because the thermodynamically favorable oxidation state, Fe(III), is strongly hydrolyzed at seawater pH and is relatively insoluble (Waite, 2001). In reducing environments, by

contrast, Fe levels are high, because Fe(II) is highly soluble. However, in the presence of the slightest oxygen, Fe(II) is thermodynamically unstable in seawater, and it is rapidly oxidized by oxygen at seawater pH. Nevertheless, there are reports of significant concentrations of Fe(II) in surface waters, particularly in cold waters where oxidation is slow, arising from non-equilibrium processes such as photoreduction (Croot and Laan, 2002; Croot et al., 2001, 2005). The presence of even small amounts of Fe(II) is of great interest because it is more readily assimilated by organisms than Fe(III), which predominantly exists as stable organic complexes and oxides (Sunda, 2001). Reductive processes, both biological and photochemical have been identified as important pathways for the uptake of

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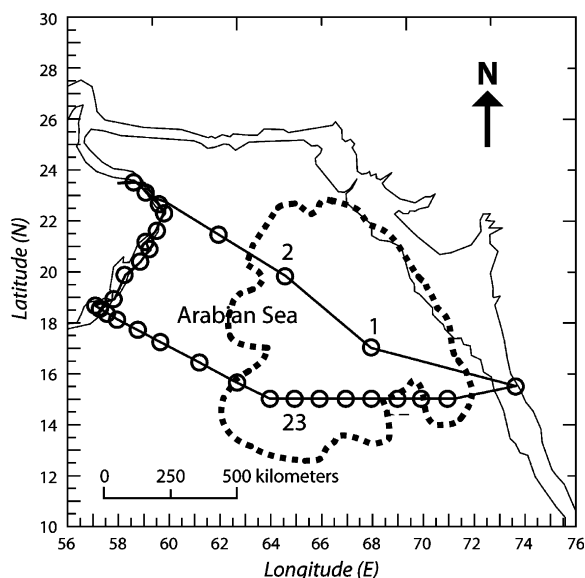


Fig. 1. Map of the Arabian Sea showing Stations 1, 2, and 23 which were sampled for Fe(II). Dotted line demarcates the permanently suboxic zone where nitrite concentrations exceeded  $0.5 \mu\text{M}$  (Bange et al., 2005).

Fe from non-available forms (Sunda, 2001; Maldonado and Price, 2001; Shaked et al., 2004).

The Arabian Sea contains one of the ocean's three major oxygen minimum zones (OMZs) where oxygen levels become low enough (colorimetric  $\text{O}_2 < 1 \mu\text{mol L}^{-1}$ ) for denitrification to occur, but not fully anoxic to support sulfate reduction. Intense monsoon-driven upwelling and vertical mixing sustain high productivity over most of the region almost throughout the year (Barber et al., 2001), which in conjunction with poor oxygen supply to intermediate waters results in the formation of the OMZ. A well-defined portion of the OMZ, characterized by elevated nitrite (the secondary nitrite maximum) and covering a maximal depth range of  $\sim 150\text{--}700\text{ m}$ , exhibits production of  $\text{N}_2$  through denitrification and probably also through anaerobic ammonium oxidation that accounts for at least one-third of the global pelagic  $\text{N}_2$  production rate (Codispoti et al., 2001; Bange et al., 2005). Moreover, while the rate of sedimentary denitrification is 3–4 times that of water column denitrification (Brandes and Devol, 2002; Codispoti, 2006), the latter is still the predominant pathway of  $\text{N}_2$  production in the Arabian Sea (Bange et al., 2005).

Fe chemistry within the OMZ, which regulates the bioavailability of the element, is important because of the high Fe requirement for denitrification

(Milligan and Harrison, 2000). Previous work on Fe in the Arabian Sea revealed an increase in total dissolved Fe concentration within the secondary nitrite maximum (Saager et al., 1989). However, the speciation of Fe was not studied by these workers. We report here results of the first ever measurements of Fe(II) together with those of total dissolved Fe in the region. The observations were made on a cruise of the research vessel *Sagar Kanya* toward the end of the SW monsoon/beginning of fall intermonsoon (September 2004) at two stations in the northern Arabian Sea (1 and 2) and one in the southern Arabian Sea (23), which were all located within the permanently suboxic zone (Fig. 1).

## 2. Methods

### 2.1. Reagents for Fe(II) analysis

Reagents were carefully selected and/or purified to avoid contamination. For Fe(II) analysis, trace metal clean ammonia and hydrochloric acid were prepared via isothermal distillation. A  $4 \times 10^{-3} \text{ mol L}^{-1}$  ferrous iron stock was prepared by dissolving ferrous ammonium sulfate hexahydrate (Fisher S80014) in  $0.2 \text{ mol L}^{-1}$  HCl to prevent oxidation to Fe(III). A  $5 \times 10^{-6} \text{ mol L}^{-1}$  stock was prepared daily from the  $4 \times 10^{-3} \text{ mol L}^{-1}$  stock and adjusted to pH 3 for standard additions. Luminol (Sigma A4685) was used to make a  $5 \times 10^{-4} \text{ mol L}^{-1}$  luminol reagent with  $0.1 \text{ mol L}^{-1}$   $\text{Na}_2\text{CO}_3$  in  $1.0 \text{ mol L}^{-1}$  ammonia with pH adjusted to 11.0 with trace metal grade (SupraPure) sulfuric acid. Reaction pH for this reagent with the seawater matrix and mixing ratio was between 10.3 and 10.5. Occasionally, the reaction pH exceeded an ideal range circa 10.3 (Rose and Waite, 2001) and excessive precipitation resulted; however, frequent flushing and a switch to an artificial saline carrier reduced this issue. Elevated reaction pH did result in larger signals, but also more precipitation. Consequently, it is advisable for future work to carefully optimize the reaction pH such that maximum signal with minimum or no precipitation is obtained; for most sample matrix conditions this range will be as proposed by previous investigations (Croot and Laan, 2002; Rose and Waite, 2001). A fresh luminol solution was prepared 24–72 h before intended analysis to allow for equilibration while minimizing degradation.

Ferrous iron analysis involved standard additions to sample and a plug flow plumbing with trace metal-clean seawater carrier that was collected from

a previous cruise. At Stations 1 and 2,  $\text{Mg}(\text{OH})_2$  precipitation occurred in the flow cell at the high reaction pH; this problem was partly resolved by frequently flushing the system with 10% HCl and Milli-Q water. For Station 23 we used a saline carrier of  $0.7 \text{ mol L}^{-1}$  sodium chloride in  $5 \times 10^{-3} \text{ mol L}^{-1}$   $\text{NaHCO}_3$  at pH 7.0 made with shipboard Milli-Q Water.

## 2.2. Sample collection and shipboard Fe(II) analysis

Samples were collected using acid-cleaned 10 L Teflon-coated General Oceanics Go-Flo bottles deployed on Duravet (a Kevlar substitute) hydro-wire and tripped with PVC messengers. After recovery, the headspace of the Go-Flo bottle was pressurized with  $0.2 \mu\text{m}$  filtered ultra high pure (UHP) nitrogen forcing the seawater through a 142 mm diameter  $0.2 \mu\text{m}$  polycarbonate filter inside a laminar flow bench. All tubing was Teflon, inserted into Teflon compression fittings. All tubing, fittings, and filters were acid cleaned before processing seawater. The filtered sample water was transferred directly to the Fe(II) analytical system with no contact with atmospheric oxygen.

A Waterville Analytical (WA) “FeLume” flow injection analysis (FIA) system with chemiluminescence detection was employed for ferrous iron measurements (King et al., 1995). Samples were run shortly after sample bottle recovery to minimize potential oxidation; typically no more than 20 min from the time the GoFlo was retrieved. Following filtration, the sample was passed directly into a seven-port Savillex Teflon mixing jar purged with UHP nitrogen (Fig. 2). The mixing jar was positioned on a stir plate with a 3 cm stir bar and was plumbed with (1) a pressure balance port which also functioned as an overflow, (2) a filtered sample input, (3) a nitrogen gas purge input, (4) a port for standard additions, and (5) a sub-sample port which drew from the bottom of the jar and ran directly to the FeLume. The standard addition port was closed with a Teflon plug when not in use, as were the remaining two ports. For a given sample the mixing jar was filled to 0.75 L and typical standard additions were 100, 200, 400, and  $800 \text{ pmol L}^{-1}$ , adjusting for volume depletion during the process. The FeLume was fitted with a standard quartz flow cell (circa 2003, WA) and a Hamamatsu HC135 photon counter configured with the following settings: pump speed: 15 rpm; photon counter

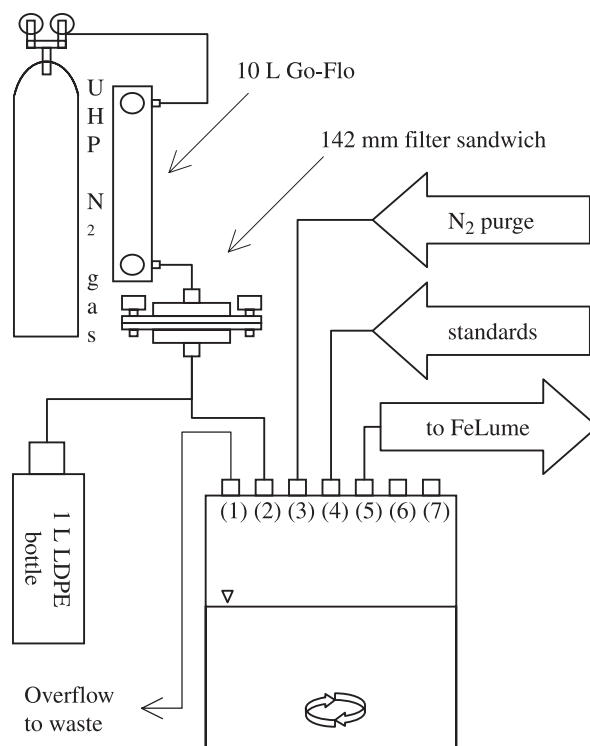


Fig. 2. Oxygen free sample collection and filtration apparatus—sample is purged from Go-Flo with nitrogen gas and passed through a  $0.2 \mu\text{m}$  filter into the sample jar upstream of the FeLume, or directly into sample bottles for sample archival.

integration time: 200 ms; load time: 20 s; No. of data pts.: 100; sample rate: 2.

## 2.3. Standard additions and FeLume settings

The FeLume comes equipped with software from WA that controls the instrument load and injection times as well as other parameters previously outlined. The WA software written in National Instruments™ LabView™ includes data analysis routines and did peak integration on the raw signal data files from standard addition runs.

Standard additions were measured in triplicate (Fig. 3) and intermediate peaks between additions were often not included. Mean values of the integrated peaks, when plotted against Fe(II) were non-linear. The  $x$ -axis intercept of the second-order fit to the peak areas plotted against standard addition values gave the initial ferrous iron concentration (Fig. 4). These calculations were done in Microsoft Excel™. The non-linearity we observed is a characteristic response of chemiluminescence reported by previous investigators (King et al.,

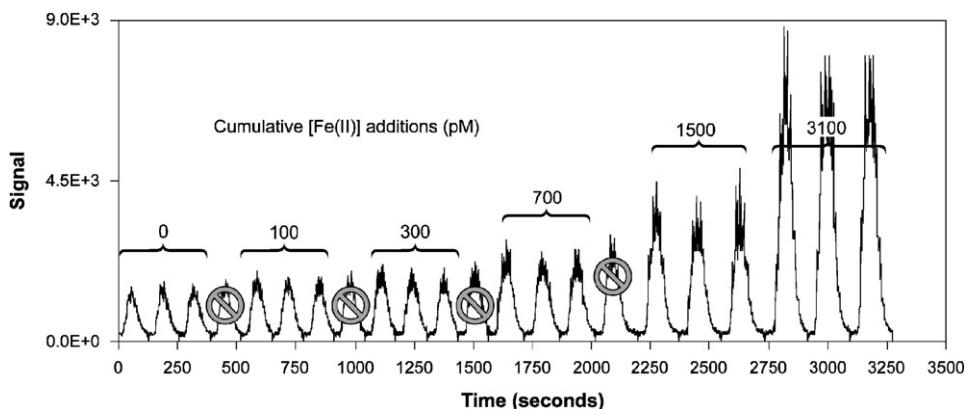


Fig. 3. Data output for a sample showing standard additions of Fe(II). Each data point was run in triplicate.

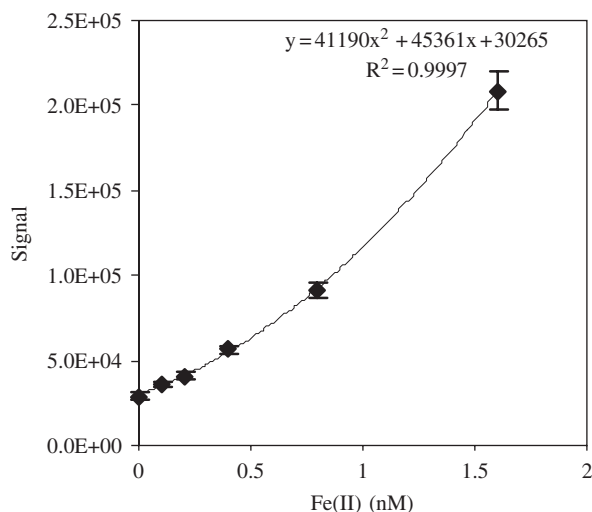


Fig. 4. Peak areas versus added Fe(II). Plots are typically non-linear and are fit using a polynomial.

1995; Rose and Waite, 2001). Detection limits were determined for surface samples where ferrous iron was negligible based on a standard  $3\sigma$  evaluation where the standard deviation of baseline signal is added back to the average of our baseline. This was done for each station and luminol reagent batch and compared with standard additions to determine the minimum detection level. Detection limit values were  $200 \text{ pmol L}^{-1}$  at Station 1, where we had electrical noise problems, but were  $50\text{--}100 \text{ pmol L}^{-1}$  elsewhere, comparable to Hopkinson and Barbeau (2006).

#### 2.4. Total Fe analysis

After Fe(II) sampling, seawater from the Go-Flo bottles was passed through the filter rig and into

0.5 L acid-cleaned conventional polyethylene bottles, stored double bagged and later acidified for total iron analysis at the Woods Hole Oceanographic Institution. The acidified samples were left to equilibrate for several months as it has been found that following acidification sample bottle walls release iron that was adsorbed prior to acidification. Total Fe was determined by ICP-MS analysis using the  $\text{Mg}(\text{OH})_2$  method of Wu and Boyle (1998), modified by Saito and Schneider (2006).

#### 2.5. Nitrite and oxygen

Dissolved oxygen was measured in samples from the CTD casts taken at the same position and within 24 h of the trace metal hydrocasts. Sodium azide was not added to the reagents to suppress the nitrite interference, and Winkler titrations were performed manually. As a result, the oxygen values measured by us in samples from the secondary nitrite maximum were  $1\text{--}2 \text{ } \mu\text{mol L}^{-1}$ . Previous colorimetric, micro  $\text{O}_2$  measurements have shown that the true oxygen concentrations within this zone are  $\sim 0.5 \text{ } \mu\text{mol L}^{-1}$  (Naqvi, 1987; Morrison et al., 1999); so our oxygen values in the low range are probably somewhat overestimated and suffer from slight nitrite interference. Among other nutrients, nitrite was measured in samples from the CTD casts on board ship with an automated nutrient analyzer.

### 3. Results

Hydrographic conditions that existed during our survey were typical of this period. Following vigorous upwelling in the western region and intense

wind mixing throughout the Arabian Sea during the SW monsoon, a transition was taking place to the calmer fall intermonsoon. However, the period was still characterized by widespread phytoplankton blooms. Oxygen concentrations declined rapidly below the mixed layer and shipboard incubations indicated high rates of denitrification, presumably due to high export production (C. Tuit and B. B. Ward, unpublished). Distributions of salinity, temperature, dissolved oxygen, nitrite, Fe(II), and total dissolved Fe are shown in Fig. 5. Dissolved Fe profiles exhibit nutrient-like distribution, indicating strong biological uptake, vertical transport, and enrichment via remineralization at depth. Fe(II) profiles display subsurface maxima which coincide with the secondary nitrite maxima and the maxima

in dissolved Fe. Concentrations of Fe(II) were below our detection limit of  $0.1 \text{ nmol L}^{-1}$  elsewhere, even deeper in the OMZ, where concentrations of oxygen remained low. The high surface value at Station 1, our first measurement, could be associated with photoreduction, but is most likely a contamination artifact since we did not observe a high surface value elsewhere. Note that the maxima in nitrite and Fe(II) were not associated with a distinct water mass. At Station 1, for example, the broad nitrite maximum and the associated Fe(II) maximum covered the salinity maximum of the Persian Gulf Water (PGW) as well as the salinity minimum found just above this feature. In contrast, at Station 2, which was located closer to the PGW source, the nitrite and Fe(II) maxima were narrower

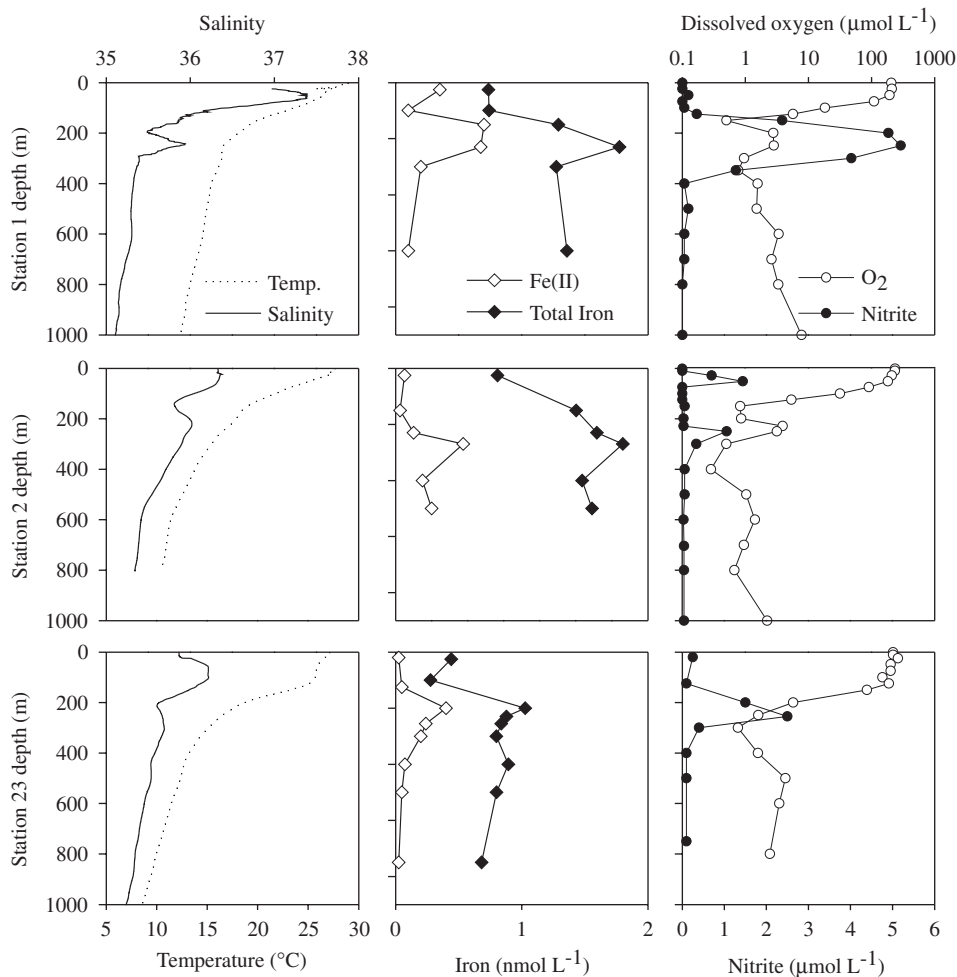


Fig. 5. Hydrographic data and depth profiles of Fe(II), total dissolved Fe,  $\text{O}_2$  and nitrite at Stations 1, 2, and 23. Note slight interference from nitrite in  $\text{O}_2$  data.



and less pronounced and occurred beneath the PGW core layer (Fig. 5).

Within the secondary nitrite maximum, Fe(II) comprised up to 50% of the dissolved Fe. The similarities in location and magnitude of the Fe(II) maxima and the local maxima in dissolved Fe are remarkable. These similarities are not the result of an experimental or computational artifact since the measurements were made in completely different ways. At Station 2, a pronounced primary nitrite maximum was observed at 60 m, presumably due to nitrification. However, it was not associated with detectable Fe(II). This indicates that nitrite did not interfere with the chemiluminescence assay for Fe(II). Follow-up lab work and tests in the Pacific with the same system did not show a nitrite interference, even with additions of several times the environmentally relevant concentrations of this potential interference. Cu(I) was tested in the lab with Sargasso Seawater and did not prove to be of concern. It may be advisable that any future work include confirmation on location of potential signal response to key interferences, particularly, nitrite, V(IV), and Cu(I). Other potential interferences, particularly V(IV), have been ruled out for this methodology in an OMZ in the eastern tropical Pacific with very similar redox characteristics (Hopkinson and Barbeau, 2006). Oxidation of standard additions was notable in surface waters, as well as oxygenated OMZ samples, however, rates were not calculated as has been done by Hopkinson and Barbeau (2006).

All samples reported here were filtered prior to analysis to eliminate concern for clogging valves and tubing. Filters were changed frequently, and their large size (142 mm diameter) makes it unlikely that the signal arises from biological reduction due to cells trapped on the filter as shown by Maldonado and Price (2001).

Hopkinson and Barbeau (2006) reported a single Fe(II) profile in OMZ waters off southern Mexico. Their profile exhibited elevated concentrations within the OMZ but no sharp maximum associated with the secondary nitrite maximum. We do not have an explanation for this, but denitrification rates measured on our cruise were higher than those measured on a cruise off Mexico by the same investigators, following the same technique (C. Tuit, personal communication), so the coupling between chemistry and biology may have been tighter in the Arabian Sea. It is also possible that the sample resolution in Hopkinson and Barbeau (2006) was not sufficient to see a sharp maximum.

Hong and Kester (1986) reported Fe(II) in waters off Peru. However, they determined Fe(II) spectrophotometrically, with a detection limit more than  $100\times$  higher than ours. These investigators found the highest Fe(II) in near-bottom waters over the continental shelf, suggesting a benthic source. The features being reported in our study would be undetectable with their method.

The strong scavenging of Fe from surface waters indicated by the profiles is consistent with measurements made in 1995 as a part of the Arabian Sea Process Study of JGOFS (Measures and Vink, 1999). These data showed a two-fold decrease in surface water Fe over most of the study region from the peak of the SW monsoon (Cruise TN049) to late SW monsoon/beginning of the fall intermonsoon (Cruise TN050). Dissolved Fe concentrations in surface waters at Stations 1 and 2 (Fig. 5) are comparable to those measured on TN050 ( $\sim 0.7\text{ nmol L}^{-1}$ ), but at Station 23, the concentration observed on SK209 was significantly lower relative to TN050. This was possibly because Station 23 was worked near the end of our cruise, and the biological uptake in the water column that had been stratified longer could have further lowered the dissolved Fe level in surface waters.

#### 4. Discussion

The emergence of a local maximum in dissolved Fe at the secondary nitrite maximum probably arises from Fe inputs due to remineralization of sinking particulate matter and/or lateral advection from reducing margin sediments. Although our data do not allow us to evaluate the relative importance of these sources, the high rates of denitrification reported within this OMZ (Bange et al., 2000; Naqvi and Shailaja, 1993; Devol et al., 2006) suggest that in situ remineralization associated with denitrification is a plausible source. The coincidence of the dissolved Fe and nitrite maxima in the Arabian Sea, also observed previously by Saager et al. (1989), does not appear to be fortuitous, and had the Fe maximum been of an advective origin, a similar mechanism should exist for the nitrite maximum as well. Such is evidently not the case, as the perennial open-ocean suboxic zone is not contiguous with the seasonal suboxic zone, which develops over the western continental shelf of India, due to the presence of a relatively oxygenated pole ward undercurrent off the Indian margin (Naqvi et al., 2000, 2006). Thus, denitrification in the Arabian Sea

intensifies offshore (Naqvi, 1991). This situation is different from that existing off Peru where, as stated above, the nitrite-bearing, dissolved Fe-rich zone seems to emanate from the continental shelf. Regardless of source, the presence of soluble Fe(II) must be a major contributor to the maxima. The secondary nitrite maximum in the Arabian Sea is associated with a pronounced nepheloid layer (Morrison et al., 1999; Naqvi et al., 1993) that probably consists of bacteria. That feature, coupled with high fluxes of settling particles characteristic of this region, would probably lead to a shorter residence time for Fe if it was entirely Fe(III).

Dissolved Mn(II) and iodide also exhibit pronounced subsurface maxima coincident with nitrite in the Arabian Sea (Saager et al., 1989; Lewis and Luther, 2000; Farrenkopf et al., 1997). These phenomena have been attributed to the utilization of iodate and Mn(IV) oxides as terminal electron acceptors by heterotrophs, which may also be the case for Fe(III), although it seems unlikely from a thermodynamic perspective. Under typical conditions in the secondary nitrite maximum, where  $[O_2]$  is  $\sim 1 \mu\text{mol L}^{-1}$  and the pH is 7.6, control by the  $O_2/H_2O$  couple yields a pe of 12.5, not much lower than in surface seawater. The concentration of Fe(II) predicted at equilibrium with the  $O_2/H_2O$  couple is  $2 \times 10^{-18} \text{mol L}^{-1}$ ,  $\sim 8$  log units lower than we observed. Clearly, the presence of any oxygen renders Fe(II) thermodynamically unfavorable at this pH. The presence of strong Fe(III) chelators measured previously in the Arabian Sea (Witter et al., 2000) further stabilizes Fe(III) relative to Fe(II), lowering the predicted Fe(II) concentration at equilibrium to  $10^{-22} \text{mol L}^{-1}$ . Calculations assuming pe is controlled by the nitrite/nitrate couple also do not account for the Fe(II) we observed. Therefore, Fe(II) must be maintained by a kinetic process. The rate of Fe(II) oxidation in the OMZ by oxygen was calculated using the rate data of Millero et al. (1987), assuming a pH of 7.6, a temperature of  $16^\circ\text{C}$  and an oxygen concentration of  $1 \mu\text{mol L}^{-1}$ . A pseudo-first-order rate constant of  $1.4 \times 10^{-5} \text{min}^{-1}$  was calculated, corresponding to a half-life of Fe(II) of about 200 h. Although that rate is approximately  $10^5 \times$  slower than at the surface, it would require unreasonably high advective velocities on the order of 2–5 km per h within the OMZ in order for isopycnal transport from reducing sediments to be important. Even if local maxima in total dissolved Fe reflect benthic sources, the redox balance of Fe

must be maintained (for Fe(II) to be a major species) by in situ processes.

While the above considerations argue against Fe(III) being used as a terminal electron acceptor for heterotrophic respiration (especially given its complexation by organic ligands) there is evidence that microbial Fe(III) reduction nevertheless occurs under microaerobic conditions. DiChristina (1992) reported simultaneous reduction of Fe(III), nitrite and nitrate by *Shewanella putrefaciens* grown under microaerobic conditions comparable to the OMZ. McKinzi and DiChristina (1999) showed that the simultaneous reduction of Fe and oxygen by *Shewanella* under low ( $< 10 \mu\text{mol L}^{-1}$ ) oxygen conditions leads to the production of OH radical, a powerful oxidant, via Fenton chemistry. Evidently, oxygen reduction leads to hydrogen peroxide via disproportionation of superoxide. Hydrogen peroxide reacts with Fe(II) to yield OH via the Fenton reaction. These results show that Fe(III) reduction in the presence of oxygen can be important, even if not (apparently) thermodynamically favored. While we do not know if *Shewanella* physiology is representative of denitrifiers or other heterotrophs in the Arabian Sea, it has been referred to by previous workers to account for redox phenomena observed there (Farrenkopf et al., 1997; Lewis and Luther, 2000).

Eukaryotic phytoplankton have been shown to reduce Fe(III) to Fe(II) as an Fe acquisition strategy (Maldonado and Price, 2001). This enables them to convert strongly complexed forms of Fe(III) to labile forms of Fe(II) that are readily taken up via an Fe(II) transport system. In this way, they can access strongly complexed forms of Fe without the benefit of the siderophore production and acquisition mechanisms found in prokaryotes. Fe(III) reduction was measured in the euphotic zone at a station in the Bering Sea (Shaked et al., 2004) at rates of around  $6 \times 10^{-14} \text{mol L}^{-1} \text{min}^{-1}$ . By comparison, we calculated an oxidation rate of  $7 \times 10^{-15} \text{mol L}^{-1} \text{min}^{-1}$  at an Fe(II) concentration of  $0.5 \text{nmol L}^{-1}$ , nearly 10-fold lower, suggesting that an in situ reduction rate substantially lower than in the Bering Sea could still give rise to the features we observed. At present, there is no evidence that denitrifiers utilize a reductase strategy to access Fe. Nevertheless, this is a plausible scenario to account for our findings. Denitrifiers, with their high Fe requirement associated with nitrate reductase and nitrite reductase would benefit from the presence of Fe(II). Moreover, Witter et al.

(2000) showed that Fe(III) was more strongly complexed by organic ligands in the Arabian Sea OMZ than in many other oceanic environments. Excess ligand concentrations were up to  $20 \times$  higher than in overlying surface waters, and conditional stability constant estimates were  $10\text{--}100 \times$  greater as well. Under such conditions, where  $[\text{Fe}^{3+}]$  is very low, a reductase strategy might be more efficient than a siderophore acquisition strategy.

It has been argued that Fe acquisition by reduction is most efficient for large cells, such as large diatoms because the thicker diffusion boundary layers around larger cells minimize the loss of Fe(II) by diffusion to the surrounding media (Sunda, 2001). However, in the OMZ, where Fe(II) oxidizes slowly, production of Fe(II) by a dense microbial community might benefit the entire community, particularly if it is regulated by a quorum sensing mechanism. In that case, reduction rates might be higher when the cell densities of the organism involved reach a certain level. There is an example of siderophore production, induced by Fe stress, being regulated by a quorum sensing mechanism (Stintzi et al., 1998).

Our estimates of the rates of these processes are subject to revision. Previous reports suggest that Fe(II) oxidation at nanomolar concentrations may be retarded by the formation of stable Fe(II) complexes (Millero et al., 1987). While we have no evidence for this, ligands could mean that the dynamics of Fe redox cycling are slower than we have proposed. Conversely, hydrogen peroxide could be the predominant oxidant of Fe(II). Under the prevailing conditions at the OMZ, concentrations of  $\text{H}_2\text{O}_2$  as low as  $1 \text{ nmol L}^{-1}$  could dominate Fe(II) oxidation, leading to more rapid dynamics than our estimates (Moffett and Zika, 1987). While published data show that  $\text{H}_2\text{O}_2$  is negligible below the photic zone,  $1 \text{ nmol L}^{-1}$  is below detection by any current analytical technique.

When concentrations of oxygen in OMZs drop below  $1 \times 10^{-6} \text{ mol L}^{-1}$ , there is a drastic shift in microbial community structure, associated with the development of a diverse, dense population around the secondary nitrite maximum (Naqvi et al., 2003). Our results suggest that this transition is also associated with an abrupt change in chemical regime. Microbial manipulation of the prevailing Fe redox equilibria, leading to pronounced subsurface maxima in total dissolved Fe, has important implications for Fe transport and biogeochemistry in the Arabian Sea. Recently, it has been argued

that Fe limitation may be important in the Arabian Sea during and after the southwest monsoon owing to limited eolian inputs of Fe (Wiggert et al., 2006). Factors controlling Fe transport within the basin are therefore of general interest. These studies suggest that the cycling of Fe and N in the Arabian Sea may be closely linked.

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## References

- Bange, H.W., Rixen, T., Johansen, A.M., Siefert, R.L., Ramesh, R., Ittekkot, V., et al., 2000. A revised nitrogen budget for the Arabian Sea. *Global Biogeochemical Cycles* 14, 1283–1297.
- Bange, H.W., Naqvi, S.W.A., Codispoti, L.A., 2005. The nitrogen cycle in the Arabian Sea. *Progress in Oceanography* 65, 145–158.
- Barber, R.T., Marra, J., Bidigare, R.R., Codispoti, L.A., Halpern, D., Johnson, Z., Latasa, M., Goericke, R., Smith, S.L., 2001. Primary productivity and its regulation in the Arabian Sea during 1995. *Deep-Sea Research II* 48, 1127–1172.
- Brandes, J.A., Devol, A.H., 2002. A global marine-fixed nitrogen isotopic budget: implications for Holocene nitrogen cycling. *Global Biogeochemical Cycles* 16, 1120–1129.
- Codispoti, L.A., 2006. An oceanic fixed nitrogen sink exceeding  $400 \text{ Tg N a}^{-1}$  vs. the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences Discussions* 3, 203.
- Codispoti, L.A., Brandes, J.A., Christensen, J.P., Devol, H., Naqvi, S.W.A., Paerl, H.W., Yoshinari, T., 2001. The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene? *Scientia Marina* 65, 85–105.
- Croot, P.L., Laan, P., 2002. Continuous shipboard determination of Fe(II) in polar waters using flow injection analysis with chemiluminescence detection. *Analytica Chimica Acta* 466, 1–13.
- Croot, P.L., Bowie, A.R., Frew, R.D., Maldonado, M.T., Hall, J.A., Safi, K.A., La Roche, J., Boyd, P.W., Law, C.S., 2001. Retention of dissolved iron and Fe-II in an iron induced Southern Ocean phytoplankton bloom. *Geophysical Research Letters* 28 (18), 3425–3428.
- Croot, P.L., Laan, P., Nishioka, J., Strass, V., Cisewski, B., Boye, M., Timmermans, K.R., Bellerby, R.G., Goldson, L., Nightingale, P., de Baar, H.J.W., 2005. Spatial and temporal distribution of Fe(II) and  $\text{H}_2\text{O}_2$  during EisenEx, an open ocean mesocoscale iron enrichment. *Marine Chemistry* 95 (1–2), 65–88.
- de Baar, H.J.W., de Jong, J.T.M., 2001. Distributions, sources and sinks of iron in seawater. In: Hunter, K., Turner, D. (Eds.), *The Biogeochemistry of Iron in Seawater*, IUPAC



- Series on Analytical and Physical Chemistry of Environmental Systems, vol. 7. Wiley, pp. 123–254 Chapter 5.
- Devol, A.H., Uhlenhopp, A.G., Naqvi, S.W.A., Brandes, J.A., Jayakumar, D.A., Naik, H., Gaurin, S., Codispoti, L.A., Yoshinari, T., 2006. Denitrification rates and excess nitrogen gas concentrations in the Arabian Sea oxygen deficient zone. *Deep-Sea Research I* 53, 1533–1547.
- DiChristina, T.J., 1992. Effects of nitrate and nitrite on dissimilatory iron reduction by *Shewanella putrefaciens*. *Journal of Bacteriology* 174, 1891–1896.
- Farrenkopf, A.M., Luther III, G.W., Truesdale, V.W., Van Der Weijden, C.H., 1997. Sub-surface iodide maxima: evidence for biologically catalyzed redox cycling in Arabian Sea OMZ during the SW intermonsoon. *Deep-Sea Research II* 44, 1391–1409.
- Hong, H., Kester, D.R., 1986. Redox state of Fe in the offshore waters of Peru. *Limnology and Oceanography* 31 (3), 512–524.
- Hopkinson, B.M., Barbeau, K.A., 2006. Organic and redox speciation of iron in the eastern tropical North Pacific suboxic zone. *Marine Chemistry*, in press, doi:10.1016/j.marchem.2006.02.008.
- King, D.W., Lounsbury, H.A., Millero, F.J., 1995. Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentrations. *Environmental Science and Technology* 29, 818–823.
- Lewis, B.L., Luther III, G., 2000. Processes controlling the distribution and cycling of manganese in the oxygen minimum zone of the Arabian Sea. *Deep-Sea Research II* 47, 1541–1561.
- Maldonado, M.T., Price, N.M., 2001. Reduction and transport of organically bound iron by *Thalassiosira oceanica* (Bacillariophyceae). *Journal of Phycology* 37, 298–309.
- McKinzi, A.M., DiChristina, T.J., 1999. Microbially driven Fenton reaction for transformation of pentachlorophenol. *Environmental Science and Technology* 33, 1886–1891.
- Measures, C.I., Vink, S., 1999. Seasonal variations in the distribution of Fe and Al in the surface waters of the Arabian Sea. *Deep-Sea Research II* 46, 1597–1622.
- Millero, F.J., Sotolongo, S., Izaguirre, M., 1987. The oxidation kinetics of Fe(II) in seawater. *Geochimica Cosmochimica Acta* 51, 793–801.
- Milligan, A.J., Harrison, P.J., 2000. Effects of non-steady-state iron limitation on nitrogen assimilatory enzymes in the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae). *Journal of Phycology* 36 (1), 78–86.
- Moffett, J.W., Zika, R.G., 1987. The reaction kinetics of hydrogen peroxide with copper and iron in seawater. *Environmental Science and Technology* 21, 804–810.
- Morrison, J.M., Codispoti, L.A., Smith, S.L., Wishner, K., Flagg, C., Gardner, W.D., Gaurin, S., Naqvi, S.W.A., Manghnani, V., Prosperie, L., Gundersen, J.S., 1999. The oxygen minimum zone in the Arabian Sea during 1995. *Deep-Sea Research II* 46, 1903–1931.
- Naqvi, S.W.A., 1987. Some aspects of the oxygen-deficient conditions and denitrification in the Arabian Sea. *Journal of Marine Research* 45, 1049–1072.
- Naqvi, S.W.A., 1991. Geographical extent of denitrification in the Arabian Sea in relation to some physical processes. *Oceanologica Acta* 14, 281–290.
- Naqvi, S.W.A., Shailaja, M.S., 1993. Activity of the respiratory electron transport system and respiration rates within the oxygen minimum layer of the Arabian Sea. *Deep-Sea Research II* 40, 687–695.
- Naqvi, S.W.A., Kumar, M.D., Narvekar, P.V., de Sousa, S.N., George, M.D., D' Silva, C., 1993. An intermediate nepheloid layer associated with high microbial metabolic rates and denitrification in the Northwest Indian Ocean. *Journal of Geophysical Research* 98, 16469–16479.
- Naqvi, S.W.A., Jayakumar, D.A., Narvekar, P.V., Naik, H., Sarma, V.V.S.S., D'Souza, W., Joseph, S., George, M.D., 2000. Increased marine production of N<sub>2</sub>O due to intensifying anoxia on the Indian continental shelf. *Nature* 408, 346–349.
- Naqvi, S.W.A., Naik, H., Narvekar, P.V., 2003. The Arabian Sea. In: Black, K., Shimmield, G., (Eds.), *Biogeochemistry of Marine Systems*, Sheffield, pp. 156–206.
- Naqvi, S.W.A., Naik, H., Jayakumar, D.A., Shailaja, M.S., Narvekar, P.V., 2006. Seasonal oxygen deficiency over the western continental shelf of India. In: Neretin, L.N. (Ed.), *Past and Present Water Column Anoxia*, NATO Sci. Ser. IV: Earth and Environmental Science, vol. 64. Springer, pp. 195–224.
- Rose, A.L., Waite, T.D., 2001. Chemiluminescence of luminol in the presence of iron(II) and oxygen: oxidation mechanism and implications for its analytical use. *Analytical Chemistry* 73 (24), 5909–5920.
- Saager, P.M., de Baar, H.J.W., Burkill, P.H., 1989. Manganese and iron in Indian Ocean waters. *Geochimica Cosmochimica Acta* 53, 2259–2267.
- Shaked, Y., Kustka, A.B., Morel, F.M.M., Erel, Y., 2004. Simultaneous determination of iron reduction and uptake by phytoplankton. *Limnology and Oceanography: methods* 2, 137–145.
- Stintzi, A., Evans, K., Meyer, J.M., Poole, K., 1998. Quorum-sensing and siderophore biosynthesis in *Pseudomonas aeruginosa*: lasRllasI mutants exhibit reduced pyoverdine biosynthesis. *FEMS Microbiology Letters* 166, 341–345.
- Sunda, W.G., 2001. Bioavailability and bioaccumulation of iron in the sea. In: Hunter, K., Turner, D. (Eds.), *Biogeochemistry of Iron in Seawater*, IUPAC Series on Analytical and Physical Chemistry of Environmental Systems, Vol. 7. Wiley, pp. 41–84 Chapter 3.
- Waite, T.D., 2001. Thermodynamics of Fe in seawater. In: Hunter, K., Turner, D. (Eds.), *Biogeochemistry of Iron in Seawater*, IUPAC Series on Analytical and Physical Chemistry of Environmental Systems, vol. 7. Wiley, pp. 250–284 Chapter 7.
- Wiggert, J.D., Murtugudde, R.G., Christian, J.R., 2006. Annual ecosystem variability in the tropical Indian Ocean: results of a coupled bio-physical ocean general circulation model. *Deep-Sea Research II* 53, 644–676.
- Witter, A.E., Lewis, B.L., Luther, G.W., 2000. Iron speciation in the Arabian Sea. *Deep-Sea Research II* 47, 1517–1539.
- Wu, J., Boyle, E.A., 1998. Determination of iron in seawater by high-resolution isotope dilution inductively coupled plasma mass spectrometry after Mg(OH)<sub>2</sub> coprecipitation. *Analytica Chimica Acta* 267, 183–191.