Nutrient limitation by major and trace metals

Peter Morton, Kristie Dick, Lauren Hearn
National High Magnetic Field Laboratory/Florida State University
Tallahassee, Florida 32310

Sara Rauschenberg
Bigelow Laboratory for Ocean Sciences
W. Boothbay Harbor, ME 04575

Shannon Burns
University of South Florida-St. Petersburg
St. Petersburg, Florida 33701

In waters of the southwest Indian and Southern Oceans, limiting concentrations of major and trace nutrients can inhibit primary productivity and/or influence community composition. During the 2020 SAMW research expedition, uncontaminated seawater samples were drawn from regular deployments of nine Teflon coated 5-L Niskin-X bottles suspended from Kevlar line at varying depths between 30 m and 750 m. The addition of a deck-mounted snatch block and increased weights up to ~160 lbs. proved to be ideal for deploying the Niskin-X bottles, after the ~1600 m of Kevlar line were intentionally cut at about 850 m after the line jumped the A-frame mounted block and became stuck in the block axle (station 3). Subsequent casts using this system experienced no similar issues, even in heavier seas.

In general, unfiltered samples were drawn from each Niskin-X for salinity and major nutrient measurements (ODF). The remainder of the volume was filtered using 47 mm, 0.4 μm pore-size Isopore membrane filters installed in Advantec filter cartridges. The filtrant was stored in unused Millipore petri slides and stored frozen at -20^{o}C. The filtrate was collected into trace metal-cleaned (acid-washed) bottles for dissolved trace metal concentrations (125 mL), barium concentrations and isotopic composition (250 or 1000 mL), and organically complexed iron (500 mL).

As is customary, trace metal analysis will occur at the home institutions under strictly controlled environments to prevent contamination of precious seawater samples.

Dissolved trace metals

Dissolved trace metal samples will be extracted using solid-phase extraction (Nobias Chelate PA-1 or Toyopearl AF-Chelate 650-M) to remove metals from the high salinity matrix and concentrate the metals into a pure nitric acid matrix suitable for direct injection and analysis by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). The analysis will produce total dissolved concentrations of Fe, Mn, Cd, Co, Cu, Ni, and Zn, which describes the standing inventories of bioactive metal nutrients. In addition, dissolved concentrations of Al and Pb can be used as tracers of mineral dust and anthropogenic aerosols, respectively. Based on previous analyses of this region, we expect dissolved Fe concentrations to be sparingly low (<0.2 nM), dissolved Mn to be <0.5 nM unless enriched in surface waters by recent dust deposition events, and

concentrations of the other bioactive elements to be low unless enriched by cross-frontal intrusions of enriched subantarctic waters where surface dissolved trace metal concentrations are much higher than in oligotrophic subtropical waters.
Dissolved barium analysis will be conducted by Julia Middleton at WHOI (see section on Ba isotopic composition).

Organically complexed Fe will be measured by two techniques. Iron-ligand concentrations and binding strengths will be measured at University of South Florida-St. Petersburg by Shannon Burns using competitive ligand equilibrium-adsorptive cathodic stripping voltammetry (CLE-AdCSV) with salicylaldoxime and BASi hanging mercury drop electrode (see section on organic complexation of metals). The analysis will produce organic ligand concentrations and binding strengths of these ligands for dissolved Fe, which provides evidence of active Fe sequestration by Fe-limited phytoplankton or bacteria.

Dissolved Pb, a tracer of atmospheric deposition of industrial pollutants, will be measured at all stations and depths as part of our usual multielement suite. In addition, 1-L samples were collected at the Agulhas Retroflection (station 12) to determine the isotopic composition of Pb, which can be used to qualitatively identify the source of Pb to the region. The Agulhas Current has previously been identified as a pathway for introducing Pb from the Indian Ocean into the south Atlantic Ocean (Paul et al. 2015), but there was insufficient data from the southern Indian Ocean to positively identify the source of Pb carried from the Indian Ocean. Station 12 is located within the Agulhas Current before leaking into the south Atlantic, and the Pb isotopic composition determined in these samples will provide valuable insights into the potential sources of Pb in the Agulhas (South Africa? Long-distance transport from industrial centers in India?).

Particulate trace elements

Particulate trace element samples will be subjected to two primary chemical treatments to determine the labile and refractory fractions of trace elements. First, the filtered samples will be thawed to room temperature, folded twice to contain the suspended particulate material (SPM) in the center of the filter, and submerged into a 25% acetic acid solution with the mild reducing agent hydroxylamine hydrochloride (Berger et al., 2008). This treatment releases biogenic (e.g., soft components of marine microorganisms) and lithogenic phases of trace metals (e.g., Fe and Mn oxides), providing an estimate of the total elemental SPM concentration that easily remineralizes and is likely bioavailable for nutrient recycling. The second treatment involves an aggressive mixture of nitric, hydrochloric, and hydrofluoric acids, that completely breaks down the more refractory mineral (e.g., aerosol dust and resuspended sediments) and biogenic phases (e.g., frustules and tests). When considered together, the concentrations of trace and major elements in each of the labile and refractory fractions, along with their relative ratios (Fe/P, Zn/Si), can be used to tease apart the biogenic, lithogenic, and authigenic composition of marine particles. Using HR-ICP-MS, a suite of elements can be simultaneously detected and their concentrations quantified, including the bioactive trace metals (e.g., Fe and Zn), mineral tracers (e.g., Al and Ti), biominerals (e.g., Ba and Si), and other tracers like P (biology), Pb (coal combustion), and V (diesel fuel combustion).

Vertical Profiles

Dissolved and particulate trace element samples were collected from the Niskin-X bottles at 16 stations, coordinated to complement the regular productivity casts conducted with the ship's CTD rosette system. The trace metal samples drawn from these casts are summarized in the **Station Summary** table below.

Surface sampler: modified otter boat, also known as "Big Jon"

Deckboard incubations

To estimate the potential for major or trace nutrient limitation, four series of nutrient amended whole water incubations were conducted throughout the cruise. Surface whole water was collected from the starboard side of the ship using a clean sampling hose (¾" Bev-a-line and ¾" drinking water hose) kept at sea-surface depth using a modified otter boat ("Big Jon") with a snorkel that extends below and forward of the otter boat itself.
Surface samples were collected with great attention to preventing and minimizing the possibility of accidental trace or major nutrient contamination (especially iron and nitrogen species). All cubitainers, tubing, fittings, pumps, and subsample bottles were acid-washed with dilute hydrochloric acid (~10%) and thoroughly rinsed with UHPW and/or freshly collected seawater. The surface seawater was pumped into the wet lab using an air-operated double-diaphragm Teflon pump (Ingersoll-Rand PD07P-APS-PTT) at ~ 7 L/min into two 200 L acid-cleaned tanks simultaneously through a tee that improved homogeneity of the water in each tank. The cubitainers were filled with 20 L of surface seawater directly from one tank at a time (without use of the tee) using the same Teflon pump, with the hoses configured in reverse. Eighteen cubitainers were filled per experiment and amended in triplicate with one or more nutrients or filtered SAMW, except for a set of Controls which contained only surface seawater.

Nutrient amendment stocks were carefully evaluated before the cruise for unwanted concentrations of either trace metals (especially Fe) or other major nutrients.

Nitrate

The nitrate standard was prepared at sea by dissolving approximately 2.5 g of sodium nitrate salt ($NaNO_3$), carefully weighed and bottled at NHMFL/FSU before the cruise, into ~ 125 mL of UHPW from the Thompson's UHPW system. The solution was then cleaned twice by passing over a small column of Chelex-100 that had been cleaned with freshly prepared 10% HCl (v/v; reagent grade) and thoroughly flushed with UHPW to rinse away residual acid and any associated metals released from the Chelex. The stock nitrate concentration was confirmed by the SIO-ODF team to be free of contamination from other major nutrients (e.g., ammonia or silicate), and preliminary tests at home demonstrated the efficient removal of trace metals like Fe from the stock by using the same Chelex method; nevertheless, the nitrate stock will be reevaluated at NHMFL/FSU for any potential trace metal contamination.

Silicate

Unlike nitrate, solutions of dissolved silicate salts cannot be cleaned of trace metals by Chelex resin, since dissolved silicate naturally produces a solution of pH greater than 12, which is above the effective complexation capacity of Chelex (effective at pH values 2-10). Therefore, a high-purity standard was purchased from Sigma Aldrich, certified for ion chromatography to be free of anionic contaminants such as nitrate. Two 100-mL bottles were ordered and tested for trace metal concentrations before the cruise, and all bioactive trace metal concentrations were found to be sparingly low (exact concentrations to be determined after the cruise in each relevant incubation test).

Iron

The Fe standard used to amend the +Fe and +Fe+Silicate incubations was prepared from an enriched stable isotope stock of Fe-57 (Oak Ridge National Labs) dissolved in dilute UHP HCl (0.3 M or 0.024 M). This standard has been well-calibrated in our lab at NHMFL/FSU to be free of other trace metal impurities and major nutrients, especially nitrate (e.g., from acidification by nitric acid). The use of Fe-57 enriched standard allows us to quantify the partitioning of the amendment between the dissolved fraction (apparent as dissolved Fe-57), the particulate fraction (as taken up by the ambient phytoplankton communities in the incubations), and surface adsorption on to the walls of the cubitainers (based on any differences between the sum of dissolved and particulate pools and the total moles of Fe added to each incubation). The use of an Fe-57 standard also allows us to distinguish between our intentionally added Fe and any accidental contamination of Fe.

Subantarctic Mode Water

Before each incubation experiment, a vertical Niskin-X cast was conducted to both characterize the vertical water column structure and collect subantarctic mode water (SAMW) to simulate an upwelling or mixing event into nutrient-depleted surface waters. SAMW was collected from the depth where the potential density was between 26.5 and 27.1 σ_θ. This Niskin-X bottle was subsampled differently, by using an Acropak-200 capsule filter to rapidly draw 0.2 µm filtered water into three 1-L FEP bottles. Before filling the SAMW cubitainers with surface seawater from the tanks, one of the SAMW FEP subsamples were added to each of the cubitainers, and then the tank water pumped in to thoroughly mix. If the cubitainers are assumed to contain ~20 L total volume, then the addition of 1-L of SAMW would produce a final mixture of ~5% SAMW with 95% surface seawater. While the initial nutrient and salinity concentrations from the Control experiment were assumed to be identical across all the other incubation amendments before they were amended, separate nutrient and salinity samples were drawn for each of the three SAMW amended cubitainers.

Subsampling the incubation cubitainers

Subsamples were drawn directly from the tanks for “t=0” time points, except for trace metal samples which were drawn immediately after each cubitainer was amended.

Thereafter, each cubitainer was subsampled approximately every 24-48 hours for nutrients, DIC/alkalinity, PAM fluorescence, POC/PIC, chlorophyll a, biogenic silica, dissolved and particulate trace metals, dissolved Ba concentrations and isotopes, and/or organic-metal ligand complexes. Trace metal samples were drawn into a 1-L acid-washed FEP Teflon bottle and filtered offline for dissolved trace metals, organically complexed Fe, and dissolved Ba concentrations and isotopes. Additional subsamples were drawn at the first and final time points for DNA/RNA analysis for D. Sturm, where ~200 mL from each of the three replicates was combined to produce a single "average" sample. Preliminary results showed that all features studied by incubation amendments (stations 5, 18, 28, and 56) were responsive to nitrate and the SAMW amendments, based on nitrate drawdown in both amendments and positive responses in photosynthetic efficiency parameters (see section by C. Brownlee on the topic). In contrast, very little effect was observed for amendments of Fe and/or silicate.

Issues and/or concerns

The Thompson UHP water system appeared to be compromised twice during the cruise. While in port in Cape Town, the prefilter resin cartridges and the DiamondPURE internal cartridges were all replaced. Nevertheless, the prefilter resin cartridges changed color from black to red within about one month, indicating that the cleaning capacity of the resin had rapidly been exceeded by the source water. In addition, the UHPW system resistance dropped to less than 15 MOhm-cm, which likely indicated that significant impurities had bypassed the prefilter cartridges and compromised the internal cartridges. A single 125-mL sample was collected to be analyzed at NHMFL/FSU to determine the trace metal concentrations in the compromised water. The two prefilter cartridges and internal cartridge were exchanged again, and the UHPW system resistance returned to the optimal value of 18.2 MOhm-cm.

Since this UHPW was used throughout our shipboard activities – to clean sampling components like tubing and filtration rigs; to rinse the cubitainers, subsampling bottles, and 200-L tanks; and to prepare the nitrate standard – there is the chance that the dissolved and particulate trace metal samples could be contaminated with whatever contamination was exhausting the UHPW system's resin cartridges.

References

Berger, C.J.M., Lippiatt, S.M., Lawrence, M.G., Bruland, K.W., 2008. Application of a chemical leach technique for estimating labile particulate aluminum, iron, and manganese in the Columbia River plume and coastal waters off Oregon and Washington. J. Geophys. Res. 113, C00B01. https://doi.org/10.1029/2007JC004703

Paul, M., Van De Flierdt, T., Rehkämper, M., Khondoker, R., Weiss, D., Lohan, M.C., Homoky, W.B., 2015. Tracing the Agulhas leakage with lead isotopes. Geophys. Res. Lett. 42, 8515–8521. https://doi.org/10.1002/2015GL065625

Station Summary Table

Description ***(units)***	**Stn**	**Fluor max** ***m***	**MLD** ***m***	**dTM** ***n=?***	**pTM** ***n=?***	**dBa** ***n=?***	**FeL** ***n=?***	**PbIC** ***n=?***	**SAMW** ***m***
Test	1	75 (60-90)	50						
	3	45 (30-60)	35	2	2	2			
Incubation A	5	25 (0-40)	35	7	7	7	8		346
	6	50 (0-75)	37	8	8	8			
	7	60 (0-75)	60	8	8	8			
	8	60 (0-75)	60	9	9	9			
Agulhas Retroflection	12	50 (40-80)	60	9	9	9		9	
	17	18 (40-80)	50	9	9	9			
Incubation B	18	80 (60-120)	80	9	9	9	6		524
	20	56 (40-100)	40	9	9	9			
	23	57 (40-90)	40	9	9	9			
Incubation C	28	40 (0-80)	30	8	8	8			450
	39	47 (0-60)	30	8	8	8			
	50	25 (0-40)	30	9	9	9			
Incubation D	56	80 (50-120)	50	9	9	9			365
	70	75 (40-120)	46	9	9	9			

Summary of incubation nutrient amendments

		Std conc	***Final conc***	***Volume to pipette***
White	Control	*n/a*	*n/a*	*n/a*
Blue	Nitrate	222.6 mM	~12 uM	1 mL
Purple	SAMW	*n/a*	5%	1 L
Yellow	Silicate	10.52 mM	~4 uM	6.8 mL (2x 3.4 mL)
Orange	Silicate/Fe	*(+Fe+Si)*	*(+Fe+Si)*	*(+Fe+Si)*
Green	Fe	17 µM	~4 nM	4 mL
		1700 µM	~4 nM	40 µL

Summary of SAMW added to each incubation*

Station	Depth	Temperature (^{o}C)	Salinity (psu)	Density (σ_θ)	Dissolved Oxygen (µmol/kg)	NO$_3$- (µM)	PO$_4^{3-}$ (µM)	H$_3$SiO$_4^-$ (µM)
5	346	9.0264	34.6437	26.8410	210.46	19.33	1.34	7.7
18	524	10.3168	34.7949	26.7482	219.64	17.18	1.21	7.7
28	450	7.3291	34.4768	26.9668	206.29	23.06	1.57	12.8
56	365	10.6209	34.7544	26.6601	210.93	13.15	0.98	4.2

**added at ~5% volume to surface seawater*