Iron ligand concentration in particles (1-51 µm) analyzed by liquid chromatography-mass spectrometry from samples collected on the US GEOTRACES Pacific Meridional Transect (PMT) cruise RR1814 (GP15) on R/V Roger Revelle in October 2018

Website: https://www.bco-dmo.org/dataset/929884
Data Type: Cruise Results
Version: 1
Version Date: 2024-06-18

Project
» Trace Element Organic Speciation along the US GEOTRACES Pacific Meridional Transect (PMT Organic Speciation)

Contributors	Affiliation	Role
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Abstract
This dataset includes iron ligand concentration in particles (1-51 µm) analyzed by liquid chromatography-mass spectrometry. Samples were collected on the US GEOTRACES Pacific Meridional Transect (PMT) cruises (GP15, RR1814 & RR1815) on R/V Roger Revelle from September to November 2018.

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Coverage

Location: GEOTRACES Pacific Meridional Transect (PMT)
Spatial Extent: **N**:51.994667 **E**:-151.99994 **S**:31.999968 **W**:-152.000017
Temporal Extent: 2018-10-01 - 2018-10-13

Methods & Sampling

Sample collection and processing
Suspended particulate organic matter (POM) samples were collected by McLane pumps according to GEOTRACES sampling protocols (Lam et al. 2018). Seawater was pumped through a 51-micrometer (µm) polyester prefilter and a Whatman QMA quartz filter (Cytiva). The particles collected on the QMA filter represent 1-51 µm size fraction of suspended POM. Up to 1500 liters (L) of seawater is pumped through each pair of filters, and we received a subsample equivalent to 1/16 of each QMA filter, representing 50-100 L of seawater.

Filters were frozen (-20 degrees Celsius (°C)) immediately after sample collection and returned to the laboratory for processing. Filters are first extracted with 10 milliliters (mL) MQ under ultrasonication at 35% power for 5 minutes (Branson Ultrasonics, model 102C). The MQ extract was collected and the filter was extracted again with 10 mL distilled MeOH under ultrasonication at 35% power for 10 minutes. Then, the MQ extract and MeOH extract were combined, and diluted with 200 mL MQ. The sample was pumped through a 0.2 µm PES filter capsule (P/N SLGPM33RS, MilliporeSigma) and a Bond-Elut ENV SPE cartridge (1 g, 6 mL, P/N 12255012, Agilent Technologies) that had been previously activated by passing 6 mL each of distilled methanol (MeOH, Optima LCMS grade, Fisher Scientific)

and ultrapure water (qH2O, 18.2 MΩ) through the column.

Columns were thawed and washed with 6 mL qH2O (to reduce salts) and the qH2O wash was discarded. Ligands were then eluted with 6 mL distilled MeOH into acid-cleaned 10 mL polypropylene tubes. A 10 µL stock solution of 2.2 micromoles (µM) Ga-Desferrioxamine-E (Ga-DFOE) was added to each sample as an internal standard. The sample was concentrated to about 500 microliters (µL) by vacuum centrifugation (SpeedVac, Thermo Scientific; 35 °C, 5 hours). A 100 µL aliquot of the sample was taken, mixed with 100 µL of qH2O, and immediately analyzed by LC-MS.

High pressure liquid chromatography-Inductively coupled plasma mass spectrometry
Chromatographic analyses were performed on a bioinert Dionex Ultimate 3000 LC system fitted with a loading pump, a nano pump, and a 10-port switching valve (Li et al. 2021). During the loading phase, 200 µL of sample were withdrawn into the sample loop, then pushed onto a C18 trap column (3.5 µm, 0.5 mm x 35 mm, PN 5064-8260, Agilent Technologies) by the loading pump at 25 µL/min for 10 minutes. The loading solvent is a mixture of 95% solvent A (5 mM aqueous ammonium formate, Optima, Fisher Scientific) and 5% solvent B (5 mM methanolic ammonium formate). During the elution phase, the solvent was delivered by the nano pump at 10 µL/min, and the trap column outflow directed onto two C18 columns (3.5 µm, 0.5 mm x 150 mm, PN 5064-8262, Agilent Technologies) connected in series. Samples were separated with an 80-minute linear gradient from 95% solvent A and 5% solvent B to 95% solvent B, followed by isocratic elution at 95% solvent B for 10 minutes. Meanwhile, the loading pump solvent was switched to 100% qH2O, increased to 35 µL/min and directed as a post column make-up flow, which was infused with the column eluant into the ICPMS. The high aqueous content of the combined flow serves to minimize the effect of changes in solvent composition (in this case increasing methanol content during the analysis) on the detector response to Fe and Ga.

The combined flow from the LC was analyzed using a Thermo Scientific iCAP Q quadrupole mass spectrometer fitted with a perfluoroalkoxy micronebulizer (PFA-ST, Elemental Scientific), and a cyclonic spray chamber cooled to 4 °C (Boiteau and Repeta, 2015). Measurements were made in kinetic energy discrimination (KED) mode, with a helium collision gas flow of 4-4.5 mL/min to minimize isobaric 40Ar16O+ interferences on 56Fe. Oxygen was introduced into the sample carrier gas at 25 mL/min to prevent the formation of reduced organic deposits onto the ICPMS skimmer and sampling cones. Isotopes monitored were 56Fe, 54Fe, 57Fe, 69Ga and 71Ga.

External and Internal Standards
The Fe detector response was calibrated using the siderophore ferrichrome which elutes at ~ 40 minutes in our chromatographic analysis. Stock solutions of 250 µM of ferrichrome were diluted to prepare standards with 2 nM, 5 nM, 10 nM, 20 nM, and 40 nM of the siderophore. Then, 5 µL of 2.2 µM Ga-DFOE was added to 995 µL of each standard. Next, a 100 µL aliquot was taken, mixed with 100 µL of qH2O, and analyzed by LC-ICPMS. A plot of the ratio of Fe-56 (ferrichrome):Ga-69 (Ga-DFOE) peak areas against ferrichrome/Ga-DFOE concentration yields a relationship (r2 ~0.999) between 0.2-4 pmole of ferrichrome. Calibrations and process blanks were made for every 10-20 samples analyzed, with only small changes (RSD~30%) in the slope of the calibration relationship observed over the course of the ~2 years of sample analysis. Concentrations of iron ligands in each sample were measured by plotting the FeL/Ga-DFOE peak area on the most appropriate calibration curve.

High pressure liquid chromatography-Electrospray ionization mass spectrometry
To verify the assignment of Fe-Ls to known siderophores, samples were analyzed by LC-ESIMS. The eluant from the LC, without qH2O infusion, was coupled to a Thermo Scientific Orbitrap Fusion mass spectrometer equipped with a heated electrospray ionization source. ESI source parameters were set to a capillary voltage of 3500 V, sheath, auxiliary and sweep gas flow rates of 5, 2, and 0 (arbitrary units), and ion transfer tube and vaporizer temperatures of 275°C and 20°C. MS1 scans for a m/z range of 150-1900 were collected in high resolution (450K) positive ion mode.

The LC-ESIMS data was converted from raw file format to mzXML (MSconvert, Chambers, Maclean, Burke et al. 2012). The mzXML is imported to Matlab, and aligned with ICPMS data using the retention time of Ga-DFOE, which was obtained by monitoring m/z of 667.26 by ESIMS and 69Ga by ICPMS. Then, the m/z and intensity from each scan are extracted, and ordered by scan number into a scan number/mass (m/z)/intensity matrix, which is then interrogated by mass search algorithms (Boiteau and Repeta, 2015; Li et al. 2021). The algorithms find pairs of co-eluting peaks with a difference of 1.995 amu in m/z and a ratio of 15.7 in intensity, which represent isotopologues of Fe containing complexes.

Instrumentation
We used a Gilson Aspec GX-271 to recover samples from solid phase extraction columns. Extracted samples were reduced in volume using a Thermo/Savant RVT 1505 vacuum centrifuge. Concentrated samples were analyzed by high pressure liquid chromatography using a Dionex Ultimate 3000 (liquid chromatograph) coupled to a Thermo iCap QC inductively coupled plasma mass spectrometer or a Thermo Orbitrap Fusion mass spectrometer fitted with a heated electrospray interface.

Data Processing Description

Data were processed using MSconvert, Proteowizard, and https://github.com/JingxuanJayLi/RepetaLab

(DOI: 10.5281/zenodo.12168798)

BCO-DMO Processing Description

- Imported original file "GP15_Siderophore_POM.xlsx" into the BCO-DMO system.
- Marked "not detected" as a missing data value (missing data are empty/blank in the final CSV file). Samples where values were "not detected" are GT12877, GT12878, GT13399, GT13400.
- Renamed fields to comply with BCO-DMO naming conventions.
- Added the ISO_DateTime_UTC field; removed the original, separate date and time columns.
- Rounded latitude and longitude columns to 6 decimal places.
- Saved the final file as "929884_v1_gp15_iron_ligands_in_particles.csv".

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Data Files

File	
929884_v1_gp15_iron_ligands_in_particles.csv	(Comma Separated Values (.csv), 863 bytes) MD5:333c37cecc0ac1813b21d7b1d342d496
Primary data file for dataset ID 929884, version 1	

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Related Publications

Boiteau, R. M., & Repeta, D. J. (2015). An extended siderophore suite from Synechococcus sp. PCC 7002 revealed by LC-ICPMS-ESIMS. Metallomics, 7(5), 877–884. https://doi.org/10.1039/c5mt00005j
Methods

Chambers, M. C., Maclean, B., Burke, R., Amodei, D., Ruderman, D. L., Neumann, S., … Mallick, P. (2012). A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology, 30(10), 918–920. doi:10.1038/nbt.2377
Software

Lam, P. J., Lee, J.-M., Heller, M. I., Mehic, S., Xiang, Y., & Bates, N. R. (2018). Size-fractionated distributions of suspended particle concentration and major phase composition from the U.S. GEOTRACES Eastern Pacific Zonal Transect (GP16). Marine Chemistry, 201, 90–107. doi:10.1016/j.marchem.2017.08.013
Methods

Li, J. (2024). Code to process HPLC-ICPMS and HPLC-ESIMS data. Zenodo. https://doi.org/10.5281/ZENODO.12168798 https://doi.org/10.5281/zenodo.12168798
Software

Li, J., Boiteau, R. M., Babcock-Adams, L., Acker, M., Song, Z., McIlvin, M. R., & Repeta, D. J. (2021). Element-Selective Targeting of Nutrient Metabolites in Environmental Samples by Inductively Coupled Plasma Mass Spectrometry and Electrospray Ionization Mass Spectrometry. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.630494
Methods

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Parameters

Parameter	Description	Units
Sample	GEOTRACES sample number	unitless
Station	Station number	unitless
Lat	Latitude of sampling event	decimal degrees
Lon	Longitude of sampling event; negative values = West	decimal degrees
Depth	Sample depth	meters (m)
Ferrioxamine_G	Ferrioxamine G concentration	picomolar (pM)
ISO_DateTime_UTC	Date and time (UTC) of sample collection in ISO 8601 format	unitless

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Instruments

Dataset-specific Instrument Name	Dionex Ultimate 3000
Generic Instrument Name	High-Performance Liquid Chromatograph
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Thermo iCap QC inductively coupled plasma mass spectrometer
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
Generic Instrument Description	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

Dataset-specific Instrument Name	Thermo Orbitrap Fusion mass spectrometer
Generic Instrument Name	Mass Spectrometer
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	Gilson Aspec GX-271
Generic Instrument Name	Solid Phase Extraction System
Generic Instrument Description	Solid-phase extraction (SPE) is a solid-liquid extractive technique, by which compounds that are dissolved or suspended in a liquid mixture are separated, isolated or purified, from other compounds in this mixture, according to their physical and chemical properties

Dataset-specific Instrument Name	Thermo/Savant RVT 1505 vacuum centrifuge
Generic Instrument Name	Vacuum centrifuge concentrator
Generic Instrument Description	A centrifuge that includes a vacuum chamber within which a centrifuge rotord is rotatably mounted for spinning a plurality of vials containing a solution at high speed while subjecting the solution to a vacuum condition for concentration and evaporation. Alternative names: sample concentrator; speed vacuum; speed vac.

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Deployments

RR1814

Website	https://www.bco-dmo.org/deployment/776913
Platform	R/V Roger Revelle
Report	https://datadocs.bco-dmo.org/docs/geotraces/GEOTRACES_PMT/casciotti/data_docs/GP15_Cruise_Report_with_ODF_Report.pdf
Start Date	2018-09-18
End Date	2018-10-21
Description	Additional cruise information is available from the Rolling Deck to Repository (R2R): https://www.rvdata.us/search/cruise/RR1814

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Project Information

Trace Element Organic Speciation along the US GEOTRACES Pacific Meridional Transect (PMT Organic Speciation)

Website: https://www2.whoi.edu/site/repetagroup/

Coverage: North Pacific Ocean

NSF Abstract:

In many areas of the ocean microbes live in an environment that has very little of the nutrients they need to grow and thrive. In particular, nutrients with nitrogen (N), phosphorus (P), and iron (Fe), needed for the synthesis of proteins and nucleic acids, are in short supply. Iron is supplied to the ocean by dust blowing off the continents, and in areas remote from land, microbial life is limited by the very low concentrations of iron dissolved in seawater. To extract iron from their surroundings, some microbes synthesize and release organic compounds called siderophores into their

environment. Siderophores are specifically designed to bind iron and transport it back into the cell. But only recently have we had the technology to measure siderophores in seawater, and we do not know where or when they are used, or which microbes are making and using them. The study proposed here is designed to address all of these questions. We will measure siderophores in the Pacific Ocean along a track from Alaska to Tahiti. The distribution of siderophores will be compared with data from other measurements (nutrients, cell numbers, genomics) to understand how microbes are able to live in very low iron environments, and how they can use organic compounds to extract metals from seawater. The study will also allow us test and improve the technology of measuring iron and other metals (mercury, copper, and cadmium, for example) bonded to organic compounds in other environmental samples, such as ground-waters, lakes and rivers, which is important for monitoring the toxicity of metal contaminants.

Nearly all iron dissolved in the ocean is complexed by strong organic ligands of unknown composition. The effect of ligand composition on microbial iron acquisition is poorly understood, but amendment experiments using model ligands show they can facilitate or impede iron uptake depending on their identity. Here we propose to measure the molecular speciation of a suite of bioactive trace element (iron, copper, cobalt, nickel, and zinc) ligands (TE-Ls) in particulate and dissolved organic matter across the US GEOTRACES Pacific Meridional Transect (PMT). We will use high pressure liquid chromatography coupled to inductively coupled plasma mass spectrometry to detect and quantify TE-Ls, and companion electrospray ionization mass spectra to identify and characterize organic ligands. The PMT will cross five different biogeochemical provinces: shelf/slope, subarctic high nutrient/low chlorophyll (HNLC), North Pacific oligotrophic gyre, equatorial HNLC, and South Pacific oligotrophic gyre. The cruise track further intersects at least three different subsurface features, the subarctic and equatorial particle veils, oxygen deficient waters, and mid depth hydrothermal plumes. We expect the unique physical, chemical, and biological properties that characterize these regimes and features will lead to very different TE-L distributions across and down the water column. TE-L molecular speciation measurements will enable us to better integrate datasets of trace element distribution with metagenomic datasets of nutrient-driven changes in microbial metabolism across some of the Earth's major biomes.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736280

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