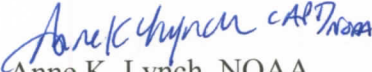




**UNITED STATES DEPARTMENT OF COMMERCE**

National Oceanic and Atmospheric Administration  
NOAA Marine and Aviation Operations  
Marine Operations Center  
439 W. York Street  
Norfolk, VA 23510-1114

MEMORANDUM FOR: Captain Robert Kamphaus, NOAA  
Commanding Officer, NOAA Ship *Ronald H. Brown*

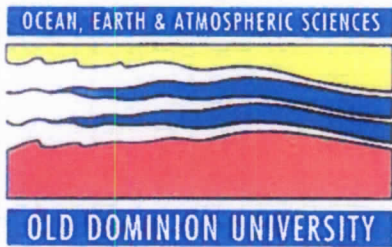
FROM:  Captain Anne K. Lynch, NOAA  
Commanding Officer, NOAA Marine Operations Center-Atlantic

SUBJECT: Project Instruction for RB-16-03  
Eastern Tropical Pacific N2 Fixation

Attached is the final Project Instruction for RB-16-03, Eastern Tropical Pacific N2 Fixation, which is scheduled aboard NOAA Ship *Ronald H. Brown* during the period of March 29 – April 25, 2016. Of the 28 DAS scheduled for this project, 28 days are Other Agency funded (NSF). This project is estimated to exhibit a Medium Operational Tempo. Acknowledge receipt of these instructions via e-mail to [ChiefOps.MOA@noaa.gov](mailto:ChiefOps.MOA@noaa.gov) at Marine Operations Center-Atlantic.

cc:  
Brian Lake





The Department of  
Ocean, Earth & Atmospheric Sciences  
Old Dominion University  
Norfolk, Virginia 23529-0276  
(757) 683-4285  
Fax (757) 683-5303

### FINAL Project Instructions

**Date Submitted:** March 10, 2016

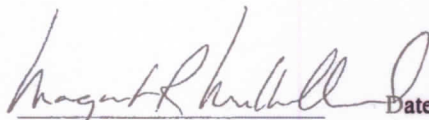
**Platform:** NOAA Ship *Ronald Brown*

**Project Number:** RB-16-03 (OMAO)

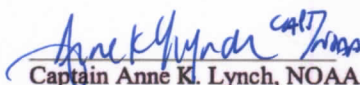
**Project Title:** Eastern Tropical Pacific N<sub>2</sub> Fixation

**Project Dates:** March 29, 2016 to April 25, 2016

Prepared by:

 Dated: 3/10/16  
Dr. Margaret R. Mulholland  
Chief Scientist  
Old Dominion University

Approved by:

 Dated: 3/14/16  
Captain Anne K. Lynch, NOAA  
Commanding Officer  
Marine Operations Center - Atlantic

## I. Overview

### A. Summary

There are three major open ocean oxygen minimum zones (OMZs) in the world: the Eastern Tropical North Pacific (ETNP), the Eastern Tropical South Pacific (ETSP), and the Arabian Sea. OMZs are important areas of denitrification (including anammox) and represent a significant loss of fixed nitrogen (N) from the ocean. However, multiple lines of evidence have recently indicated that N inputs via dinitrogen (N<sub>2</sub>) fixation and denitrification may be more closely coupled in space than previously suggested. Despite the geochemical inferences regarding the location and magnitude of N<sub>2</sub> fixation that might be associated with OMZs and the importance of these regions for removing fixed N from the ocean, it is thought that OMZs do not harbour diazotrophs. In a preliminary study, *nifH* genes and their expression were detected from within the Arabian Sea and ETNP OMZs and active N<sub>2</sub> fixation was measured in the ETNP OMZ, confirming that N<sub>2</sub> fixation occurs in oxygen deficient waters. Here we propose to measure N<sub>2</sub> fixation and the diversity of diazotrophic communities with respect to vertical gradients of oxygen, light, and dissolved nitrogen (N) concentrations. We will compare these detailed vertical profiles with similar profiles made in fully oxic waters adjacent to the OMZs. In addition, we will compare and contrast two very different OMZ regions; one that includes some of the most productive oceanic waters on Earth (ETSP), and another that is far less productive (ETNP). As part of this project, we aim to acquire a better understanding of where N<sub>2</sub> fixation occurs with respect to areas of active denitrification and the microbes involved in these processes. Armed with this more comprehensive understanding of the vertical distribution of N<sub>2</sub> fixation and active diazotrophic communities with respect to OMZ waters, we will garner a more realistic view of the N cycle within these regions and a better understanding of depth integrated rates of N<sub>2</sub> fixation that include oxic and anoxic aphotic waters.

Together, collaborators from Old Dominion University (ODU), Princeton University (PU), the University of Southern California (USC), Monterey Bay Aquarium Research Institute (MBARI), Scripps Institute of Oceanography (SIO), University of Concepcion (UC), and the Universidad Autonoma de Baja California (UABC), we will examine dinitrogen (N<sub>2</sub>) fixation rates, isotopic proxies for N<sub>2</sub> fixation and loss, and *nifH* gene diversity in the context of light, nutrient, iron, and oxygen gradients (and necessarily temperature gradients) along vertical profiles that penetrate into to the eastern tropical North Pacific (ETNP) and eastern tropical South Pacific (ETSP) oxygen minimum zones (OMZs). These oceanic realms have contrasting surface productivity which may control rates of microbial growth and processes at depth. We will compare rates of N<sub>2</sub> fixation and diazotrophic community composition in vertical profiles within the OMZs to those in water masses adjacent to OMZs. Rates will be measured using stable isotope tracer techniques that account for slow gas dissolution and that we have already applied successfully in the ETNP; we will continue to refine those methods as part of this project. We will compare rate measurements of N<sub>2</sub> fixation with the abundance and expression of *nifH* genes and *nirS* genes as a proxy for active denitrification in the region to better understand the juxtaposition of these two processes in association with OMZs.

The overarching questions that we will address as part of this project are:

1. *What is the contribution of diazotrophy to the total productivity in the euphotic zone of the ETP?*

2. Is  $N_2$  fixation occurring in and above the ETP OMZs and if so, how do the rates compare to  $N_2$  fixation rates in euphotic and aphotic  $N_2$  fixation in adjacent oxic waters?
3. How does the community composition of  $N_2$  fixing microbes vary with respect to the vertical gradients of light, oxygen, trace metals, and dissolved inorganic N concentrations with depth?
4. What is the contribution of heterotrophic  $N_2$  fixation to depth integrated  $N_2$  fixation in the ETP both in and adjacent to the OMZs?
5. Is the rate of  $N_2$  fixation ( $N$  inputs) within and above the ETP OMZs enough to partially offset denitrification ( $N$  losses) from these regions?

#### B. Days at Sea (DAS)

Of the 28 DAS scheduled for this project, 0 DAS are funded by an OMAO allocation, 0 DAS are funded by a Line Office Allocation, 0 DAS are Program Funded, and 28 DAS are Other Agency funded (NSF). This project is estimated to exhibit a medium Operational Tempo.

The days are allocated as follows:  
 DEP: 03/29/16 San Diego, CA  
 ARR: 04/25/16 San Diego, CA

#### C. Operating Area (including map)

Operations will take place in the Eastern Tropical Pacific Ocean. If we gain clearances prior to sailing, we will first conduct an along shore transect going southeast along the Mexican coastline (See Figure 1). We will then conduct an East to West transect along 15°N latitude from approximately 99°W to 115°W. We will make a South to North transect along 115°W longitude occupying approximately 6 stations, 1 station per day, starting at approximately 15°N and ending at approximately 25°N. See Figure 1. If clearances are not obtained prior to sailing, we will approximately reverse the cruise track and the coastal transect will be along the Mexican EEZ.

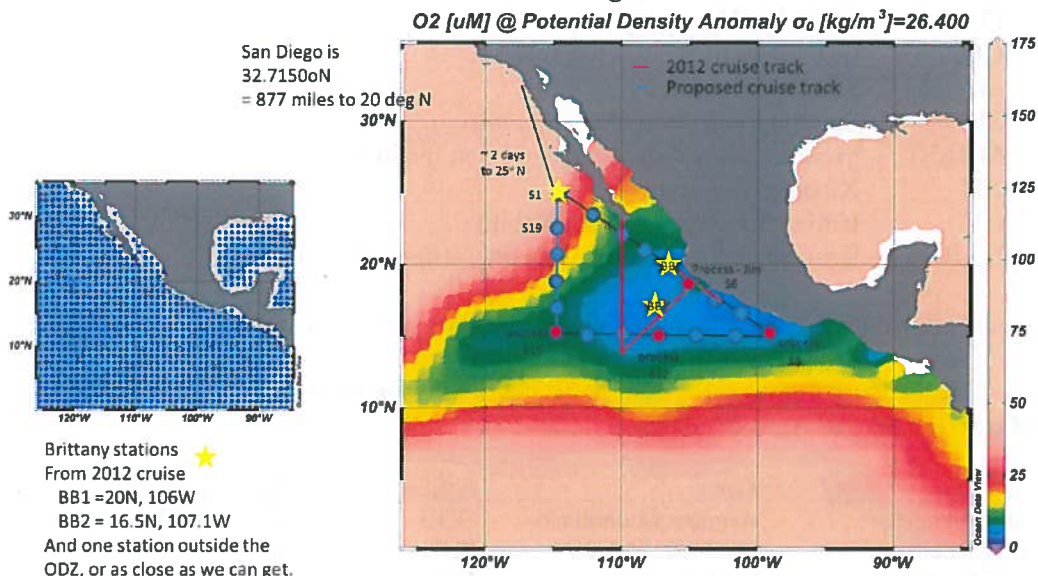


Figure 1. Approximate cruise track with estimated station locations in blue and red circles.

#### D. Summary of Objectives

To measure rates of diazotrophy relative to primary productivity in the euphotic zone and relative to rates of N loss via denitrification and anammox in oxygen deficient waters.

To determine vertical gradients of N<sub>2</sub> fixation and its isotopic proxies with respect to light, nutrients, iron, isotopic distributions, and oxygen.

To determine the dominant N<sub>2</sub> fixers and how these vary with respect to the vertical gradients of light, oxygen, trace metals, and dissolved inorganic N concentrations with depth?

To determine the contribution of heterotrophic N<sub>2</sub> fixation to depth integrated N<sub>2</sub> fixation in the ETP both in and adjacent to the OMZs.

To determine if N inputs via N<sub>2</sub> fixation (N inputs) within and above the ETP OMZs are enough to at least partially offset denitrification (N losses) from these regions.

#### E. Participating Institutions

##### a. Primary

Old Dominion University (ODU)  
Department of Ocean, Earth, and Atmospheric Sciences  
4600 Elkhorn Avenue  
Norfolk, VA 23529-0276  
Telephone: 757-683-3972  
FAX: 757-683-5303

##### b. Additional

PU Princeton University  
USC University of Southern California  
SU Stanford University  
SIO Scripps Institute of Oceanography  
MBARI Monterey Bay Aquarium Research Institute  
XU Xiamen University  
UC University of Concepcion, Chile  
UABC Universidad Autonoma de Baja California, Mexico

#### F. Personnel/Science Party: duty, name, affiliation, gender, and nationality

Table 1: Science Party Personnel. All personnel will be aboard for the entire cruise from March 28, 2016 to April 25, 2016.

Title	Name	Affiliation	Gen.	Nationality
Chief Scientist	Margaret Mulholland	ODU	F	USA
Senior Scientist	Amal Jayakumar	PU	M	USA
Senior Scientist	James Moffett	USC	M	USA
Scientist	Peter Bernhardt	ODU	M	USA
Graduate Student	Brittany Widner	ODU	F	USA
Graduate Student	Shannon Cofield	ODU	F	USA
Graduate Student	Corday Selden	ODU	F	USA

Graduate Student	Wei Yan	ODU/XU	M	PRC
Graduate Student	Qixing Ji	PU	M	PRC
Graduate Student	Xin Sun	PU	F	PRC
Graduate Student	Montserrat Aldunate	UC	F	Chile
Graduate Student	Kenneth Bolster	USC	M	USA
Post-doctoral Researcher	Alejandro Arias	USC	M	Mexico
Graduate Student	Nicole Mayu Travis	SU	F	USA
Graduate Student	Matthew Forbes	SU	M	USA
Graduate Student	Alfonso Macias-Tapia	UABC	M	Mexico
CTD/technical support	Brett Hembrough	SIO	M	USA
PPS/nutrient support	Marguerite Blum	MBARI	F	USA

## G. Administrative

### 1. Points of Contact

Chief Scientist: Dr. Margaret Mulholland  
 Old Dominion University  
 Department of Ocean, Earth and Atmospheric Sciences  
 4600 Elkhorn Avenue  
 Norfolk, VA 23529-0276 USA  
 Telephone: 757-683-3972  
 Fax: 757-683-5303  
 E-mail: [mmulholl@odu.edu](mailto:mmulholl@odu.edu)

Operations officer: Lt. Adrienne Hopper  
[ops.ronald.brown@noaa.gov](mailto:ops.ronald.brown@noaa.gov)  
 (843) 297-1835 OOD Cell

Shipping agent San Diego: NOAA Marine Operations Center, in conjunction  
 with Southwest Fisheries Science Center  
 (SWFSC), La Jolla, CA

#### Project Operation Leads:

CTD:	Brett Hembrough, Scripps
Pump profiler:	Marguerite Blum, MBARI
Nutrients:	Marguerite Blum, MBARI
	Brittany Widner, ODU
DIC/pH:	Marguerite Blum, MBARI
N <sub>2</sub> fixation/primary productivity:	Margaret Mulholland, ODU
Molecular sampling:	Amal Jayakumar, Princeton Univ.
Nitrogen/oxygen isotopes:	Matthew Forbes, Stanford
Nitrite sources/sinks:	Nicole Travis, Stanford
Nitrogen uptake:	Peter Bernhardt, ODU
Prochlorococcus isolation:	Wei Yan, ODU
Taxa specific N uptake:	Montserrat Aldunate, Concepcion
Cyanate/cyanammox:	Brittany Widner, ODU
Denitrification	Qixing Ji, Princeton
Iron/trace metals	Jim Moffett, USC

### 2. Diplomatic Clearances



This project involves Marine Scientific Research in waters under the jurisdiction of Mexico. Diplomatic clearance has been requested via the RATS system through Wendy Bradfield-Smith.

### 3. Licenses and Permits

None.

## II. Operations

The Chief Scientist is responsible for ensuring the scientific staff are trained in planned operations and are knowledgeable of project objectives and priorities. The Commanding Officer is responsible for ensuring all operations conform to the ship's accepted practices and procedures.

### A. Project Itinerary

The ETNP cruise is scheduled for 28 DAS. After departing San Diego on March 29, the vessel will steam south along 115°W. We will occupy our first station on March 31, at approximately 25°N and 115°W. Our first activity on station will be to deploy the CTD/rosette package at approximately 04:45 or 1 hour before dawn. The first CTD/rosette cast will be to approximately 600-800 m. Bottles will be fired to collect water from discrete depths on its transit up. Following water collection to set up incubation experiments and to measure nutrient and other water constituents, the CTD/rosette cast will be deployed again to collect surface and deep water for additional incubation experiments and trace constituent measurements. Subsequent to recovering the CTD/rosette, we will deploy a pump profiling system (PPS) equipped with a pump attached to a CTD/rosette to a maximum depth of 400 m (or less). This system will be deployed with a dedicated winch that will be operated by the science party (Blum). Water will be pumped to collect nutrient and incubation samples from oxygen deficient waters. Following the recovery of the PPS, a trace metal clean rosette equipped with an autofire module will be deployed to obtain trace metal samples. This system will normally be deployed to depths less than 1000m and will have a dedicated winch system to operate it. This will be the general order of operations at subsequent stations. Stations will be approximately 140 nm apart. We will first conduct a NW to SE transect along the Mexican coastline. Nine stations will be occupied along this transect (see Figure 1). Station 6 will be a "process station" and occupied for 2 days (April 5-6). At process stations there will be additional casts to sample deeper water (up to 5000m) and set up incubation experiments. In addition, there will be casts during the night to determine diel periodicity in biogeochemical processes. After Station 6, we will continue to steam Southeast and at Station 9 (at approximately 15°N and 99°W) we will conduct our second process station (April 9-10). After this station, we will conduct steam West along 15°N during which we will occupy an additional 6 stations evenly distributed from 99°W to 115°W. Stations will be equally spaced along that transect. At Station 12 we will conduct our third process station (April 13-14). At the western end of our transect (115°W), we will conduct our final process station (April 17-18). After this station we will steam North along 115°W occupying 4 additional stations along this South to North transect. On April 22 on or before 14:00 we will complete our last station operations to allow time for transit to San Diego. We estimate from our last station at approximately 22.5°N and 115°W that transit time will be on the order of 64 hours (using an estimated cruising speed of 11 knots). During transit we will wrap up experiments, pack gear and clean workspaces. See Appendix A for approximate station coordinates and steam times.

## B. Staging and Destaging

Staging of laboratory vans, equipment and gear for the cruise will be conducted in San Diego on March 25 and March 28. We request access to the ship starting on March 25 at 8:00 am for loading and equipment set-up. We have arranged for a shore crane (Bob's crane) to load the two vans. We will load two 20-foot lab containers onto the ship: a 20-foot container for trace metal work that contains equipment for collecting, extracting and analyzing samples (Moffett, USC) and an empty 20-foot refrigerated container (UNOLS van pool) that will be used to incubate samples. We would like to power up both vans on Friday to make sure they are in good working order and that the cold van is maintaining temperature. In addition, from the UNOLS equipment pool we will load a -80°C freezer, and a CTD/rosette package, a clean-water system (Hembrough, Scripps). We will also load a trace metal clean rosette system with an auto fire module (Moffett, USC) and a pump profiling/CTD system (Blum, MBARI). Both will have their own dedicated winches. They will be loaded with a shore-based crane. On March 28 beginning at 08:00 we will use the ship's crane to load pallet boxes and equipment brought by individual science groups. We will also load gas tanks that we will need to process samples onboard. See Table 2 for a list of items to be loaded, their weight and power requirements (as applicable). Some items can be hand-carried aboard.

Load	Scientist	Weight	Power requirement
Trace Metal Van	Moffett	1 x 10,000 lbs	480 single phase
Cold Storage Van	Hembrough	1 x 14,500 lbs	3 Phase ,460-480V, 20Amp
CTD	Hembrough	1 x 1100 lbs	
Trace Metal Rosette	Moffett	1 x	
Winch	Moffett	1 x 5732 lbs empty	480 V, three phase
Deck incubators	Bernhardt	4 x 100 lbs empty	continuously flowing seawater
Deep Freezer (-80°C)	Hembrough	1 x 280 lbs	110 V
Gas Cylinders	Jayakumar	14 x 80 lbs	none
Gas Cylinders	Moffett	5 x 80 lbs	none
Pump Profiling System	Blum	1 x 1500 lbs	220 single phase with a max spike of 12 amps
Winch	Blum	1 x 1500 lbs	
Pallets	Bernhardt	6-8 x <500 lbs	none
Pallets	Jayakumar	3 x 500 lbs	none
Pallets	Casciotti	2 x 500 lbs	none
Pallets	Blum	2 x 500 lbs	none
Pallet boxes	Hembrough	2 x <500 lbs	none
Pallet	Aldunate	1 x ~500 lbs	none

\*Pump profiling system includes computer, deck box, pump, CTD rosette and winch.

The two vans will be placed on the stern on either side of the centerline. The trace metal winch will be placed on the stern as well, and the PPS system and winch will be secured. Four, 4' x 4' deck incubators will be secured on the port side forward of the vans. They will be equipped with continuously flowing saltwater via a spigot and hoses.



Destaging will also take place in San Diego on April 25 and 26. We anticipate that most of the scientific gear will be off-loaded using a shore crane (for vans) and the ship's crane (for all other gear) on the 25<sup>th</sup> and shipped out that same day or on Tuesday, April 26<sup>th</sup>. Peter Bernhardt will be the point of contact from Old Dominion University to supervise shipping.

### C. Operations to be conducted.

The preliminary personnel task assignments (ship's or scientific personnel) are indicated with each operation. The Chief Scientist and the Commanding Officer will determine final responsibilities. It is of utmost importance to the success of the expedition that the ship be able to hold position at all times during the CTD and pump profiling casts, and that the CTD winch, meter wheel, hydraulic frame, conducting cable and backups function flawlessly during this expedition. Both primary and secondary winches must contain full lengths (10,000 m) of CTD conducting cable in good condition. Skilled ship personnel and adequate spare parts must be available on all legs to assure that this equipment is maintained in good working order. The ship's personnel must be skilled in CTD wire re-terminations, and adequate supplies of materials for CTD wire re-terminations must be available. The CTD/rosette will be deployed off the starboard side. A 24-position rosette system with 10 liter bottles will be used for CTD/rosette casts. This system will be obtained from Scripps (through UNOLS) along with a dedicated technician for operating and maintaining it. A secondary 12-position rosette with 5 liter water bottles will be available (RHB CTD system). The second package must be secured in a readily accessible area, and will be switched when required. A trace metal rosette package and PPS will be deployed separately using dedicated winches and should be readily accessible to quickly transition between their deployments. The trace metal rosette and PPS will be deployed off the stern using the winch provided from WHOI via the UNOLS winch pool. Brett Hembrough from Scripps will provide CTD technical support and Shannon Cofield from ODU will provide deck support for all instrument deployments. Marguerite Blum from MBARI will supervise all PPS deployments and will be assisted by Shannon Cofield or other members of the science party.

Two working winches with conducting cable in good shape (at least one new would be best) will be required for deployment of the CTD. The winch, wire and meter wheel for both winches must be capable of making 5000 meter casts with the rosette system at process stations. NSF and ODU are sending CTD watch leaders (Hembrough, Scripps; and Cofield, ODU), for data processing and quality control. CTD watch leaders will assign science party members to monitor CTD casts. The ship's electronics technician will share responsibility with the scientific party for maintaining good electrical and mechanical connections between the CTD/rosette system, the conducting cable and winch slip-rings, and to the deck unit for the CTD/rosette system. The ship's personnel will be responsible for the deployment and recovery of the CTD/rosette systems and the PPS with assistance of scientific personnel during deployment and recovery (see Tables 1 & 2 above). Many members of the scientific party have experience with CTD deployments and will be available to assist with these operations. Deployments of the trace metal rosette will be supervised by Moffett and deployments of the PPS will be supervised for Blum.

Members of the scientific party will be responsible for collecting the water samples from the rosette. Members of the scientific party will also be responsible for collecting oxygen,

nutrient, salinity and isotope samples and recording sample ID's. Particular care must be taken in the collection and analysis of water samples to assure that all properties are measured with the greatest accuracy possible. The Chief and Co-Chief scientists and CTD data processor in collaboration with the individual science teams will monitor the preliminary data measurements to ensure the highest possible quality. Many of the chemical measurements are sensitive to contamination from smoke, soot, oils, solvents, spray cleaners, lubricants, paints, hydraulic fluid, ospho (rust treatment) and other substances. The Chief Scientist must be notified prior to the use of these substances. Care must be taken to avoid contamination of the rosette system with these substances.

**Smoking is prohibited in the area around the rosettes during sampling and at all times in the laboratories and in and near the staging bay.**

Discharges from holding tanks must be secured 20 minutes before arriving on station. Tanks should not be pumped when CTD packages or the PPS system are deployed. The bridge must inform the ship's engineers in advance when discharges are to be secured. Trace metal casts are particularly sensitive to shipboard activities.

Of particular concern for this study is contamination of water samples with oxygen. Such contamination occurs through exposure to air. We will therefore be particularly sensitive to exposure of water samples to air.

The collection of CTD data relies on using the ship's CTD workstation in the computer room, consisting of a desktop computer with a recent version of Sea-Bird acquisition and processing software installed, one (preferably two) monitors, and Sea-Bird V2 deck unit (and spare) with a NMEA data feed that allows position data to be merged with CTD data. The NMEA interface is designed to decode messages that are output from ship's navigation devices supporting NMEA protocol. The deck unit automatically decodes the messages and appends latitude and longitude to the CTD data stream that is passed to the computer for storage and real-time display with the CTD data. The NMEA interface is setup and maintained by the ship.

*i. General CTD sampling*

Sampling of the rosette bottles (Scientific personnel) will be conducted in accordance with procedures used previously so as to provide the highest quality data. This means that we will avoid contamination and organize sampling so as to sample for gases first followed by nutrient sampling and collection of water for incubation experiments. Most nutrient analyses will be conducted on the ship.

*ii. Profiling PPS*

Because our research is focused on oxygen deficient waters and gradients within oxygen deficient zones, we require depth-specific sampling within narrow ranges. We are fortunate to have Marguerite Blum participating in the cruise from MBARI. She will provide valuable high resolution profiles of nutrient concentrations, dissolved oxygen concentrations, DO, and pH. At the same time, this system can pump water from discrete depths to the surface allowing us to collect samples in real time.

*iii. Trace metal rosette sampling*

Some of our research is understanding the distribution and bioavailability of trace metals (iron in particular). We will have a trace metal clean rosette equipped with an auto fire module that will be deployed daily to collect samples for trace metal analyses. Jim Moffett will supervise those operations.

iv. *Underway sampling*

Underway measurements will be made along the entire cruise track. The ship's seawater line including all branches of the lines to laboratories should be flushed with fresh water and cleaned with bleach prior to departure from San Diego. We will use 'clean' underway seawater system to collect samples concurrently with CTD casts.

v. *Automated Sea surface temperature and salinity (Ship's personnel):*

Sea surface temperature and salinity will be recorded continuously with a system accurate to within 0.02°C and 0.1 PSS-78. A copy of the calibration data will be provided to the Chief Scientist. The thermosalinograph should be calibrated no more than 3 months before the start of the cruise.

vi. *ADCP underway operations (Ship's and scientific personnel):*

Data from the ship's ADCP system will be logged continuously while underway.

D. Dive Plan

All dives are to be conducted in accordance with the requirements and regulations of the NOAA Diving Program (<http://www.ndc.noaa.gov/dr.html>) and require the approval of the ship's Commanding Officer.

Dives are not planned for this project.

E. Applicable Restrictions

See Appendix A for mitigation strategies to deal with delays.

**III. Equipment**

A. Equipment and Capabilities provided by the ship (itemized)

The following communications devices are currently on board RHB and are expected to be in working order. The chief scientist should be apprised at earliest possibility of malfunction of equipment.

- a. High Frequency SSB (SEA 330): SEA Inc. 300-watt high frequency transceiver. The transceiver covers a frequency range from 1.6 to 29.9 MHz
- b. Furuno Global Maritime Distress and Safety System (GMDSS)
- c. Satellite communication system (INMARSAT, -B, -M)
- d. Five fixed VHF radios with eight channels pre-programmed with a selection of marine band and NOAA frequencies.
- e. Cell phones

The electronic instrumentation used for navigation includes:

- f. Furuno Navigator GP-150 GPS
- g. Applanix POSMV GPS
- i. Meridian Commercial Gyro Compass SG Brown
- j. Two Furuno FAR 2xx7 Series Marine RADAR(S-band (10 cm) 30 kW radar and an X-band (3 cm) 25 kW radar)
- k. Kongsberg K-POS Dynamic Positioning System
- l. Raytheon model DSN-450 Doppler Speed/distance log
- m. NAVTEX receiving and printing the international automated medium frequency (518 KHz) weather warnings
- n. Weather maps: Medium frequency/high frequency

Ship's scientific equipment:

- o. Echo Sounder (Ocean Data Equipment Corporation (ODEC) Bathy 2000 or the Knudsen system) used in 12 kHz mode to be used while on CTD station. This will be resolved with SST and ETs prior to cruise
- p. Continuous EM122 multibeam swath bathymetric sonar system sampling while underway between stations.
- q. Barometer
- r. WOCE IMET sensors
- s. Hydrographic Winch system and readouts (using 10 km of 0.322 conducting capable for CTD operations).
- t. One backup hydrographic winch system for CTD operations with 5 km of 0.322 conducting cable.
- u. Hull mounted acoustic Doppler current profiler (RD Instruments (RDI), 75 kHz Ocean Surveyor acoustic Doppler current profiler) with gyro input.
- v. MAHRS gyro system for acquisition of heading data used by acoustic Doppler current profiler.
- w. Seapath GPS system for acquisition of heading data for testing the new MAHRS system.
- x. A 12-position, 5-liter bottle CTD rosette

**B. Equipment and Capabilities provided by the scientists (itemized):**

Two container vans will be loaded aboard RHB for this cruise. One of these containers will act as laboratory van (trace metal clean), and must be accessible at all times throughout the expedition. Compressed gas (non-flammable) cylinders will be used in this van and the ship's laboratories. The other van will serve as a cold incubator and should be accessible at all times throughout the expedition.

Extensive instrumentation to measure a variety of biogeochemical parameters in ocean water and atmosphere will be deployed during the cruise as detailed in Appendix B. In addition to the suite of oceanographic and meteorological instruments on board RHB, the science party will bring the following instruments and materials on board (in addition see Appendix B for full specifications):

- a. One 24 position rosette with 10 liter Niskin water sampling bottles and spare parts.
- b. Complete CTD recording and processing system (Sea bird 911+), sensors, spare parts, connectors, spare parts and consumables.

- c. Chemical analysis instrumentation including gas chromatographs, equilibrators, 2 autoanalyzers, fluorometers, spectrofluorometers, titrator, and spectrophotometers.
- d. Chemical reagents, compressed gases (approximately 30 cylinders), and liquid N<sub>2</sub> (4 dewars). A listing of chemicals is given in Appendix B.
- g. Milli-Q system, and replacement parts
- h. A -80°C freezer for molecular sample storage.
- i. Disposables for sample collection and storage.
- j. A pump profiling system comprised of a compact CTD rosette with 12 2-L Niskin bottles which is attached to the pump cable and with a dedicated winch and associated autoanalyzer and sensors. The pump system uses a 220 single phase plug. The weight of the winch is ~1500 lbs. The winch package is 49"x49"x53" and the aluminum cage has feet with holes 48" on the center. The start-up amps are less than 12 and it runs at 6 amps. There is also a deck box and computer to run the CTD.
- k. A trace metal rosette equipped with an auto fire module.
- l. A 480V three phase winch and block from the UNOLS winch pool that bolts onto standard 24" spaced deck bolts.  
The winch is a MacArtney model MASH4000; Serial number: H11152 V35480-2; WHOI property tag number: 114786; with an empty weight of 5732 lbs; dimensions are 66.9"x 102.4"x80.7"; drum diameter is 20.1"; roller diameter of 11.8"; drum width of 46.6"; flange diameter of 39.6"; with tension and payout monitoring and remote control.
- m. 4500m of 1/4" Amsteel
- n. 4 deck incubators

#### IV. Hazardous Materials

##### A. Policy and Compliance

The Chief Scientist is responsible for complying with FEC 07 Hazardous Materials and Hazardous Waste Management Requirements for Visiting Scientific Parties (or the OMAO procedure that supersedes it). By Federal regulations and NOAA Marine and Aviation Operations policy, the ship may not sail without a complete inventory of all hazardous materials by name and quantity, MSDS, appropriate spill cleanup materials (neutralizing agents, buffers, or absorbents) in amounts adequate to address spills of a size equal to the amount of chemical brought aboard, and chemical safety and spill response procedures. . Documentation regarding those requirements will be provided by the Chief of Operations, Marine Operations Center, upon request.

Per OMAO procedure, the scientific party will include with their project instructions and provide to the CO of the respective ship 30 days before departure:

- List of chemicals by name with anticipated quantity
- List of spill response materials, including neutralizing agents, buffers, and absorbents
- Chemical safety and spill response procedures, such as excerpts of the program's Chemical Hygiene Plan or SOPs relevant for shipboard laboratories
- For bulk quantities of chemicals in excess of 50 gallons total or in containers larger than 10 gallons each, notify ship's Operations Officer regarding quantity, packaging and chemical to verify safe stowage is available as soon as chemical quantities are known.

Upon embarkation and prior to loading hazardous materials aboard the vessel, the scientific party will provide to the CO or their designee:

- An inventory list showing actual amount of hazardous material brought aboard
- An MSDS for each material
- Confirmation that neutralizing agents and spill equipment were brought aboard sufficient to contain and cleanup all of the hazardous material brought aboard by the program
- Confirmation that chemical safety and spill response procedures were brought aboard

Upon departure from the ship, scientific parties will provide the CO or their designee an inventory showing that all chemicals were removed from the vessel. The CO's designee will maintain a log to track scientific party hazardous materials. MSDS will be made available to the ship's complement, in compliance with Hazard Communication Laws.

Scientific parties are expected to manage and respond to spills of scientific hazardous materials. Overboard discharge of hazardous materials is not permitted aboard NOAA ships.

#### B. Inventory (itemized)

Appendix A provides the inventory by group.

#### C. Chemical safety and spill response procedures

##### **A: ACID**

- Wear appropriate protective equipment and clothing during clean-up. Keep upwind. Keep out of low areas.
- Ventilate closed spaces before entering them.
- Stop the flow of material, if this is without risk. Dike the spilled material, where this is possible.
- **Large Spills:** Dike far ahead of spill for later disposal. Use a non-combustible material like vermiculite, sand or earth to soak up the product and place into a container for later disposal.
- **Small Spills:** Wipe up with absorbent material (e.g. cloth, fleece). Clean surface thoroughly to remove residual contamination.
- Never return spills in original containers for re-use.
- Neutralize spill area and washings with soda ash or lime. Collect in a non-combustible container for prompt disposal.
- J. T. Baker NEUTRASORB® acid neutralizers are recommended for spills of this product.

##### **M: Mercury**

- Spills: Pick up and place in a suitable container for reclamation or disposal in a method that does not generate dust. Sprinkle area with sulfur or calcium polysulfide to suppress mercury. Use Mercury Spill Kit if need be.

##### **F: Formalin/Formaldehyde**

- Ventilate area of leak or spill. Remove all sources of ignition.
- Wear appropriate personal protective equipment.

- Isolate hazard area. Keep unnecessary and unprotected personnel from entering. Contain and recover liquid when possible.
- Use non-sparking tools and equipment. Collect liquid in an appropriate container or absorb with an inert material (e. g., vermiculite, dry sand, earth), and place in a chemical waste container.
- Do not use combustible materials, such as saw dust.

An inventory of spill kit supplies is included in Appendix C.

#### D. Radioactive Materials

No Radioactive Isotopes are planned for this project

### V. Additional Projects

#### A. Supplementary (“Piggyback”) Projects

None

#### B. NOAA Fleet Ancillary Projects

No NOAA Fleet Ancillary Projects are planned.

### VI. Disposition of Data and Reports

Disposition of data gathered aboard NOAA ships will conform to NAO 216-101 *Ocean Data Acquisitions* and NAO 212-15 *Management of Environmental Data and Information*. To guide the implementation of these NAOs, NOAA’s Environmental Data Management Committee (EDMC) provides the *NOAA Data Documentation Procedural Directive* (data documentation) and *NOAA Data Management Planning Procedural Directive* (preparation of Data Management Plans). OMAO is developing procedures and allocating resources to manage OMAO data and Programs are encouraged to do the same for their Project data.

#### A. Data Classifications: *Under Development*

a. OMAO Data

b. Program Data

#### B. Responsibilities: *Under Development*

Individuals in charge of piggyback projects conducted during the cruise have the same responsibilities for their project's data as the Chief Scientist has for primary project data. All requests for data should be made through the Chief Scientist.

The PIs in the project are well aware of the needs for sharing of scientific information and will include multiple activities to provide unrestricted access for other researchers and the public as final results are available. Information generated by this project will be made available to other investigators through the NSF preferred archival arrangements. The project will yield valuable data



distribution, abundance and activity of diazotrophs in and above two major oxygen minimum zones (OMZs) in the Eastern Tropical North Pacific (ETNP) and the Eastern Tropical South Pacific (ETSP). We will generate environmental and genetic data as part of this study. Environmental data include salinity, temperature, pH, concentrations of dissolved oxygen and dissolved nutrients (nitrate, nitrite, ammonia, phosphate and silicate), chlorophyll *a*, pigment concentrations and particulate N and C concentrations. PIs will submit all the physical, chemical and biological data collected through this project to the designated National Data Centers (<http://www.nodc.noaa.gov/>) within the time frame suggested by NSF. These data will have different formats and will be made available through different channels. We will also work with the staff of the Biological and Chemical Oceanography Data Management Office (BCO-DMO, see below) to manage our data and data generated during this project. BCO-DMO staff will provide additional assistance to coordinate interactions with other repositories that are natural locations for archival and access of some of our expected data (e.g., NCBI, Genbank, CAMERA). We will discuss details associated with each of our data types and define protocols for producing appropriate data format, documentation of quality control, and metadata with BCO-DMO before our field campaign. **We have now registered the project with BCO-DMO and data resulting from the project will be submitted to BCO-DMO in accordance with the NSF OCE Data Policy. Both the revised plan and the Project metadata form have been sent to [info@bco-dmo.org](mailto:info@bco-dmo.org).**

Molecular data include the DNA sequences of target genes. All DNA sequence information will be released as soon as possible to the scientific community. Raw sequence data will be released monthly to the NCBI Sequence Read Archive (SRA, [www.ncbi.nlm.nih.gov/Traces/home](http://www.ncbi.nlm.nih.gov/Traces/home)) and the CAMERA database (<http://camera.calit2.net/>) as this site provides access to sequence data including rich metadata from diverse habitats. We have not proposed to generate any genome assemblies under this project, however if we do any generate any such information based on this project will be released to the international sequence databases following NSF guidelines. We are committed to providing complete and open access to our sequence data.

Recently, a database of diazotrophs in the global ocean was created to collect data on the abundance of diazotrophs, their biomass, and nitrogen fixation rates from the global ocean (Luo et al. 2012). We will contribute data generated as part of this project to that database. The database is stored in PANGAEA (doi: 10.1594/PANGAEA.774851).

All the data will be stored in PI's computers and backed up automatically on a daily basis. Results will be made available through multiple outlets, including PI websites, which have links to presentations and publications and contact information for specific requests. PIs will meet annually to discuss research progress including data sharing activities. Annual reports will include the progress on the dissemination of data and research products.

Mulholland will oversee data management for the environmental data and Jayakumar will oversee data management for gene and transcript sequence data.

*Biological and Chemical Oceanography Data Management Office (BCO-DMO)*

BCO-DMO was created in late 2006 to serve PIs funded by the NSF Geosciences Directorate (GEO) Division of Ocean Sciences (OCE) Biological and Chemical Oceanography Sections and (with augmented funding in 2010) Office of Polar Programs (OPP) Antarctic Sciences (ANT). BCO-DMO manages and serves oceanographic biogeochemical, ecological, and companion physical data and information developed in the course of scientific research and contributed by the originating investigators. The BCO-DMO data system facilitates data stewardship, dissemination, and storage on short and intermediate time-frames. See <http://bco-dmo.org> for Data, Resources and Contact information.

**VII. Meetings, Vessel Familiarization, and Project Evaluations**

A. Pre-Project Meeting:

The Chief Scientist and Commanding Officer will conduct a meeting of pertinent members of the scientific party and ship's crew to discuss required equipment, planned operations, concerns, and establish mitigation strategies for all concerns. This meeting shall be conducted before the beginning of the project with sufficient time to allow for preparation of the ship and project personnel. The ship's Operations Officer usually is delegated to assist the Chief Scientist in arranging this meeting.

B. Vessel Familiarization Meeting:

The Commanding Officer is responsible for ensuring scientific personnel are familiarized with applicable sections of the standing orders and vessel protocols, e.g., meals, watches, etiquette, drills, etc. A vessel familiarization meeting shall be conducted in the first 24 hours of the project's start and is normally presented by the ship's Operations Officer.

C. Post-Project Meeting:

The Commanding Officer is responsible for conducted a meeting no earlier than 24 hrs before or 7 days after the completion of a project to discuss the overall success and short comings of the project. Concerns regarding safety, efficiency, and suggestions for future improvements shall be discussed and mitigations for future projects will be documented for future use. This meeting shall be attended by the ship's officers, applicable crew, the Chief Scientist, and members of the scientific party and is normally arranged by the Operations Officer and Chief Scientist.

D. Project Evaluation Report:

Within seven days of the completion of the project, a Customer Satisfaction Survey is to be completed by the Chief Scientist. The form is available at <http://www.oma.noaa.gov/fleeteval.html> and provides a "Submit" button at the end of the form. Submitted form data is deposited into a spreadsheet used by OMAO management to analyze the information. Though the complete form is not shared with the ships', specific concerns and praises are followed up on while not divulging the identity of the evaluator.

**VIII. Miscellaneous**

## A. Meals and Berthing

The ship will provide meals for the scientists listed above. Meals will be served 3 times daily beginning one hour before scheduled departure, extending throughout the project, and ending two hours after the termination of the project. Since the watch schedule is split between day and night, the night watch may often miss daytime meals and will require adequate food and beverages (for example a variety of sandwich items, cheeses, fruit, milk, juices) during what are not typically meal hours. Special dietary requirements for scientific participants will be made available to the ship's command at least seven days prior to the project.

Berthing requirements, including number and gender of the scientific party, will be provided to the ship by the Chief Scientist. The Chief Scientist and Commanding Officer will work together on a detailed berthing plan to accommodate the gender mix of the scientific party taking into consideration the current make-up of the ship's complement. The Chief Scientist is responsible for ensuring the scientific berthing spaces are left in the condition in which they were received; for stripping bedding and linen return; and for the return of any room keys which were issued. The Chief Scientist is also responsible for the cleanliness of the laboratory spaces and the storage areas utilized by the scientific party, both during the project and at its conclusion prior to departing the ship.

All NOAA scientists will have proper travel orders when assigned to any NOAA ship. The Chief Scientist will ensure that all non NOAA or non-Federal scientists aboard also have proper orders. It is the responsibility of the Chief Scientist to ensure that the entire scientific party has a mechanism in place to provide lodging and food and to be reimbursed for these costs in the event that the ship becomes uninhabitable and/or the galley is closed during any part of the scheduled project.

All persons boarding NOAA vessels give implied consent to comply with all safety and security policies and regulations which are administered by the Commanding Officer. All spaces and equipment on the vessel are subject to inspection or search at any time. All personnel must comply with OMAO's Drug and Alcohol Policy dated May 17, 2000 which forbids the possession and/or use of illegal drugs and alcohol aboard NOAA Vessels.

## B. Medical Forms and Emergency Contacts

The NOAA Health Services Questionnaire (NHSQ, NF 57-10-01 (3-14)) must be completed in advance by each participating scientist. The NHSQ can be obtained from the Chief Scientist or the NOAA website

<http://www.corporateservices.noaa.gov/noaforms/eforms/nf57-10-01.pdf>.

All NHSQs submitted after March 1, 2014 must be accompanied by [NOAA Form \(NF\) 57-10-02](#) - Tuberculosis Screening Document in compliance with [OMAO Policy 1008](#) (Tuberculosis Protection Program).

The completed forms should be sent to the Regional Director of Health Services at the applicable Marine Operations Center. The NHSQ and Tuberculosis Screening Document should reach the Health Services Office no later than 4 weeks prior to the start of the project to allow time for the participant to obtain and submit additional information should health services require it, before clearance to sail can be granted. Please contact

MOC Health Services with any questions regarding eligibility or completion of either form. Ensure to fully complete each form and indicate the ship or ships the participant will be sailing on. The participant will receive an email notice when medically cleared to sail if a legible email address is provided on the NHSQ.

The participant can mail, fax, or email the forms to the contact information below.

Participants should take precautions to protect their Personally Identifiable Information (PII) and medical information and ensure all correspondence adheres to DOC guidance ([http://ocio.os.doc.gov/ITPolicyandPrograms/IT\\_Privacy/PROD01\\_008240](http://ocio.os.doc.gov/ITPolicyandPrograms/IT_Privacy/PROD01_008240)).

The only secure email process approved by NOAA is [Accellion Secure File Transfer](#) which requires the sender to setup an account. [Accellion's Web Users Guide](#) is a valuable aid in using this service, however to reduce cost the DOC contract doesn't provide for automatically issuing full functioning accounts. To receive access to a "Send Tab", after your Accellion account has been established send an email from the associated email account to [accellionAlerts@doc.gov](mailto:accellionAlerts@doc.gov) requesting access to the "Send Tab" function. They will notify you via email usually within 1 business day of your approval. The "Send Tab" function will be accessible for 30 days.

Contact information:

Regional Director of Health Services

Marine Operations Center – Atlantic

439 W. York Street

Norfolk, VA 23510

Telephone 757-441-6320

Fax 757-441-3760

Email [MOA.Health.Services@noaa.gov](mailto:MOA.Health.Services@noaa.gov)

Prior to departure, the Chief Scientist must provide an electronic listing of emergency contacts to the Executive Officer for all members of the scientific party, with the following information: contact name, address, relationship to member, and telephone number.

#### C. Shipboard Safety

Hard hats are required when working with suspended loads. Work vests are required when working near open railings and during small boat launch and recovery operations. Hard hats and work vests will be provided by the ship when required.

Wearing open-toed footwear or shoes that do not completely enclose the foot (such as sandals or clogs) outside of private berthing areas is not permitted. At the discretion of the ship CO, safety shoes (i.e. steel or composite toe protection) may be required to participate in any work dealing with suspended loads, including CTD deployment and recovery. The ship does not provide safety-toed shoes/boots. The ship's Operations Officer should be consulted by the Chief Scientist to ensure members of the scientific party report aboard with the proper attire.

#### D. Communications

A progress report on operations prepared by the Chief Scientist may be relayed to the program office. Sometimes it is necessary for the Chief Scientist to communicate with another vessel, aircraft, or shore facility. Through various means of communications, the



ship can usually accommodate the Chief Scientist. Special radio voice communications requirements should be listed in the project instructions. The ship's primary means of communication with the Marine Operations Center is via email and the Very Small Aperture Terminal (VSAT) link. Standard VSAT bandwidth at 128kbs is shared by all vessels staff and the science team at no charge. Increased bandwidth in 30 day increments is available on the VSAT systems at increased cost to the scientific party. If increased bandwidth is being considered, program accounting is required and it must be arranged through the ship's Commanding Officer at least 30 days in advance.

#### E. IT Security

Any computer that will be hooked into the ship's network must comply with the *OMAO Fleet IT Security Policy 1.1* (November 4, 2005) prior to establishing a direct connection to the NOAA WAN. Requirements include, but are not limited to:

- (1) Installation of the latest virus definition (.DAT) file on all systems and performance of a virus scan on each system.
- (2) Installation of the latest critical operating system security patches.
- (3) No external public Internet Service Provider (ISP) connections.

Completion of the above requirements prior to boarding the ship is required.

Non-NOAA personnel using the ship's computers or connecting their own computers to the ship's network must complete NOAA's IT Security Awareness Course within 3 days of embarking.

#### F. Foreign National Guests Access to OMAO Facilities and Platforms

All foreign national access to the vessel shall be in accordance with NAO 207-12 and RADM De Bow's March 16, 2006 memo (<http://deemedexports.noaa.gov>). National Marine Fisheries Service personnel will use the Foreign National Registration System (FNRS) to submit requests for access to NOAA facilities and ships. The Departmental Sponsor/NOAA (DSN) is responsible for obtaining clearances and export licenses and for providing escorts required by the NAO. DSNs should consult with their designated Line Office Deemed Export point of contact to assist with the process.

Full compliance with NAO 207-12 is required.

Responsibilities of the Chief Scientist:

1. Provide the Commanding Officer with the email generated by the Servicing Security Office granting approval for the foreign national guest's visit. (For NMFS-sponsored guests, this email will be transmitted by FNRS.) This email will identify the guest's DSN and will serve as evidence that the requirements of NAO 207-12 have been complied with.
2. Escorts – The Chief Scientist is responsible to provide escorts to comply with NAO 207-12 Section 5.10, or as required by the vessel's DOC/OSY Regional Security Officer.
3. Ensure all non-foreign national members of the scientific party receive the briefing on Espionage Indicators (NAO 207-12 Appendix A) at least annually or as required by the Servicing Security Office.
4. Export Control - Ensure that approved controls are in place for any technologies that are subject to Export Administration Regulations (EAR).

The Commanding Officer and the Chief Scientist will work together to implement any access controls necessary to ensure no unlicensed export occurs of any controlled technology onboard regardless of ownership.

Responsibilities of the Commanding Officer:

1. Ensure only those foreign nationals with DOC/OSY clearance are granted access.
2. Deny access to OMAO platforms and facilities by foreign nationals from countries controlled for anti-terrorism (AT) reasons and individuals from Cuba or Iran without written approval from the Director of the Office of Marine and Aviation Operations and compliance with export and sanction regulations.
3. Ensure foreign national access is permitted only if unlicensed deemed export is not likely to occur.
4. Ensure receipt from the Chief Scientist or the DSN of the FNRS or Servicing Security Office email granting approval for the foreign national guest's visit.
5. Ensure Foreign Port Officials, e.g., Pilots, immigration officials, receive escorted access in accordance with maritime custom to facilitate the vessel's visit to foreign ports.
6. Export Control - 8 weeks in advance of the project, provide the Chief Scientist with a current inventory of OMAO controlled technology onboard the vessel and a copy of the vessel Technology Access Control Plan (TACP). Also notify the Chief Scientist of any OMAO-sponsored foreign nationals that will be onboard while program equipment is aboard so that the Chief Scientist can take steps to prevent unlicensed export of Program controlled technology. The Commanding Officer and the Chief Scientist will work together to implement any access controls necessary to ensure no unlicensed export occurs of any controlled technology onboard regardless of ownership.
7. Ensure all OMAO personnel onboard receive the briefing on Espionage Indicators (NAO 207-12 Appendix A) at least annually or as required by the Servicing Security Office.

Responsibilities of the Foreign National Sponsor:

1. Export Control - The foreign national's sponsor is responsible for obtaining any required export licenses and complying with any conditions of those licenses prior to the foreign national being provided access to the controlled technology onboard regardless of the technology's ownership.
2. The DSN of the foreign national shall assign an on-board Program individual, who will be responsible for the foreign national while on board. The identified individual must be a U.S. citizen and a NOAA or DOC employee. According to DOC/OSY, this requirement cannot be altered.
3. Ensure completion and submission of Appendix C (Certification of Conditions and Responsibilities for a Foreign National

## **APPENDICES**

### **Appendix A**

#### ***Strategies for dealing with delays:***

Delays will be offset by changing the spacing of stations or dropping them altogether. Planned station locations are identified in Figure 1. The Chief Scientist will work with the Chief Officer daily to establish coordinates of stations given weather and ship-permitted steam time between stations.

Table 3. Approximate station coordinates based on conservative steaming speed of 10 knots and the cruise track shown in Figure 1.

Station number	Date	Latitude	Longitude	Distance
1*	3/31/16	25°N	115°W	~550 nm (~50 h)
2	4/01/16	23.75°N	112.5°W	~140 nm (~14 h)
3	4/02/16	22.5°N	110°W	~140 nm (~14 h)
4	4/03/16	21.25°N	108°W	~140 nm (~14 h)
5	4/04/16	20°N	106°W	~140 nm (~14 h)
6	4/05/16	18.75°N	104.4°W	~140 nm (~14 h)
6	4/06/16	18.75°N	104.4°W	
7	4/07/16	17.5°N	102.7°W	~140 nm (~14 h)
8	4/08/16	16.25°N	101°W	~140 nm (~14 h)
9	4/09/16	15°N	99°W	~131.3 nm (~14 h)
9	4/10/16	15°N	99°W	
10	4/11/16	15°N	101.5°W	~131.3 nm (~14 h)
11	4/12/16	15°N	104°W	~131.3 nm (~14 h)
12	4/13/16	15°N	107°W	~105 nm (~12 h)
12	4/14/16	15°N	107°W	~131.3 nm (~14 h)
13	4/15/16	15°N	110°W	~105 nm (~12 h)
14	4/16/16	15°N	112.5°W	~105 nm (~12 h)
15	4/17/16	15°N	115°W	~138 nm (~14 h)
15	4/18/16	15°N	115°W	
16	4/19/16	17°N	115°W	~138 nm (~14 h)
17	4/20/16	19°N	115°W	~138 nm (~14 h)
18	4/21/16	21°N	115°W	~138 nm (~14 h)
19	4/22/16	23°N	115°W	~138 nm (~14 h)
20**	4/23/16	25°N	115°W	~140 nm (~14 h)
San Diego	4/25/16			550 nm (45-55 h)

\*if we can't get to 25°N before sunrise, we will stop where we are at approximately 04:30 on March 31.

\*\*we will likely need to drop this repeat station to get back on the morning of the 25<sup>th</sup>.

1°latitude is about 69.1 nm

1°longitude is about 52.5 nm at 15°N

If clearances aren't obtained, the cruise track will be reversed and modified to account for the Mexican EEZ (see figure 2).



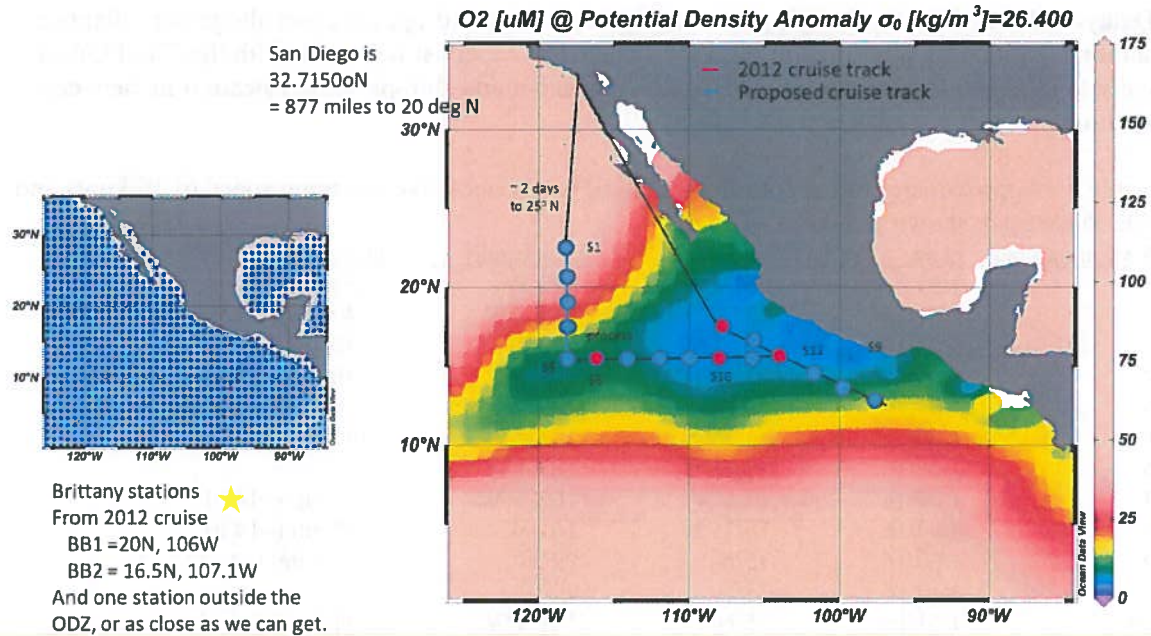


Figure 2. Approximate cruise track if clearances to sample in Mexican coastal waters are not obtained. Exact coordinates will be identified in consultation with NOAA personnel in the event that clearances are NOT issued so that we can consult current charts and avoid the Mexican EEZ.

## Appendix B. Equipment list including Hazmats

### 1. ODU: Mulholland

- 6-8 pallets/pallet boxes containing scientific equipment including deckboard incubators.
- See appendix C for hazmats.

### 2. Princeton: Jayakumar

- 3 pallets containing scientific equipment
- Gases
- See appendix C for hazmats

### 3. USC VAN: Moffett

- This will contain equipment for processing trace metal samples.
- See appendix C for hazmats

### 4. Stanford gear: Casciotti

- 2 pallet boxes containing scientific equipment
- See appendix C for hazmats

### 5. Scripps gear: Hembrough

- CTD
- -80 freezer
- Milli-Q water system
- UNOLS cold van
- 2 pallets with gear

**6. MBARI gear: Blum**

- Pump profiler system including CTD rosette, pump and winch
- 2 pallets

**Appendix C. List of Chemicals**

Table 4. List of Chemicals

Group	Common Name of Material	Qty	Notes	Trained Individual	Spill control
ODU	Gluteraldehyde (10%)	1 x 500ml	liquid	Peter Bernhardt	F
ODU	Mercuric Chloride	1 x 100ml	Alkalinity, Stored in ship chem. lkr	Peter Bernhardt	M
ODU	Hydrochloric Acid, 12N	2x 2.5L	Acid Stored in ship chem. lkr	Peter Bernhardt	A
ODU	Sulfuric acid	1x 2.5L	Acid stored in the ship chem. lkr	Peter Bernhardt	A
ODU	Ethanol	1x 2.5L	Liquid stored in flammable locker	Peter Bernhardt	
ODU	Acetone	2x 4L	Liquid stored in flammable locker	Peter Bernhardt	
ODU	OPA (o-Phthalaldehyde)	2x 4g	Solid stored in lab	Peter Bernhardt	
ODU	Ammonium molybdate		Solid stored in lab	Peter Bernhardt	
ODU	Cadmium coil	4	Solid stored in lab	Peter Bernhardt	
ODU	Diacetyl monoxime		Solid stored in lab	Peter Bernhardt	
ODU	Hydrazine sulfate		Solid stored in lab	Peter Bernhardt	
ODU	Imidazole		Solid stored in lab	Peter Bernhardt	

Group	Common Name of Material	Qty	Notes	Trained Individual	Spill control
ODU	N-(1-Naphthyl)-ethylenediamine (NED)		Solid stored in lab	Peter Bernhardt	
ODU	Ammonium chloride		Solid stored in lab	Peter Bernhardt	
ODU	Sodium sulfite		Solid stored in lab	Peter Bernhardt	
ODU	Sodium tetraborate		Solid stored in lab	Peter Bernhardt	
ODU	Sulfanilamide		Solid stored in lab	Peter Bernhardt	
ODU	Zinc chloride, 7M solution	1 x 100ml	liquid	Peter Bernhardt	
MBARI	Imidazole	1 x 50g	Granular solid	Marguerite Blum	
MBARI	NED (ninaptylethylendiam Di-HCl)	1 x 7g	Granular solid	Marguerite Blum	
MBARI	Sulfanilimide	1 x 70g	Granular solid	Marguerite Blum	
MBARI	Hydrochloric acid	8 x 100 mL	liquid	Marguerite Blum	A
MBARI	OPA (o-Phthalaldehyde)	8L	Liquid	Marguerite Blum	
Stanford	Acetic Acid	1 x 200ml	Liquid	M. Forbes & N. Travis	A
Stanford	Hydrochloric Acid	2 x 100ml	Liquid	M. Forbes & N. Travis	A
Stanford	NED N(-1-Naphthyl)ethylene-diamine dihydrochloride	1 x 10g	granualr solid	M. Forbes & N. Travis	
Stanford	SAN Sulfanilamide	1 x 10g	granualr solid	M. Forbes & N. Travis	

Group	Common Name of Material	Qty	Notes	Trained Individual	Spill control
Stanford	Sodium Azide	1 x 7gram	granualr solid	M. Forbes & N. Travis	A
Stanford	Sodium Hydroxide	4 x 50ml	liquid	M. Forbes & N. Travis	
Stanford	Sodium Nitrite	6 x 50ml	Liquid	M. Forbes & N. Travis	
Stanford	Potassium Nitrate	1 x 10g	granular solid	M. Forbes & N. Travis	
Stanford	Ammonium Chloride	1 x 10g	granular solid	M. Forbes & N. Travis	
Princeton	UHP Helium	4 x 300	gas	Jayakumar,	
Princeton	UHP N2	8 x 300	gas	Jayakumar	
Princeton	800ppm CO2 in UHP He	2 x 200	gas	Aldunate	
Princeton	Glutaraldehyde 25%	1 x 500 ml	liquid	Amal Jayakumar	F
Princeton	Potassium Chloride	1 x 500 g	Granular solid	Amal Jayakumar	
Princeton	Zinc Chloride	1 x 250 g	Granular solid	Amal Jayakumar	
Princeton	Hydrochloric acid	2 x 2.5 L	liquid	Amal Jayakumar	A
Princeton	Mercuric Chloride	1 X 500 g	Granular solid	Amal Jayakumar	
Concepcion	Hydrochloric acid	3 L	Acid	Montserrat Aldunate	A
Concepcion	Ethanol	3 L	Solvent	Montserrat Aldunate	
Concepcion	Acetate-13	1 g	Solid	Montserrat Aldunate	
Concepcion	Glucose-13	1 g	Solid	Montserrat Aldunate	
Concepcion	Sodium bicarbonate-13	1 g	Solid	Montserrat Aldunate	

Group	Common Name of Material	Qty	Notes	Trained Individual	Spill control
Concepcion	Algal aminoacid mixture-13	1 g	Solid	Montserrat Aldunate	
Concepcion	Cyanate-13	1 g	Solid	Montserrat Aldunate	
Concepcion	Glutaraldehyde	20 ml	Fixative/Liquid	Montserrat Aldunate	F
Concepcion	Paraformaldehyde	10 g	Fixative/Solid	Montserrat Aldunate	F
Concepcion	RNA later	200 ml	RNA stabilization solution	Montserrat Aldunate	
USC	99.999% Nitrogen gas	4x300	Gas	Jim Moffett	
USC	Nitrogen (98%), carbon dioxide (2%) mixture	1x300	Gas	Jim Moffett	
USC	Hydrochloric Acid, 12N	1500 mL	Liquid	Moffett	A
USC	Luminol	10 g	solid	Moffett	
USC	Ammonia 30%	3 L	Liquid	Moffett	
USC	Copper chloride solution 0.01M	100 ml	Liquid	Moffett	
USC	Nitric Acid 10N	500 ml	Liquid	Moffett	A
USC	MOPS Buffer	5g	Solid	Moffett	
USC	Ferrozine	10g	Solid	Moffett	
USC	Hydrogen Peroxide 30%	50ml	Liquid	Moffett	
USC	Iron sulfate solution 0.01M	100ml	Liquid	Moffett	
USC	Methanol	1 L	Liquid	Moffett	F