Cruise Report: A16N

(May 2023)

Highlights

Cruise Summary information

Section Designation	**A16N (Leg 1)**
Expedition designation (ExpoCode)	**33RO20230306**
Chief Scientists	**Zachary Erickson / PMEL** **Katelyn Schockmann / AOML**
Virtual Chief Scientists	**Leticia Barbero / AOML** **Denis Pierrot / AOML**
Dates	**2023 MAR 6 – 2023 APR 7**
Ship	*NOAA SHIP RONALD H. BROWN*
Ports of call	Port Saupe, Brazil – Rota, Spain
Geographic Boundaries	28°N 29°W 24.5°W 6°S
Stations	75
Core Argo Floats Deployed	4
Biogeochemical Argo Floats Deployed	3
Surface Drifters Deployed	5

Contact information

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GO-SHIP A16N (Leg 1) Cruise Report

NOAA Ship Ronald H. Brown
06 February 2013 – 07 March 2013
Port Saupe, Brazil – Rota, Spain

Chief Scientist:
Dr. Zachary K. Erickson
NOAA / PMEL

Co-Chief Scientist:
Dr. Katelyn Schockmann
NOAA / AOML

Virtual Chief Scientists:
Drs. Leticia Barbero and Denis Pierrot
NOAA / AOML

Preliminary CTD data submitted by:
Zachary K. Erickson
NOAA / PMEL

Preliminary bottle data submitted by:
Zachary K. Erickson
NOAA / PMEL

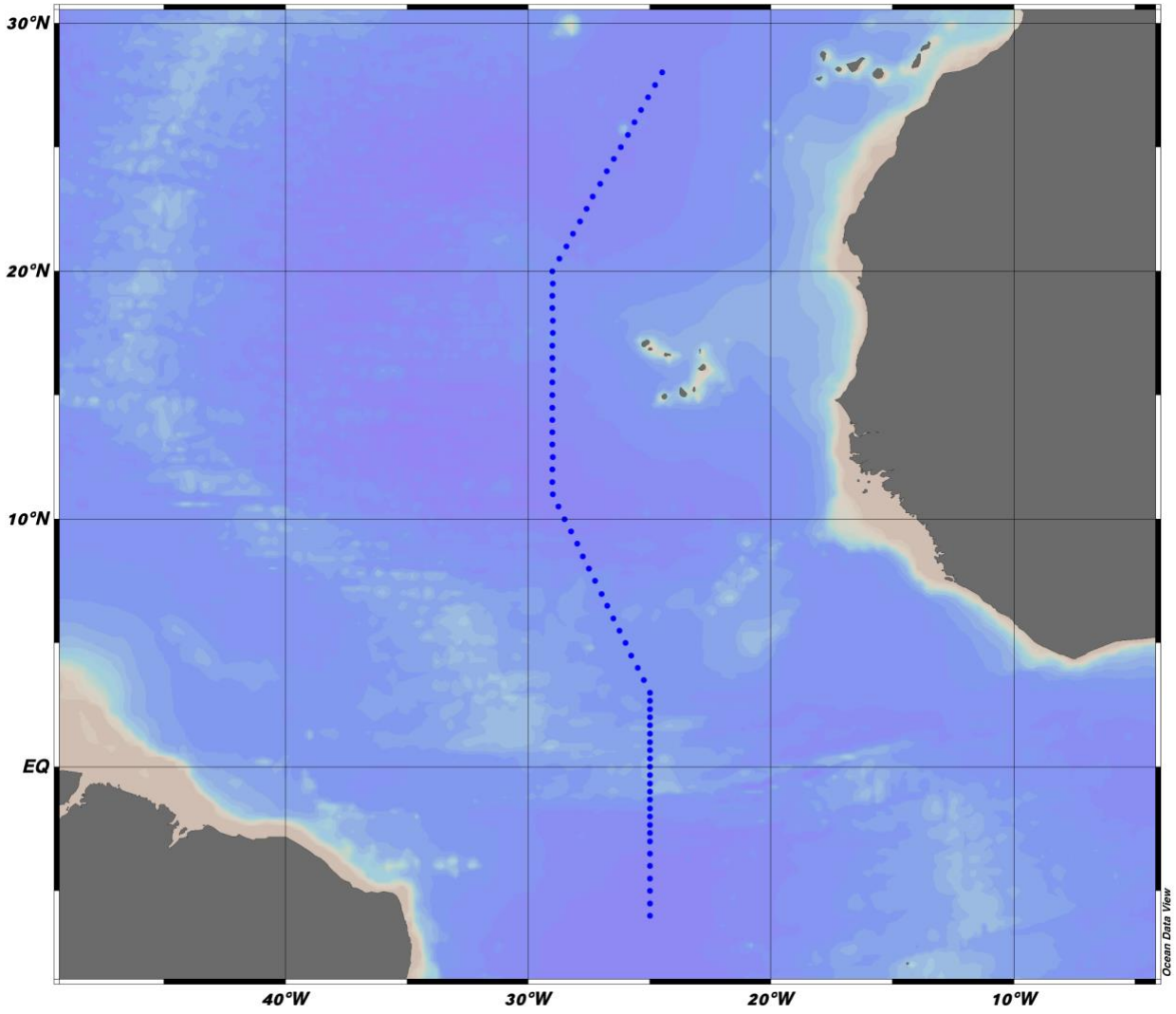

Figure 1. Station locations for A16N (Leg 1).

1. Introduction

Hydrographic measurements were carried out along transect A16N in the Atlantic Ocean on the NOAA Ship *Ronald H. Brown* in support of the Global Ocean Ship-based Hydrographic Investigation Program (GO-SHIP), funded primarily by NOAA and NSF. The goals of this program are to occupy a set of hydrographic transects, such as A16N, at approximately decadal resolution to with full water column measurements to study physical, hydrographic, and chemical changes over time. This transect ran from south to north and was split into two legs. This report details measurements taken during the first leg, starting in Port Saupe, Brazil and ending in Rota, Spain. 75 stations were accomplished during this cruise, with latitudes between 6˚S and 28˚N at a spacing of ½˚ except for within 3˚of the equator, where the spacing was reduced to 1/3˚. Measurements from approximately 1800 bottles were taken as part of this transect Leg, analyzing a variety of parameters including salinity, dissolved oxygen, chlorofluorocarbons, sulfur hexafluoride, dissolved inorganic carbon, dissolved organic carbon, total alkalinity, pH, and carbon isotopes (13C and 14C). This cruise is a re-occupation of the A16N line, with previous transects in 1993, 2003, and 103.

For the 2023 re-occupation, a substantial biological component was also included, with dedicated "Bio" casts to 1000 m every day supporting measurements of parameters such as particulate organic carbon and phosphorus (POC and POP), pigment analysis through high performance liquid chromatography (HPLC), flow cytometry (FCM), environmental DNA (eDNA), and RNA.

Underway measurements were also made throughout the cruise of horizontal velocities through a ship-board ADCP, sea surface temperature, salinity, and pCO2 from the ship's underway clean water intake, and bathymetry data, as well as discrete measurements of other parameters listed above during transit periods between Port Saupe, Brazil and the first station and between the last station and the port in Rota, Spain.

This report details the scientific objectives, operations carried out, and issues encountered during this cruise. More information can be found at the dedicated cruise website, https://www.aoml.noaa.gov/ocd/gcc/A16N_2023/.

1.1 GO-SHIP A16N 2023 Leg 1 Participating Institutions

Abbreviation	Institution
AOML	Atlantic Oceanographic and Meteorological Laboratory (NOAA)
Bigelow	Bigelow Laboratory for Ocean Sciences
CICOES	Cooperative Institute for Climate, Ocean, and Ecosystem Studies/U. Washington
CIMAS	Cooperative Institute for Marine and Atmospheric Studies/U. Miami
FAU	Florida Atlantic University
LDEO	Lamont-Doherty Earth Observatory/Columbia University
MIT	Massachusetts Institute of Technology
MLML	Moss Landing Marine Laboratory
NGI	Northern Gulf Institute
ODU	Old Dominion University
OSU	Oregon State University
PMEL	Pacific Marine Environmental Laboratory (NOAA)
RSMAS	Rosenstiel School of Marine and Atmospheric Science/U. Miami
SIO	Scripps Institute of Oceanography/University of California San Diego
TAMU	Texas A&M University
UCI	University of California Irvine
UCSB	University of California Santa Barbara
U. Colorado	University of Colorado Boulder
U. Delaware	University of Delaware
U. Georgia	University of Georgia
U. Guam	University of Guam
U. Hawaii	University of Hawaii
USF	University of South Florida
U. Washington	University of Washington
U. Wisconsin	University of Wisconsin Madison
WHOI	Woods Hole Oceanographic Institution

1.2 GO-SHIP A16N 2023 Leg 1 Principal Investigators

Table 1. List of Principal Investigators

Parameter	Lead PI(s)	Affiliation(s)	Email Address(es)
SADCP	Jules Hummon Eric Firing	U. Hawaii U. Hawaii	hummon@hawaii.edu efiring@hawaii.edu
pCO_2 (underway)	Denis Pierrot Rik Wanninkhof	AOML AOML	denis.pierrot@noaa.gov rik.wanninkhof@noaa.gov
CTD/O_2	Zachary Erickson Rick Lumpkin	PMEL AOML	zachary.k.erickson@noaa.gov rick.lumpkin@noaa.gov
Argo floats	Steve Jayne Pelle Robbins Susan Wijffels	WHOI WHOI WHOI	sjayne@whoi.edu probbins@whoi.edu swijffels@whoi.edu
GO-BGC floats	David Nicholson Susan Wijffels	WHOI WHOI	dnicholson@whoi.edu swijffels@whoi.edu
NOAA Drifters	Shaun Dolk	AOML	shaun.dolk@noaa.gov
LADCP	Andreas Thurnherr	LDEO	ant@ldeo.columbia.edu
CFCs/SF_6	Rolf Sonnerup	PMEL/CICOES	rolf@uw.edu
Dissolved O_2	Chris Langdon	RSMAS	clangdon@earth.miami.edu
pH	Chris Langdon	RSMAS	clangdon@earth.miami.edu
Alkalinity	Chris Langdon	RSMAS	clangdon@earth.miami.edu
pCO_2 (discrete)	Rik Wanninkhof	AOML	rik.wanninkhof@noaa.gov
DIC	Richard Feely Rik Wanninkhof	PMEL AOML	richard.a.feely@noaa.gov rik.wanninkhof@noaa.gov
$\delta^{13}C$	Wei-Jun Cai	U. Delaware	wcai@udel.edu
^{14}C	Rolf Sonnerup Roberta Hansman	PMEL/CICOES WHOI	rolf@uw.edu rhansman@whoi.edu
DOC	Dennis Hansell	RSMAS	dhansell@miami.edu
Nutrients	Calvin Mordy Jia-Zhong Zhang	PMEL/CICOES AOML	calvin.w.mordy@noaa.gov jia-zhong.zhang@noaa.gov
Salinity (discrete)	Zachary Erickson Rick Lumpkin	PMEL AOML	zachary.k.erickson@noaa.gov rick.lumpkin@noaa.gov
Bio	Harriet Alexander Sophie Clayton Jason Graff Adam Martiny Nicole Poulton Luke Thompson	WHOI ODU OSU UCI Bigelow AOML	halexander@whoi.edu sclayton@odu.edu jason.graff@oregonstate.edu amartiny@uci.edu npoulton@bigelow.org luke.thompson@noaa.gov
Sargassum	Dennis McGillicuddy	WHOI	dmcgillicuddy@whoi.edu

1.3 GO-SHIP A16N 2023 Leg 1 Scientific Participants

Table 2. List of scientific participants.

Position	Name	Affiliation	Email address
Chief scientist	Zachary Erickson	PMEL	zachary.k.erickson@noaa.gov
Co-chief sci./pCO_2	Katelyn Schockman	AOML/CIMAS	katelyn.schockman@noaa.gov
CTD processing	Kristy McTaggart	PMEL	kristene.e.mctaggart@noaa.gov
CTD Watchstander	Taydra Low	U. Wisconsin	tlow2@wisc.edu
CTD Watchstander	Sam Mogen	U. Colorado	samuel.mogen@colorado.edu
Salts/CTD/LADCP	Jay Hooper	AOML/CIMAS	james.hooper@noaa.gov
Salts/LADCP	Christian Saiz	AOML/CIMAS	christian.saiz@noaa.gov
Nutrients	Eric Wisegarver	PMEL	eric.wisegarver@noaa.gov
Nutrients	Alexandra Fine	AOML/CIMAS	alexandra.fine@noaa.gov
Dissolved O_2	Emma Ponte	RSMAS	epontes@earth.miami.edu
Dissolved O_2	Riley Palmer	RSMAS	rileypalmer@rsmas.miami.edu
CFCs	David Cooper	CICOES	davidcooper59@gmail.com
CFCs	Melissa Miller	CICOES	melissatruth@gmail.com
CFCs	Rachel Bramblett	U. Georgia	rachel.bramblett@uga.edu
pCO_2	Patrick Mears	AOML/CIMAS	patrick.mears@noaa.gov
DIC	Chuck Featherstone	AOML	charles.featherstone@noaa.gov
DIC	Alison MacLeod	AOML/CIMAS	alison.macleod@noaa.gov
Alkalinity/pH	Bo Yang	RSMAS	bxy189@miami.edu
Alkalinity/pH	Jessica Leonard	RSMAS	jxl2967@miami.edu
Alkalinity/pH	Caroline Branan	RSMAS	cebranan@alumni.ncsu.edu
Alkalinity/pH	Mackenzie Blanusa	RSMAS	mackenzie.blanusa@uconn.edu
^{14}C/DOC	Victoria Dina	RSMAS	victoria.dina@earth.miami.edu
^{13}C	Bo Dong	U. Delaware	bodong@udel.edu
^{13}C	Najid Hussain	U. Delaware	nhussain@udel.edu
LADCP	Kieran Claassen	SIO	lyndsey.claassen@sjsu.edu
Floats	Ellen Park	WHOI	epark@whoi.edu
Bio	Star Dressler	U. Guam	dresslerc@gotritons.uog.edu
Bio	Tyler Christian	AOML/CIMAS	tyler.christian@noaa.gov

1.4 GO-SHIP A16N 2023 Leg 1 Crew

Table 3. List of crew.

Department	Position	Name
Bridge	Commanding Officer	Capt. Marc Moser
Bridge	Executive Officer	LT Caroline Wilkinson
Bridge	Operations Officer	LT Sony Vang
Bridge	Operations	LT Dale Gump
Bridge	JO	ENS Gemma Venuti
Bridge	JO	ENS Jacob Alvey
Medical	Medic	LCDR Michael Reed
Electronics	Electronic Technician	Mike Peperato
Survey	Survey Technician	Heather Spillane
Survey	Survey Technician	Stephanie Stabile
Deck	Chief Bosun	Michael Lastinger
Deck	BGL	Bruce Harrison
Deck	AB	Nick Granozio
Deck	AB	Jared von Bargen
Deck	AB	Frank Forbell
Deck	AB	Jeff Greeley
Deck	AB	Michael Gornto
Deck	GVA	Mike Burke
Deck	GVA	Michael Wise
Steward	Chief Steward	Arnold Dones
Steward	2C	Ashley Pape
Steward	2C	Jude Reyes
Steward	CC	Ricco Speight
Engineering	Chief Engineer	Alan Currie
Engineering	First Engineer	Lee Blume
Engineering	Second Engineer	David Perez
Engineering	Third Engineer	Sarah Ellenberger
Engineering	EU	Mark Watson
Engineering	GVA	Kim Robbins
Engineering	O	Derrick Mitchell

2. Cruise Narrative

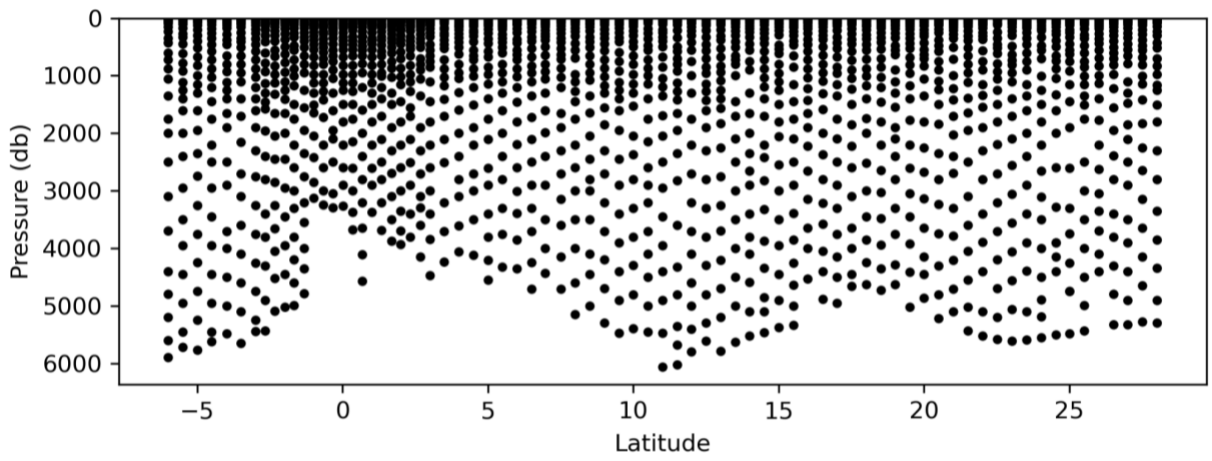

Figure 2. A16N 2023 Leg 1 bottle sample distribution.

NOAA Ship *Ronald H. Brown* departed Port Saupe, Brazil on March 6, 2023 after a somewhat tumultuous three day loading period. Access to the port was highly controlled and led to several complications, especially with scientists getting out of the port. Future cruises with port calls in Port Saupe, Brazil should carefully think through how scientists will arrive and depart from the port, especially during loading days. For this cruise, drivers had to be individually cleared for port access prior to arrival. A recommendation is therefore to either have the science party rent cars or to enter into a multi-day contract with a local driver(s) who can be cleared to access the port through to the pier for the NOAA Ship *Ronald H. Brown*. A medical emergency led to a change in personnel roles, with Zachary Erickson (PMEL) and Katelyn Schockmann (AOML/CIMAS) assuming the duties of Chief and Co-Chief Scientist, respectively. Denis Pierrot (AOML; formerly Chief Scientist) and Leticia Barbero (AOML/CIMAS) became Virtual Chief Scientists for the duration of the cruise.

Transit to the first station, at 6°S, 25°W, took approximately 54 hours. Underway measurements began once out of the Brazilian Exclusive Economic Zone (EEZ), with continuous monitoring of temperature, salinity, pCO2, horiztonal velocities from ADCP, and bathymetry and discrete measurements of dissolved oxygen, total alkalinity, pH, pCO2, and nutrients every 4 hours. A test station to 1000 m was successfully completed during transit to the first station at approximately 7.2°S, 30.3°W.

At the first CTD station a number of problems with the CTD were encountered. At 5000 db on the first downcast current readings on the CTD deck unit spiked and the sensors went offline. The CTD was immediately turned off to prevent damage. Power was restored twice to the CTD unit but both times the current meter spiked and the instrument was again turned off. The cast was aborted and the package returned to the surface. At the surface the connection to the rosette package was checked and confirmed to be good, suggesting the problem was in the

winch cable. Approximately 30 m of cable was cut from the winch cable and the cable was reterminated. On the second cast the Valport altimeter did not register the bottom. Upon reaching near the target depth of 5900 db with no altimeter reading the package was slowed to 30 m/min (compared to a normal speed of 60 m/min). Confirmation that the CTD had hit the bottom came only when the wire tension decreased and the pressure stopped increasing. The CTD was returned to the surface and bottles were fired at target pressures. Sediment had infiltrated the CTD sensors and upcast data for this cast are therefore unusable, but otherwise no harm to the instruments or the CTD frame was observed. The sensors were subsequently flushed out with DI water.

Stations 2-25 proceeded with only minor complications. Station spacing was reduced to 1/3˚ from 7 to 25 (3°S to 3°N) to more fully capture the smaller-scale features in this region. The forward winch began shaking severely at speeds of 60 m/min, which was the target speed for the water column below 200 db on every cast. The source of this shaking was not able to be determined, but appeared to be related to a physical coupling within the winch box and often happened in the 1000-2000 m depth. This reduced the top speed of the winch in the 1000-2000 m depth range, often to around 50-55 m/min.

The CTD frame was lost during recovery operations at Station 26. The cast had otherwise proceeded as normal, with no problems noted. During recovery, at approximately 10:30 GMT on March 16, 2023, the CTD was winched upwards to a target position above the water line, where it was snagged by “CTD wranglers” from the ship’s deck department, who attached ropes to the frame to guide it in to its landing platform on the deck. After the ropes were attached, the boom is supposed to be brought inward simultaneously with the winch restarting, keeping the CTD frame at a constant height above the deck. Due to operator error the CTD rose too quickly and impacted the blocks at the top of the winch, which appeared to cause the cable to snap. The CTD frame dropped into the ocean and sank. Sensors lost included two CTDs, a reference temperature sensor, two oxygen sensors, our only working Valport altimeter, and two LADCPs. Fortunately, nobody on the crew or the scientific party was physically harmed.

The CTD team was able to efficiently prepare the spare rosette for operations, with the next cast in the water after only about an 8 hour delay. Prior to this second cast of Station 26, the ship held a “safety stand-down” to review procedures for all aspects of a CTD station. The second cast revealed a problem with the secondary CTD sensor, and the package was recovered again after the preliminary soak to switch out this sensor. The third and final cast of Station 26 therefore started at 19:30 GMT and ended at 22:35 GMT. The most major impact of this lost package was a decrease in morale and confidence on the ship, as prior to this loss we had been conducting stations for a number of days without any significant problems. The next major impact was financial, as the lost systems were worth over $300K. The third impact was on time, as we lost about 12 hours as a result. The fourth was a loss of sensor redundancy. This was especially important for our altimeter, as we did not have another working sensor. On the next casts we experimented with using different combinations of Valport altimeters, as well as a Benthos supplied by the ship. We were not able to get any Valport altimeter to work, and eventually stopped putting it on the package. For the rest of the cruise we used the ship’s

Benthos, which was reliable but did not “kick in” until 35-40 m above the bottom. In an abundance of caution, as we no longer had a back-up rosette, for the rest of the cruise our target depth was 15 m above bottom, and occasionally 20 m in areas of rough bathymetry.

Stations 27-75 proceeded without major issues. The aft winch was used for the secondary CTD frame. The cable on this winch had a number of level-wind problems, which would sometimes necessitate inserting spacers into the cable to prevent too many rolls from mis-wrapping. The impact to the cruise was lost time in many of the casts, as the winch would often temporarily need to be halted in the middle of an upcast. On several occasions during station upcasts tension would need to come off of the winch to re-adjust the winding of the cable. However, on no occasion did pressure ever increase during a CTD upcast.

Early on March 31, 2023, between Stations 73 and 74, winds picked up to 25 knots (gusting to 30 knots) and swell increased to 6-9 feet. While normally well within operating parameters for the NOAA Ship *Ronald H. Brown*, the starboard Z-drive thruster encountered issues subsequently hypothesized by the Chief Engineer to be due to a loose wire in the engine that would get knocked out of alignment when a wave hit the starboard side. Starboard thruster drop-outs occurred several times throughout March 31 and April 1, 2023, along with occasional port thruster drop-outs that were not diagnosed. This led to significantly slower speeds and worry about reaching our scheduled port call in Rota, Spain.

After Station 75, the decision was made to transit to Station 76 and attempt to repair the engine. Once at Station 76 maneuvering tests were performed on the starboard thruster, but the sea state had calmed down and it was therefore not possible to recreate the conditions that led to thruster drop-outs. Given the uncertainty in the stability of the starboard thruster and the likelihood of rough seas on the transit to Rota, Spain, a decision was made by the Commanding Officer, at approximately 9:45 GMT on April 1, 2023, to immediately curtail science operations and transit directly to Rota, Spain. Station 75 was therefore the last station sampled. Net loss to science time as a result of these issues was estimated at 36 hours, or approximately 5 stations.

Underway discrete samples were taken every 4 hours during the transit to Rota, Spain. Due to the new ending point of the cruise, the ship trajectory to Rota, Spain went within the Spanish EEZ near the Canary Islands from approximately 23:00 GMT, April 1 to 18:30 GMT, April 2, 2023. After exiting this EEZ, we re-started underway sampling until reaching the Spanish EEZ near Rota, Spain.

Upon arriving at Rota, Spain on April 6, 2023 the *Ronald H. Brown* rendezvoused with a water taxi to offload three foreign nationals who were not able to enter the Navy Base. We were unable to dock at the pier on April 6 due its use by another ship which had priority. We anchored off-shore until the morning of April 7, 2023 when a space opened up and we were able to dock and offload.

3. Underway Data Acquisition

Underway data collection included meteorological parameters, upper ocean current measurements from the shipboard ADCP, surface oceanographic measurements (temperature, salinity, pCO_2) from the ship's underway clean seawater intake, bathymetric data, and discrete measurements of CFCs, SF_6, dissolved oxygen, pH, total alkalinity, and nutrients. Discrete measurements were collected every 4 hours during the initial transit to the first station and the final transit from the last station, except when in a Spanish or Brazilian EEZ. All other underway sensors were always collecting data except within a Spanish or Brazilian EEZ. Navigation data were acquired at 1-second intervals from the ship's Furuno Marine Touch Screen navigational radar. In addition, centerbeam depth data, with a correction for hull depth included in each data line, were acquired directly from the ship's Multibeam/Kongsberg EM122 system. These data were used to determine the position and ocean depth information for each station and deployment. The centerbeam depths were also continuously displayed, and data were recorded at cast start/bottom/end on CTD Cast Logs.

3.1 Acoustic Doppler Current Profiler (ADCP) Measurements

*Principal Investigator*s**:** *Jules Hummon (U. Hawaii) and Eric Firing (U. Hawaii)*

The NOAA Ship *Ronald H. Brown* has a permanently mounted 75 kHz acoustic Doppler current profiler ("ADCP" Teledyne RDI) for measuring ocean velocity in the upper water column. The ADCP is a Phased Array instrument, capable of pinging in broadband mode (for higher resolution), narrowband mode (lower resolution, deeper penetration), or interleaved mode (alternating). On this cruise, data were collected with 8 m broadband pings and 16 m narrowband pings. The data were collected for the entire duration of A16N except when the ship was within the Brazilian or Spanish EEZ.

The shipboard ADCP data are acquired and processed by specialized software developed at the University of Hawaii and installed on the Brown. The acquisition system ("UHDAS", University of Hawaii Data Acquisition System) acquires data from the ADCPs, gyro heading (for reliability), position and orientation systems for marine vessels (POSMV) headings (for increased accuracy), and GPS positions from various sensors. Single-ping ADCP data are automatically edited and combined with ancillary feeds, averaged, and disseminated via the ship's web, as regularly-updated figures on a web page and as Matlab and netCDF files.

3.2 Underway pCO_2 Analyses

Principal Investigators: Denis Pierrot (AOML), Rik Wanninkhof (AOML)
Analysts: N. Patrick Mears (AOML/CIMAS), Kevin Sullivan (AOML/CIMAS)

During the GO-SHIP A16N cruise, there was an automated underway pCO_2 system from AOML situated in the hydrolab, as it has been since 2007. The design of the instrumental system is

based on Wanninkhof and Thoning (1993) and Feely et al. (1998), while the details of the instrument and of the data processing are described in Pierrot et al. (2009).

The repeating cycle of the system includes 4 gas standards, 5 ambient air samples, and 66 headspace samples from its equilibrator within 3.3 hours. The concentrations of the standards range from 232 to 541 ppm CO_2 in compressed natural air. They were purchased from NOAA Earth System Research Laboratories (ESRL) in Boulder, CO and are directly traceable to the WMO scale. The system includes an equilibrator where approximately 0.6 liters of constantly refreshed surface seawater from the bow intake is equilibrated with 0.8 liters of gaseous headspace. The water flow rate through the equilibrator was 2.0 - 2.5 liters/min.

The equilibrator headspace is circulated through a non-dispersive infrared analyzer (IR), a LI-COR™ 6262, and then returned to the equilibrator. When ambient air or standard gas is analyzed, the gas leaving the analyzer is vented to the lab. A KNF pump constantly draws 6-8 liter/min of marine air through 100 m of 0.95 cm (= ⅜ in) OD Dekoron™ tubing from an intake on the bow mast. The intake has a rain guard and a filter of glass wool to prevent water and larger particles from reaching the pump. The headspace and marine air gases are dried before flushing the IR analyzer.

A custom program developed using LabView™ controls the system and graphically displays the air and water results. The program records the output of the infrared analyzer, the GPS position, water and gas flows, water and air temperatures, internal and external pressures, and a variety of other sensors. The program records all of this data for each analysis.

Since the beginning of the cruise, a non-functioning 3-way solenoid caused standard and atmospheric gas measurements analyzed by the LI-COR™ to be returned to the headspace gas of the equilibrator via the equilibrator return line instead venting out of the system. This results in several data points collected from the headspace gas being discarded when taken directly after standard and atmospheric sample analysis. In addition, this causes an excess of salt crystals to form in the return line that required cleaning to prevent restricted gas flow.

Standard Gas Cylinders

Cylinder#	ppm CO_2
CB09731	232.26
CA08234	399.36
CC720367	430.75
CA06355	541.86

References

Feely, R.A., R. Wanninkhof, H.B. Milburn, C.E. Cosca, M. Stapp, and P.P. Murphy, 1998: A new automated underway system for making high precision pCO_2 measurements onboard research ships. Analytica Chim. Acta, v. 377, pp. 185-191.

Pierrot, D., C. Neill, K. Sullivan, R. Castle, R.Wanninkhof, H. Luger, T. Johannessen, A. Olsen, R.A.

Wanninkhof, R., and K. Thoning, 1993: Measurement of fugacity of CO_2 in surface water using continuous and discrete sampling methods. Mar. Chem., v. 44, no. 2-4, pp. 189-205.

4. Stations

4.1 Main Stations

Stations were distributed at regular intervals of latitude, every ½° from 6°S-3°S, every 1/3° from 3°S-3°N, and every ½° from 3°N-31.5°N, for a total of 75 stations occupied. On the upcast, 24 'Bullister' bottles were fired. The first was always at the deepest depth, 10-15 m above the sea floor, and the last was always at the surface, at 3-5 m depth. The rest were arranged throughout the water column, always with about half in the upper 1000 m. Bottle spacing in the upper 100 m was generally 15-30 m, and at depth was frequently 400-700 m. Bottle locations were generally similar to those done during the 2013 A16N transect, and modified so that subsequent stations did not sample at exactly the same depths.

At the end of each rosette deployment water samples were drawn from the rosette bottles in the following order:

- Chlorofluorocarbons (CFCs) and SF_6
- Oxygen
- pH/Total Alkalinity (TA)
- Discrete pCO_2
- Dissolved Inorganic Carbon (DIC)
- ^{13}C DIC
- ^{14}C DIC
- Dissolved Organic Carbon (DOC)
- Nutrients
- Salinity

The correspondence between individual sample containers and the rosette bottle position (1-24) from which the sample was drawn was recorded on the sample log for the cast. This log also included any comments or anomalous conditions noted about the rosette and bottles. One member of the sampling team was designated the sample cop, whose sole responsibility was to maintain this log and ensure that sampling progressed in the proper drawing order. Normal sampling practice included opening the drain valve and then the air vent on the bottle, indicating an air leak if water escaped. This observation together with other diagnostic comments (e.g., 'lanyard caught in lid', 'valve left open") that might later prove useful in determining sample integrity were routinely noted on the sample log. Drawing oxygen samples also involved taking the draw temperature from the bottle. The temperature was noted on the sample log and was sometimes useful in determining leaking or mis-tripped bottles.

4.2 Bio Stations

During a once-daily "bio-cast" station, the CTD rosette was casted twice (22 total stations). The first cast only collected Bio-GO-SHIP samples to 1000 m, and the second cast only collected core GO-SHIP samples. For bio-casts, Niskin bottles were fired at depths of 1000 m, 500 m, 200 m, 150 m, 100 m, 75 m, 40 m, and 5 m. The surface bottles (5 m) were set to "rapid fire" all remaining

bottles in the rosette at one time point as a time-saving measure. Water was divided for appropriate sampling of eDNA, RNA, particulate organic matter (POM), high performance liquid chromatography (HPLC), and flow cytometry (FCM). Further details on bio-cast sampling protocols are detailed in the sections below.

At each bio-cast station correlating with a BGC Argo float deployment (3 total stations), additional water was collected alongside the standard bio-cast sampling (with the exception of standard HPLC sampling depths, which was not gathered during float casts). For BGC Argo Floats, water was gathered at five depths (surface, base of mixed layer, between deep chlorophyll maximum and base of mixed layer, deep chlorophyll maximum, deep chlorophyll maximum + 50 m) and processed for small volume particulate organic carbon (POC) and HPLC. Due to the lack of a fluorometer and transmissometer on the CTD, the deep chlorophyll maximum was estimated at 15 m below the base of the mixed layer. At each depth, two liters of water were gathered for POC and two liters for HPLC, with one duplicate for each parameter set at a random depth. Water samples were filtered through pre-combusted, 25 mm GF/F filters secured on a filtration manifold attached to a vacuum pump. Filtering took place immediately following Niskin sampling. Filtered HPLC samples were placed in labeled cryovials, POC samples in labeled aluminum foil envelopes, and stored at -80º C for later analysis. Wet and dry blanks were included for both POC and HPLC. Twelve samples were processed for each float (36 total samples), not including one dry blank and one wet blank filter for each parameter at each deployment station.

4.3 CTD Data Acquisition

Principal Investigator: Zachary Erickson (PMEL)
Analytical Personnel: Kristy McTaggart (PMEL)

The CTD data acquisition system consisted of the ship's SBE-llplus (V2) deck unit s/n 11P98520367 and a networked Dell Optiplex 755 PC workstation running Windows XP Professional. SBE Seasave v.7.21d software (c.2011) was used for data acquisition and to close bottles on the rosette. Real-time digital data were backed up by the data manager, and raw data files were archived immediately after each cast on a thumb drive as well as on Survey and PMEL networked PCs.

CTD deployments were initiated by Survey after the Bridge advised that the ship was on station. The computer console operator maintained a CTD Cast log recording position and depth information at the surface, depth, and end of each cast; a record of every attempt to close a bottle, and any pertinent comments. After the underwater package entered the water, the winch operator would lower it to 10 meters and stop. The CTD pumps are configured with a 60 second startup delay, and were usually on by this time. The console operator checked the CTD data for reasonable values, waited an additional three minutes for sensors to stabilize, instructed the winch operator to bring the package to the surface, paused for 10 seconds, and descended to a target depth. The profiling rate was nominally 30 m/min to 50 m, 45 m/min to 200 m, and 60 m/min deeper than 200 m. These rates could vary depending on sea cable tension and the sea state. For the first part of the cruise, when using the forward winch, speed was reduced to around 50-55 m/min in the 100-2000 m depth range because of excessive shaking of the winch box.

The console operator monitored the progress of the deployment and quality of the CTD data through interactive graphics and operational displays. The Chief or co-Chief created a sample log for the cast that would be used to record the water samples taken from each rosette sample bottle. The altimeter channel, CTD depth, wire-out, and EM122 bathymetric depth were all monitored to determine the distance of the package from the bottom allowing a safe approach to within 10 meters. Rosette sample bottles were closed on the upcast through the software, and were tripped 30 seconds after stopping at a bottle depth to allow the rosette wake to dissipate and the bottles to flush. The winch operator was instructed to proceed to the next bottle stop 15 seconds after closing bottles to ensure that stable CTD and reference temperature data were associated with the trip. Near the surface, Survey directed the winch to stop the rosette just beneath the surface. After the surface bottle was closed, the package was recovered.

Once on deck, the console operator terminated data acquisition, turned off the deck unit, and assisted with rosette sampling. At the end of each cast, primary and secondary CTDO sensors were flushed with a solution of dilute Triton-X in de-ionized water using syringes fitted with tubing. The syringes were left attached to the temperature ducts between casts, with the temperature and conductivity sensors immersed in the solution to guard against airborne contaminants.

4.4 CTD Data Processing

Principal Investigator: Zachary Erickson (PMEL)
Analytical Personnel: Kristy McTaggart (PMEL)

The reduction of profile data began with a standard suite of processing modules using Sea-Bird Data Processing Version 7.21d software (Version 7.23.1 post-cruise) in the following order:

DATCNV converts raw data into engineering units and creates a ROS bottle file. Both down and up casts were processed for scan, elapsed time(s), pressure, tO, tl, cO, cl, oxvol, oxvo2, oxl and ox2. Optical sensor data were converted to voltages and also carried through the processing stream. MARKSCAN was used to skip over scans acquired on deck and while priming the system under water.

ALIGNCTD aligns temperature, conductivity, and oxygen measurements in time relative to pressure to ensure that derived parameters are made using measurements from the same parcel of water. Primary and secondary conductivity were automatically advanced in the V2 deck unit by 0.073 seconds. No further alignment was warranted. It was not necessary to align temperature or oxygen.

BOTTLESUM averages burst data over an 8-second interval (+1- 4 seconds of the confirm bit) and derives both primary and secondary salinity, potential temperature (0), and potential density anomaly (). Primary and secondary oxygen (in umol/kg) were derived in DATCNV and averaged in BOTTLESUM, as recommended recently by Sea-Bird.

FILTER applies a low pass filter to pressure with a time constant of 0.15 seconds. In order to produce zero phase (no time shift) the filter is first run forward through the file and then run backwards through the file.

CELLTM uses a recursive filter to remove conductivity cell thermal mass effects from measured conductivity. In areas with steep temperature gradients the thermal mass correction is on the order of 0.005 PSS-78. In other areas the correction is negligible. Nominal values of 0.03 and 7.0 s were used for the thermal anomaly amplitude (α) and the thermal anomaly time constant (β-1), respectively, as suggested by Sea-Bird.

LOOPEDIT removes scans associated with pressure slowdowns and reversals. If the CTD velocity is less than 0.25 m s-1 or the pressure is not greater than the previous maximum scan, the scan is omitted.

DERIVE uses 1-dbar averaged pressure, temperature, and conductivity to compute primary and secondary salinity, as well as more accurate oxygen values.

BINAVG averages the data into 1-dbar bins. Each bin is centered on an integer pressure value, e.g. the 1-dbar bin averages scans where pressure is between 0.5 dbar and 1.5 dbar. There is no surface bin. The number of points averaged in each bin is included in the data file.

STRIP removes oxygen that was derived in DATCNV.

TRANS converts the binary data file to ASCII format.

Package slowdowns and reversals owing to ship roll can move mixed water in tow to in front of the CTD sensors and create artificial density inversions and other artifacts. In addition to Seasoft module LOOPEDIT, MATLAB program deloop.m computes values of density locally referenced between every 1 dbar of pressure to compute the square of the buoyancy frequency, N2, and linearly interpolates temperature, conductivity, and oxygen voltage over those records where N2 is less than or equal to -1 x 10-5 s-2. Some profiles failed the criteria in the top 9 dbars. These data were retained by program deloop_post.m and will be flagged as questionable in the final WOCE formatted files.

Program calctd.m reads the delooped data files and applies preliminary calibrations to temperature, conductivity, and oxygen; and computes calibrated salinity.

4.5 Water Budgets

4.5.1 Main Cast

Table 4. Water budget for main casts.

Parameter	Budget (L)
CFCs/SF_6	0.75
Oxygen	0.7
pH/TA	0.6
pCO_2	1.5
DIC	0.65
^{13}C	0.5
^{14}C	0.2
DOC	0.1
Nutrients	0.1
Salinity	0.5
Total	5.6

4.5.2 Bio Cast

Table 5. Water budget for Bio casts

Parameter	Depth	Budget (L)
HPLC	surface	3
LV POC	surface	27
POP	surface	27
eDNA	surface	10
RNA	surface	10
FCM	surface	0.25
sub-total	surface	77.25
HPLC	40 m	3
FCM	40 m	0.25
FCM	75 m	0.25
HPLC	100 m	3
eDNA	100 m	10
FCM	100 m	0.25
FCM	150 m	0.25
eDNA	200 m	10
FCM	200 m	0.25
FCM	500 m	0.25
eDNA	1000 m	10
FCM	1000 m	0.25
Total		115

4.5.3 Bio Cast with BGC Float Deployment (additional water)

Table 6. Additional water budget for Bio casts with Biogeochemical Argo float deployments (note that some of these may overlap with the budget on Table 5).

Parameter	Depth	Budget (L)
POC	surface	3
HPLC	DCM	3
POC	DCM	3
HPLC	ML base	3
POC	ML base	3
HPLC	between ML base and DCM	3
POC	between ML base and DCM	3
HPLC	50 m below DCM	3
POC	50 m below DCM	3

5. Deployments

5.1 Core Argo Floats

Principal Investigators: Steven Jayne (WHOI), Pelle Robbins (WHOI), and Susan Wijffels (WHOI)
Shipboard personnel: Ellen Park (MIT/WHOI)

On the first leg of this cruise, three Core Argo floats were deployed (see Table 7 and Figure 3). All floats deployed were WHOI Solo floats. Floats were deployed boxed and with water releases from the stern of the ship. 50% of the water releases worked successfully.

Figure 3. Core Argo float (SN: 7804) being deployed. Photo by Alexandra Fine (AOML/CIMAS).

Table 7. Float deployment information for Core Argo floats

Dep. #	Serial #	Lat (°N)	Lon (°W)	Date and Time (GMT)	Notes
1	7792	-7.00	29.18	2023-03-07 22:30	Water release didn't work
2	7712	11.00	29.00	2023-03-21 19:52	
3	7791	23.02	27.35	2023-03-28 21:21	Water release didn't work
4	7804	27.52	24.78	2023-03-31 18:42	

5.2 Biogeochemical Argo Floats

Principal Investigators: David Nicholson (WHOI), Susan Wijffels (WHOI)
Shipboard personnel: Ellen Park (MIT/WHOI)

On the first leg of this cruise, four biogeochemical (BGC) Argo floats were deployed as a part of the Global Ocean Biogeochemistry (GO-BGC) program, which is funded by NSF Award OCE-1946578.

All floats deployed were Seabird NAVIS floats, which were provided by the WHOI Float Lab. Floats were deployed by hand lowering each float with line from the stern of the ship, according to the WHOI GO-BGC Navis Floats Deployment Procedures manual (see Figure 4).

Figure 4. GO-BGC WMO 4903489 (Adopt-a-float: Data Diver) being deployed. Photo by Tyler Christian (AOML/CIMAS).

All floats were deployed after stations where Bio-casts occurred and ideally after a full-suite CTD cast (see Table 8). Bio-casts were CTD casts where HPLC, POC, and other bio-optical water samples were collected by the Bio-GO-SHIP group. Full-suite CTD casts were those where all parameters (CFCs, dissolved oxygen, pH, total alkalinity, pCO_2, dissolved inorganic carbon, nutrients, salts) were measured at all depths.

HPLC and POC water samples were collected and filtered specifically for float deployments at 5 depths: surface, base of mixed layer depth (MLD), between the MLD and chlorophyll maximum, the chlorophyll maximum, and the chlorophyll maximum plus 50 m. The SIO Oceanographic Data Facility organized these measurements. The CTD did not have a fluorometer, so the chlorophyll maximum plus 15 meters. Duplicates of both HPLC and POC were taken at random depths. Wet filter blanks were collected from the filtration of the duplicate samples for both HPLC and POC. Dry filter blanks were saved for HPLC and POC were saved for each float. We received assistance from Bio-GO-SHIP group (Star Dressler (U. Guam) and Tyler Christian (AOML/CIMAS)) for the collection and filtration of the HPLC and POC samples. The HPLC samples will be run by NASA and the POC samples will be run by the SIO Aluwihare lab.

All floats were adopted by schools and programs as a part of the Adopt-a-float program. Posts were written following each floats deployment for the GO-BGC Expedition log, which was managed by George Matsumoto and Jennifer Magnusson (MBARI).

Table 8. Float deployment information for GO-BGC floats.

Dep. #	Serial #	WMO	Lat (°N)	Lon (°W)	Date and Time (GMT)	Station #	Full Suite Cast?
1	1475	4903489	-4.48	25.00	2023-03-10 18:18	4	Y*
2	1356	4903486	4.52	25.75	2023-03-17 19:58	28	Y+
3	1361	4903487	24.52	26.47	2023-03-29 20:37	68	Y+

* All parameters samples at all depths, except for pCO_2, which sampled at almost all depths

+ Dissolved organic carbon measured at all depths at this station

5.3 Surface Velocity Program (SVP) Drifters

Principal Investigator: Shaun Dolk (AOML)

SVP drifters were deployed from the back deck as the ship left each station, except for the first which was deployed during the initial transit. Each drifter was unwrapped and thrown off the back deck by two people, typically one scientist and one crew member. Information for each SVP drifter can be found in the Table below.

Table 9. Information for SVP Drifter deployments.

Dep. #	Serial #	Lat (°N)	Lon (°W)	Date and Time (GMT)	Station #	Speed (knots)
1	61594030	-6.43	26.80	2023-03-08 12:04	N/A	10.5
2	61458810	6.97	26.99	2023-03-19 06:10	33	4.8
3	61458820	11.01	29.00	2023-03-21 19:59	41	5.3
4	61458850	15.01	29.01	2023-03-24 05:54	49	5.6
5	61595080	18.02	29.00	2023-03-25 22:35	55	6.2

6. Cruise Measurements

6.1 Lowered Acoustic Doppler Current Profiler (LADCP)

Principal Investigator: Andreas Thurnherr (LDEO)
Watchstanders: Kierran Claasen (UCSD), Jay Hooper (AOML/CIMAS), Christian Saiz (AOML/CIMAS)

Data acquisition and QC

Initial Setup
Full depth profiles of horizontal and vertical velocities were obtained using two Acoustic Doppler Current Profilers (ADCPs) supplied by the Lamont-Doherty Earth Observatory. One ADCP was mounted in the upward position (to function as the uplooker), while the other was mounted below it in the downward position (to function as the downlooker; Figure 5). These devices were mounted on the CTD rosette along with a 48V battery provided by NOAA/AOML, completing the LADCP package. Because the package is self-contained, deck cables were required to connect the system from the sampling bay door through the outer wall of the Wet Lab, where the acquisition computer and charger were housed. A single charger with an eisco voltage meter was connected by NOAA/AOML to help monitor the power supply to the LADCP battery before deployment. While the CTD was on deck it was connected to the acquisition computer and charger, consistently receiving power until the next cast. This meant that there was voltage on the LADCP package cables, and that extra care was taken when dummying them.

The deck cables were permanently connected to the acquisition computer using RS232-to-USB adapters. Between 10-15 minutes before a cast, the LADCPs were woken via commands from the data acquisition computer and previous cast data was deleted. The LADCPs were then disconnected from the battery charger via a switch on the benchtop. In the CTD bay, the deck cables were disconnected from the permanently installed star cable on the rosette package, and all terminal ends of the cables were dummied up. During this action, confirmation of pinging from the ADCPs was confirmed, and the terminal ends were secured using a velcro strap to avoid whipping. The survey technician on shift was then informed that the package was ready for deployment, and relevant log information was recorded. Immediately after the CTD was secured in the bay after recovery, the terminal ends on the package were rinsed with fresh water, dummies removed, and then connected to the deck cables. Because there is voltage on the cables, this action took place before any sampling occurred and the cable was set up out of the way to avoid potential damage.

At the bench the battery charger was switched on, the time was recorded, and commands were run to begin downloading the LADCP data. The system stayed in this configuration until the next cast. Once data finished downloading (~20 minutes for casts >4000m), the files were checked using an integration of vertical velocities in time to identify the zmax (deepest depth recorded

by the downlooker) and zend (shallowest depth recorded by the uplooker). Both values were then recorded on the corresponding log sheet for each profile.

There were three watch standers for the cruise, and the overlap in shifts occasionally resulted in errors for station numbers on the logsheets. The LADCP numbers corresponding to each CTD Station can be found in Table 14 in Section 7.

Final setup

The first CTD package was unfortunately lost to the sea at CTD station 026.01 during this cruise. For the second CTD package, all benchtop configurations and deck cables were consistent with initial setup-though only a downlooker was used for the remainder of Leg 1 (Figure 6).

Note: The LADCP package was used during 'bio casts', which occurred prior to regular CTD casts once on station. The bio cast was always sent to a depth of 1000m, and all have been noted in Table 14 (Section 7).

Instrumentation

Initial setup

Both the uplooker (s/n 3441) and downlooker (s/n 24477) used for this cruise (A16) were 300kHz TRDI Workhorse Monitor ADCP. The downlooker was fitted with a custom accelerometer package.

Final setup

When a new CTD package was constructed, only one ADCP was available to replace the lost two. This was installed as the downlooker (s/n 754). Occasionally an error would occur during downloading or starting the LADCPs, but resending the commands was sufficient to avoid issues. There were no obvious instrumentation problems during the cruise.

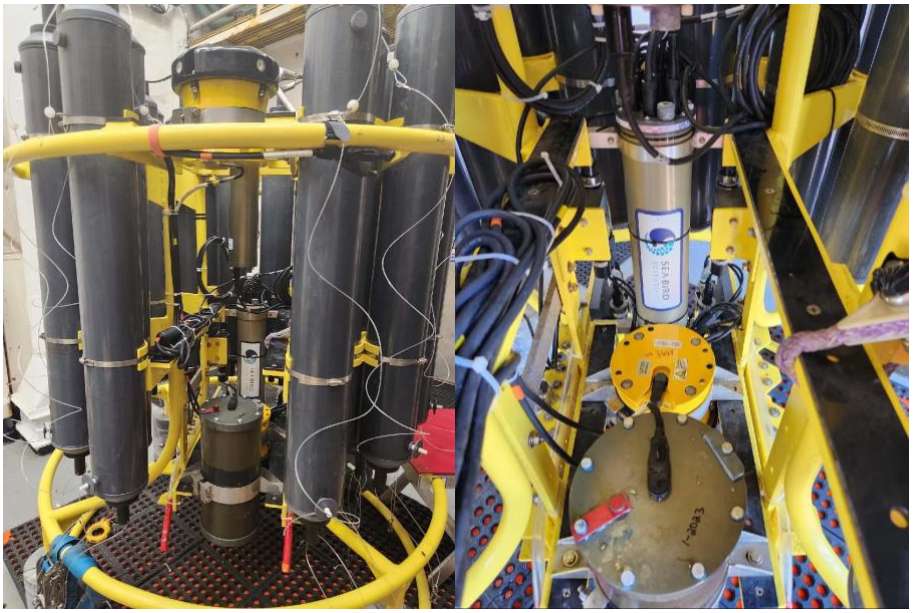

Figure 5. Complete LADCP package setup for Part I of A16N Leg 1. The left image shows the entire package, while the right highlights the position of the downlooker.

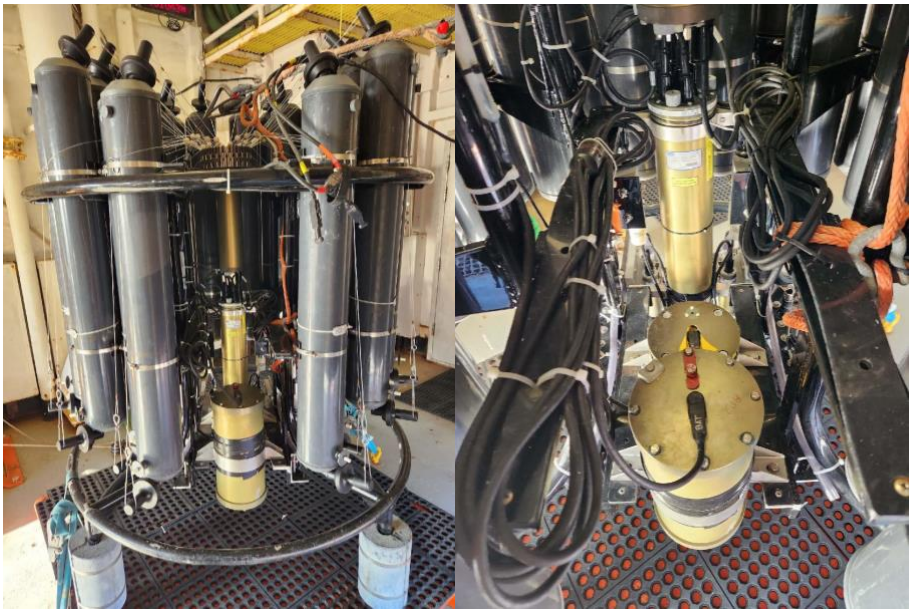

Figure 6. Complete LADCP package setup for Part II of A16N Leg 1. The left image shows the entire package, while the right highlights the position of the downlooker.

6.2 Chlorofluorocarbons (CFCs) and sulfur hexafluoride (SF_6)

Principal Investigator: Rolf Sonnerup (CICOES)
Analysts: David Cooper (CICOES), Melissa Miller (CICOES), and Rachel Bramblett (U. Georgia)

Samples for the chlorofluorocarbons (CFCs, freons) F11 and F12, sulfur hexafluoride (SF_6) and nitrous oxide (N_2O) were collected and analyzed. Seawater samples were taken from all casts, with full profiles taken from most casts and strategically determined bottles sampled from the remaining casts. These measurements are complemented by periodic measurements of air samples.

Seawater samples were drawn from 10 L Niskin bottles. Samples for CFC and SF_6 were the first samples drawn, taking care to check the integrity of the sample and coordinate the sampling analysts to minimize any time between the initial opening of each bottle and the completion of sample drawing. To minimize contact with air, the CFC samples were drawn directly through the stopcocks of the Niskin bottles into 250 mL precision glass syringes. Syringes were rinsed and filled via three-way plastic stopcocks. The syringes were subsequently held at 0-5 degrees C until 30 minutes before being analyzed. At that time, the syringe was placed in a bath of water heated at approximately 30˚C.

For atmospheric sampling, a ~90 m length of 3/8" OD Dekaron tubing was run from the forward tower on the bow of the ship. A flow of air was drawn through this line into the analytical van using an air-cadet pump. The air was compressed in the pump, with the downstream pressure held at ~1.4 atm. using a backpressure regulator. A tee allowed a flow (100 mL/min) of the compressed air to be directed to the gas sample valve of the CFC analytical system, while the bulk flow of the air (>7 L/min) was vented through the backpressure regulator. Analysis of bow air was performed at several locations along the cruise track. Approximately five measurements were made at each location to increase the precision. Atmospheric data were not submitted to the database, but were found to be in excellent agreement with current global databases.

Concentrations of CFC-11, CFC-12, SF_6 and N_2O in air samples, seawater samples and gas standards were measured by shipboard electron capture gas chromatography (ECD-GC) using techniques described by Bullister and Wisegarver (2008). This method has been modified with the addition of an extra ECD to accommodate N_2O analysis. For seawater analyses, water was transferred from a glass syringe to a glass sparging chamber (~200 mL). The dissolved gases in the seawater sample were extracted by passing a supply of CFC-free purge gas through the sparging chamber for a period of 6 minutes at 140 - 150 mL/min. Water vapor was removed from the purge gas by passage through a Nafion drier, backed up by a 18 cm long, 3/8" diameter glass tube packed with the desiccant magnesium perchlorate. This tube also contained a short length of Ascarite to remove carbon dioxide, a potential interferent in N_2O analysis. The sample gases were concentrated on a cold-trap consisting of a 1/16" OD stainless steel tube with a ~5 cm section packed tightly with Porapak Q (60-80 mesh), a 22 cm section packed with Carboxen 1004 and a 2.5 cm section packed with molecular sieve MS5A. A neslab cryocool was used to cool the trap, to approximately -70°C. After 6 minutes of purging, the trap was isolated, and it

was heated electrically to ~170°C. The sample gases held in the trap were then injected onto a precolumn (~60 cm of 1/8" O.D. stainless steel tubing packed with 80-100 mesh Porasil B, held at 80°C) for the initial separation of CFC-12 and CFC-11 from later eluting peaks. After the F12 had passed from the pre-column through the second pre-column (22 cm of 1/8" O.D. Stainless steel tubing packed with Molecular Sieve 5A, 100/120 mesh) and into the analytical column #1 (~170 cm of 1/8" OD stainless steel tubing packed with MS5A and held at 80°C) the outflow from the first precolumn was diverted to the second analytical column (~150 cm 1/8" OD stainless steel tubing packed with Carbograph 1AC, 80-100 mesh, held at 80°C). After F11 had passed through the first precolumn, the flow was diverted to a third analytical column (1/8" stainless steel tube with 30cm Molecular Sieve 5A, 60/80 mesh) for N_2O analysis. The first pre-column was then backflushed and vented. The first two analytical columns and precolumn 1 were held isothermal at 80 degrees C in an Agilent (HP) 6890N gas chromatograph with two electron capture detectors (250°C). The third analytical column and second pre-column were held at 160C in a Shimadzu GC-8A gas chromatogram, with the detector held at 250C.

The analytical system was calibrated using a blended standard gas (seawater ratio, PMEL 72611), with available further reference to a second atmospheric ratio standard. Gas sample loops of known volume were thoroughly flushed with standard gas and injected into the system. The temperature and pressure was recorded so that the amount of gas injected could be calculated. The procedures used to transfer the standard gas to the trap, precolumn, main chromatographic column, and EC detector were similar to those used for analyzing water samples. Four sizes of gas sample loops were used. Multiple injections of these loop volumes could be made to allow the system to be calibrated over a relatively wide range of concentrations. Air samples and system blanks (injections of loops of CFC-free gas) were injected and analyzed in a similar manner. The typical analysis time for seawater, air, standard or blank samples was ~12 minutes. Concentrations of the CFCs in air, seawater samples, and gas standards are reported relative to the SIO98 calibration scale (e.g. Bullister and Tanhua, 2010). Concentrations in air and standard gas are reported in units of mole fraction CFC in dry gas, and are typically in the parts per trillion (ppt) range. Dissolved F11 and F12 concentrations are given in units of picomoles per kilogram seawater (pmol/kg), SF_6 concentrations are given in femtomoles per kilogram of seawater (fmol/kg). N_2O concentrations are given in nanomoles per kilogram of seawater (nmol/kg). The analytical system was calibrated by fitting their chromatographic peak areas to multi-point calibration curves, generated by injecting multiple sample loops of gas from the working standard into the analytical instrument. The response of the detector remained relatively constant during the cruise. Due to limited time before sampling began, partial-range calibration curves were used when possible during the cruise and a full calibration was run at the conclusion of Leg 1. Single injections of a fixed volume of standard gas at one atmosphere were run much more frequently (at intervals of ~90 minutes) to monitor and normalize short-term changes in detector sensitivity.

The purging efficiency of the stripper was estimated by re-purging a water sample in the upper concentration range and measuring the residual signal. At a flow rate of 120 cc/min for 6 minutes, the purging efficiency for SF_6 and F12 was greater than 99% and the efficiency for F11 was about 99%. The purging efficiency for N_2O was about 95%, but subject to some degree of

variability due to changes in flow rate and purging temperature. Correction is made for this variability, together with correction for any measured stripper blank value.

Results of 1771 seawater samples have been submitted from the 75 stations of Leg 1. Duplicates were taken from 74 stations to estimate precision and variability. These duplicates are divided between lower level CFC/SF_6 samples from deeper water (F11 < 0.4 pmol/kg) and higher level samples taken from the upper water column (F11 > 0.4 pmol/kg). N_2O samples were not divided in this manner due to its ubiquity in the water column. From the higher level samples, we calculate the average deviation to be less than 0.6% from the mean of the pairs for F12, F11 and N_2O measurements, and 3% from the mean for SF_6 measurements. Deviation from the mean of pairs from the lower concentration CFC/SF_6 samples averaged less than 4% from the mean for F12, less than 1% from the mean for F11, and is not calculable for SF_6 due to the exceedingly low levels of this gas present in deeper water, frequently at or below the limit of detection (approximately 0.02 fmol/kg). Due to current software limitations, many of the extremely low SF_6 data were unresolved from baseline noise. It is anticipated that some of the flagged data will be replaced with more accurate values.

The atmospheric samples were run whenever time permitted, often during CTD sampling. Data are limited for this reason, but serve as a check for our system by comparing with the global database, and as a basis for calculating the surface saturation state in future analysis of our water data. In summary, the atmospheric data agree very well with the expected global data set (current data in parentheses), with mean concentrations of 11.6 ppt SF_6 (11.6 ppt), 497 ppt CFC12 (485 ppt), 222 ppt CFC11 (219 ppt) and 334 ppb N_2O (336 ppt).

A small number of water samples had anomalous SF_6 or CFC concentrations relative to adjacent samples. These samples occurred sporadically during the cruise, were not clearly associated with other features in the water column (e.g., anomalous dissolved oxygen, salinity, or temperature features) and are omitted from the reported data.

References

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Bullister, J.L. and D.P. Wisegarver. 2008. The shipboard analysis of trace levels of sulfur hexafluoride, chlorofluorocarbon-11 and chlorofluorocarbon-12 in seawater. Deep-Sea Res. I, v. 55, pp. 1063-1074.

6.3 Dissolved Oxygen

Principal Investigator: Chris Langdon (RSMAS)
Analysts: Emma Pontes (RSMAS), Riley Palmer (RSMAS)

Equipment and Techniques

Dissolved oxygen analyses were performed with an automated titrator using amperometric end-point detection (Langdon, 2010). Sample titration, data logging, and graphical display were performed with a PC running a LabView program written by Ulises Rivero of AOML. Lab temperature was maintained at 16.3-18.7^{o}C. The temperature-corrected molarity of the thiosulfate titrant was determined as given by Dickson (1994). Thiosulfate was dispensed by a 2 mL Kloehn syringe driven with a stepper motor controlled by the titrator. The whole-bottle titration technique of Carpenter (1965), with modifications by Culberson et al. (1991), was used. Three to four replicate 10 mL iodate standards were run every 1-6 days (Average SD = 0.8 uL) when a new thiosulfate bottle was used and when the current thiosulfate bottle was half full. The reagent blank calculated as the difference between V1 and V2, the volumes of thiosulfate required to titrate 1-mL aliquots of the iodate standard, was determined at the beginning of leg one.

Sampling and Data Processing

Dissolved oxygen samples were drawn from Niskin bottles into calibrated 125-150 mL iodine titration flasks using silicon tubing to avoid contamination of DOC and CDOM samples. Samples were drawn by counting while the flask was allowed to fill at full flow from the Niskin. This count was then doubled and repeated thereby allowing the flask to be overflowed by two flask volumes. At this point the silicone tubing was pinched to reduce the flow to a trickle. This was continued until a stable draw temperature was obtained on the Digi-sense Thermistor Meter. During sampling, the thermistor was stored in a plastic bag with 1 cup of uncooked rice to reduce moisture. After every cast, the thermistor was removed from the bag, and the male-connection of the sensor was submerged in a bowl of uncooked rice anytime it was not in use to further reduce moisture. Draw temperatures were used to calculate umol/kg concentrations, and provide a diagnostic check of Niskin bottle integrity. 1 mL of $MnCl_2$ and 1 mL of NaOH/NaI were added immediately after drawing the sample using a SOCOREX Calibrex 520 dispenser. The flasks were then stoppered and shaken well. DIW was added to the neck of each flask to create a water seal. 24 samples were drawn at each station. At stations 1-27, only one duplicate was drawn from one Niskin. At stations 28-80, a second duplicate was added. The total number of samples collected from the rosette was 1949. The samples were stored in the lab in plastic totes at room temperature for 30-40 minutes before analysis. The data were incorporated into the cruise database shortly after analysis. Thiosulfate normality was calculated for each standardization and corrected to the laboratory temperature. This temperature ranged between 16.3 and 18.7 C. Reagent blanks were run at the beginning (1.5±1.0 uL) of leg one.

Volumetric Calibration

The dispenser used for the standard solution (SOCOREX Calibrex 520) and the burette were calibrated gravimetrically just before the cruise. Oxygen flask volumes were determined gravimetrically with degassed deionized water at AOML. The correction for buoyancy was applied. Flask volumes were corrected to the draw temperature.

Duplicate Samples

Duplicate samples were initially drawn at one depth per station (up to Station 27). Starting at Station 28, two duplicates were drawn: the first from Niskin 1 (deepest depth of that station) and the second from a different depth. The Niskins selected for the second duplicate, and hence the oxygen flasks, were changed for each cast. A total of 127 sets of duplicates were run. One set of duplicates were removed from analysis (Station 30, Niskin 6) and coded with quality flag #3 due to sampling error.

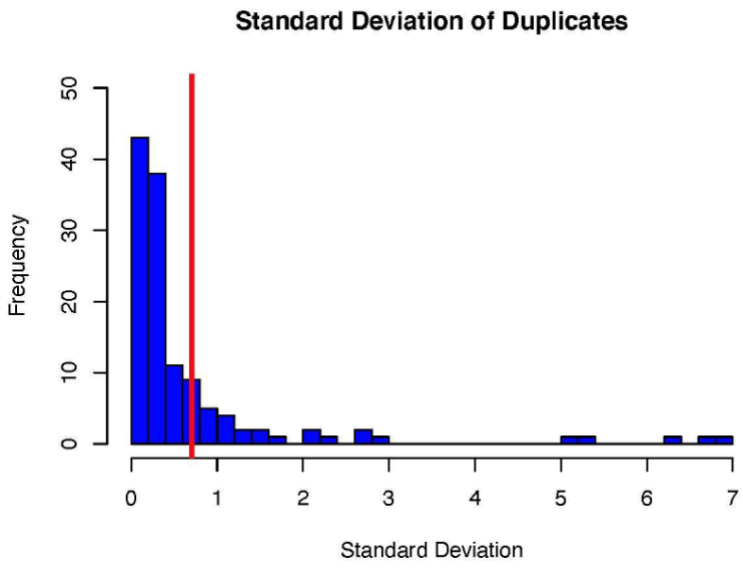

Figure 7. Standard deviation of duplicate oxygen analyses performed during A16N 2023. Average is 0.71 umol/kg (red line), median is 0.30 umol/kg, IQR is 0.66 - 0.16 = 0.5 umol/kg, and n = 126.

Quality Coding

Preliminary quality code flags have been assigned to the oxygen data. A summary of the quality coding can be found in Table 10.

Table 10. Summary of quality code flags for leg one of A16N 2023.

O2 Quality Flag	Number	Reason
3	26	sample value high or low compared to CTD value, potential sampling error, endpoint not reached during titration
4	2	known sampling error
6	116	average of duplicates
9	14	missing value: Niskin not sampled due to leak, sample discarded when flask broke

Problems

NaI/NaOH dispenser

At two points in the cruise (Stations 34 and 35) the NaI/NaOH dispenser was found to be sticking and was flushed with 10% H_2SO_4 followed by DIW. Since then, the NaI/NaOH dispenser was regularly flushed every 2 days to reduce sticking.

Slope increase

A total of 8 samples exceeded the number of data points during titration (n>30). All occurrences of a sample exceeding data points occurred very close to the end of titration (endpoint #7 or 8). When this happened, O_2 umol/kg was recalculated based on temperature, salinity, closest endpoint, thiosulfate temperature and molarity, and density of seawater. The first occurrence of a sample exceeding data points occurred at Station 29 (Niskin 5). At Station 40, after 6 samples had exceeded the number of data points, the slope was increased from 6 to 6.5. After this increase, 1 sample exceeded the number of data points and the slope was raised again from 6.5 to 7.

New electrode

The electrode (accumet) was replaced at Station 60 due to inaccurate titration curves. Upon replacing the inaccurate electrode with a new one, the titrations curves were reliable. A series of underway samples (surface water) were titrated at different slopes (m=4 - 6.5 increasing in 0.5 increments) to determine the most accurate slope for the new electrode (15 < number of data points < 30) and a slope of 6 was used for the remainder of leg one.

Broken flasks

Flask 12: broken at Station 18; sample recovered and run. Replaced with flask 26 on Station 19.
Flask 4: broken at Station 19 with sample discarded. Replaced with flask 34 on Station 20.
Flask 19: broken at Station 37; sample recovered and run. Replaced with flaks 49 at Station 38.
Flask 16: broken during sampling at Station 65, replaced with flask 46 immediately.
Flask 20: broken during sampling at Station 68, replaced with flask 50 at Station 69.

References

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6.4 pH

PI: Chris Langdon (RSMAS)
Analysts: Jessica Leonard (RSMAS), Mackenzie Blanusa (RSMAS), Caroline Branan (RSMAS)

Sampling
Samples were collected in 250 mL narrow mouth borosilicate glass bottles using silicone tubing that fit over the stopcock. Bottles were rinsed a minimum of 2 times and overflowed by a minimum of the bottle's volume. Samples were sealed by overflowing to eliminate headspace and using a glass stopper and a plastic cap. Samples were stored in a water bath kept at 25.0°C until measurement. From station 39 onwards, two duplicates were collected from each station. Samples were collected in the same bottles used by total alkalinity. Multiple samples per day were analyzed with double the amount of indicator to correct for pH changes as a result of adding the indicator.

Analysis
pH (total scale) was measured spectrophotometrically using a HP8453 spectrophotometer and in accordance with the methods outlined by Carter et al. (2013). A recirculating Thermo Scientific Haake A10 water bath maintained the spectrophotometric cell temperature at 25.0°C. Sample temperature was measured using a Hart Scientific FLUKE 1523 Reference Thermometer. The sample was drawn into the system using a silicon tube connected to a 10 mL syringe controlled using a Kloehn 6v syringe pump. The first pull of the sample rinsed the syringe. The second pull provided a blank for the spectrophotometer. During the third pull, the indicator meta-cresol purple (mCP) was injected automatically by the Kloehn 6v pump. A jacketed 100 mm quartz flow cell (Starna Cells 583.65-Q-100) was filled automatically using the Kloehn 6v syringe pump. The absorbance of light was measured at four different wavelengths (434 nm, 578 nm, 730 nm, and 488 nm). The ratios of absorbency at the different wavelengths (434 nm and 578 nm), temperature, and salinity were used to calculate pH on the total pH scale with wavelength 730 nm used to correct any disturbances ($R = (A_{578} - A_{730})/(A_{434} - A_{730})$). The equations outlined in Liu et al. (2011) were used for calculations. The isosbestic point (488 nm) will be used for the indicator correction. Salinity data were obtained from the conductivity sensor on the CTD. These data were later corrected by shipboard measurements.

Reagants
Purified mCP indicator was obtained from Dr. Robert Byrne's lab at the University of South Florida, and prepared as 2 mM solution with ion strength of 0.7 M (ion strength adjusted using NaCl).

Data Processing
pH was calculated using R-ratio, temperature, and salinity with equations from Liu et.al (2011). mCP perturbation correction was performed using the 'double dye' method. Briefly, for each station we chose 1-2 samples with different pH and measured R-ratio twice (with the dye addition doubled at the 2nd time) to get difference in R-ratio (ΔR). And then the mCP perturbation can be corrected with a linear regression between R-ratio and ΔR. We used the

same batch of mCP throughout Leg 1, therefore we combined all the data from 'double dye' experiments to get one correction curve.

Addition of the indicator affects the pH of the sample, and the degree to which pH is affected is a function of the pH difference between the seawater and the indicator. Therefore, a correction is applied for each batch of dye. Multiple samples from each day were measured twice, one normally, and one with double the amount of indicator. The measured absorbance ratio (R) and an isosbestic absorbance (A_{iso}) were determined for each measurement.

$$R = \frac{A_{578} - A_{488}}{A_{434} - A_{488}}$$

$$A_{iso} = A_{488} - A_{base}$$

The change in R for a given change in , was then plotted against the measured R ratio for the normal amount of dye and fitted with a linear regression. From this fit the slope and intercept are determined by:

$$\Delta R / \Delta A_{iso} = bR + a$$

From this the corrected ratio (*R'*) corresponding to the measured absorbance ratio if no indicator dye were present can be determined by:

$$R' = R - A_{iso}(bR + a)$$

The correction has not yet been applied to the samples. This data should be treated as preliminary. The standard deviation of the duplicates was 0.0007 (N = 65). The preliminary quality controls are given in Table 11.

Table 11. 2023 A16N Leg 1 pH quality control code assignment.

Number of Samples	**1800**
Acceptable (QC = 2)	1669
Questionable (QC = 3)	3
Duplicate (QC = 6)	65
Missing Value (QC = 9)	63

Problems

In the beginning of Leg 1 during transit to the first station, the indicator bag in use suffered a leak. The connector on the mCP reagent bag disconnected and ~ 100 mL of mCP solution was lost. There was still enough of this indicator batch for Leg 1. The Kloehn 6v syringe pump failed in the 2nd week and was immediately replaced.

References

Carter, H.A., Ceballos-Osuna, L., Miller, N.A. and Stillman, J.H., 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab Petrolisthes cinctipes. *Journal of Experimental Biology*, *216*(8), pp.1412-1422.

Liu, X., Patsavas, M.C. and Byrne, R.H., 2011. Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements. *Environmental science & technology*, *45*(11), pp.4862-4868.

6.5 Total Alkalinity (TA)

Principal Investigators: Chris Langdon (RSMAS)
Analysts: Bo Yang (RSMAS), Jessica Leonard (RSMAS), and Caroline Branan (RSMAS)

Sampling
We used the leftover from pH measurements for TA measurements. A custom-made sample dispenser with a glass pipette was used to volumetrically measured out an accurate amount (96.083 ml) of sample for titration.

Analysis
An automatic open-cell titration system built by Dr. Andrew Dickson's lab was used for the TA measurements, which consists of a Metrohm 876 Dosimat titrator (controlled by the PC via a NI USB-6501 digital I/O), a Keysight DAQ970A data acquisition system, a pH Metrohm glass electrode (6.0262.100), a Sierra SmartTrak 50 mass flow controller, a Tetra air pump, and a custom-made amplifier power by two 9v batteries. A custom-made LabView software was used for system control and TA calculation.

During the titration, an initial aliquot of approximately 2.5-2.6 mL of standardized hydrochloric acid (~0.1M HCl in ~0.6M NaCl solution) is first delivered and the sample is stirred and purged (with air) for 5 minutes at a rate of 200 scc/m to remove any CO_2 generated during this process. After that, a series of aliquots of 0.05 ml HCl were added and the pH after each addition was measured by the pH glass electrode. The total alkalinity is computed from the titrant volume and pH values using a non-linear least-squares approach over the pH range of 3.5 to 3.0 (Dickson 2007). Salinity data from CTD was used for TA calculation.

The mean difference between two duplicate samples (sample 2 – sample 1) is -0.05 +- 1.42 umol/kg (n=65).

Reagants
Hydrochloric acid (~ 0.1 M) prepared in ~ 0.6 M NaCl solution was used for titration.

Standardization
HCl solution was standardized in the lab before the cruise using the certified reference material (CRM) Batch 197 from Dr. Andrew Dickson's lab at SIO. During the cruise, the acid concentration was checked several times by measuring CRM Batch 202 (leftover from DIC measurements).

Data Processing
A custom-made LabView software was used for system control and TA calculation, which automatically calculated the TA with inputs of salinity, temperature, and acid concentration. TA is computed with the sample's mass (measured volumetrically), salinity, the mass of HCl added, the HCl concentration (standardized with CRM), and the cell temperature. A non-linear least square fitting is used to get the end point of the titration and the TA of the sample. For the details, see Dickson et al. (2007).

Problems

We brought two TA units on this cruise. One unit was faulty and we could not get it work during the transit to Station 1. Therefore all the measurements were done with the second unit. Electromagnetic fields (EMFs) were hypothesized as a possible source of interference for measurements. For future TA group, it would be better to set up the TA system in a space that is relatively stable (safer to operate the fragile glass electrode), far from known EMF sources (e.g. motor, UPS), and without AC vents blasting at the instrument.

References

Dickson, A.G.; Sabine, C.L. and Christian, J.R. (eds) (2007) Guide to best practices for ocean CO2 measurement. Sidney, British Columbia, North Pacific Marine Science Organization, 191pp. (PICES Special Publication 3; IOCCP Report 8). DOI: https://doi.org/10.25607/OBP-1342

6.6 pCO2

Principal Investigator: Rik Wanninkhof (AOML)
Analysts: N. Patrick Mears (AOML/CIMAS), Katelyn Schockman (AOML/CIMAS)

Sampling:
Samples were drawn from 11-L Niskin bottles into 500 mL glass bottles using nylon tubing with a Silicone adapter that fit over the drain cock. Bottles were first rinsed three times with ~25 mL of water. They were then filled from the bottom, overflowing a bottle volume while taking care not to entrain any bubbles. About 5 mL of water was withdrawn to allow for expansion of the water as it warms and to provide space for the stopper and tubing of the analytical system. Saturated mercuric chloride solution (0.24 mL) was added as a preservative. The sample bottles were sealed with glass stoppers lightly covered with grease and were stored at room temperature for a maximum of seven hours prior to being run.

The analyses for pCO_2 were done with the discrete samples at 20°C. A primary water bath was kept within 0.02°C of the analytical temperature; a secondary bath was kept within 0.3°C the analytical temperature. The majority of the samples were analyzed in batches of twelve bottles, which took approximately 3.5 hours including the six standard gases. When twelve bottles were moved into the primary water bath for analyses, the next twelve bottles were moved into the secondary water bath. No sample bottle spent less than two hours in the secondary water bath prior to being moved to the analytical water bath. Duplicate samples from the same Niskin were drawn to check the precision of the sampling and analysis.

1712 unique samples were drawn from 75 CTD casts covering 95% of all unique depths. Seventy-five sets of duplicate bottles were drawn at numerous depths. The average relative standard error was 0.08%, while the median relative error was 0.04%.

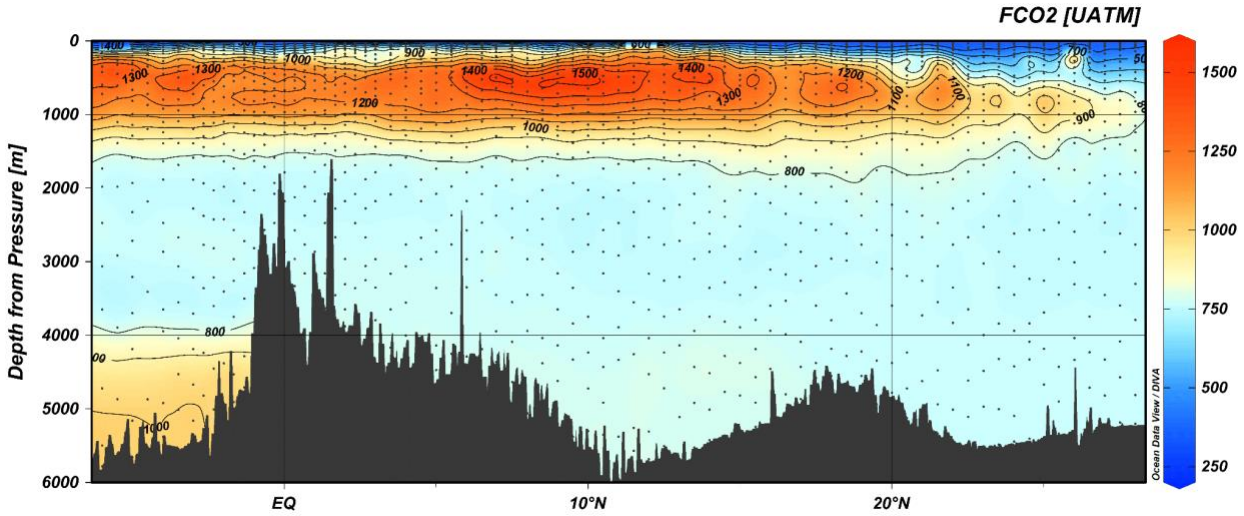

Figure 8. Measured fCO_2 from bottle data collected along GO-SHIP A16N line from 6°S to 28°N.

One sample was lost due to a stuck stopper that resulted in being unable to safely fit the bottle to the instrument.

Underway Sampling

Underway samples were collected every 4 hours from the underway seawater line located in the hydrolab that is connected to the same seawater line as the underway pCO_2 system located in the same space. The seawater is pumped from a bow seawater inlet located approximately 5.3 meters below the waterline through a sea chest where instruments measure and record temperature and salinity.

A total of 32 underway stations were collected with duplicate samples collected every 4 stations during the transit from Port Suape, Brazil to Station 1 and from Station 76 to Rota, Spain.

Analyzer Description:
The principles of the discrete pCO_2 system are described in Wanninkhof and Thoning (1993) and Chipman et al. (1993). The major difference in the current system is the method of equilibrating the sample water with the constantly circulating gas phase. This system uses miniature membrane contactors (Micromodules from Memrana, Inc.), which contain bundles of hydrophobic micro-porous tubes in polycarbonate shells (2.5 x 2.5 x 0.5 cm). The sample water is pumped over the outside of the tubing bundles in two contactors in series at approximately 25 mL/min and to a drain. The gas is recirculated in a vented loop, which includes the tubing bundles and a non-dispersive infrared analyzer (LI-COR™ model 840) at approximately 30 mL/min.

The flow rates of the water and gas are chosen with consideration of competing concerns. Faster water and gas flows yield faster equilibration. A slower water flow would allow collection of smaller sample volume; plus a slower gas flow would minimize the pressure increase in the contactor. Additionally, the flow rates are chosen so that the two fluids generate equal pressures at the micro-pores in the tubes to avoid leakage into or out of the tubes. A significant advantage of this instrumental design is the complete immersion of the miniature contactors in the constant temperature bath. Also in the water bath are coils of stainless steel tubing before the contactors that ensure the water and gas enter the contactors at the known equilibration temperature.

The instrumental system employs a large insulated cooler (Igloo Inc.) that accommodates twelve sample bottles, the miniature contactors, a water circulation pump, a copper coil connected to a refrigerated circulating water bath, an immersion heater, a 12-position sample distribution valve, two thermistors, and two miniature pumps. The immersion heater works in opposition to the cooler water passing through the copper coil. One thermistor is immersed in the water bath, while the second thermistor is in a sample flow cell after the second contactor. The difference between the two thermistor readings was consistently less than 0.02°C during sample analyses. In a separate enclosure are the 8-port gas distribution valve, the infrared analyzer, a barometer,

and other electronic components. The gas distribution valve is connected to the gas pump and to six standard gas cylinders.

To ensure analytical accuracy, a set of six gas standards (ranging from 288 to 1534 ppm) was run through the analyzer before and after every sample batch. The standards were obtained from Scott-Marin and referenced against primary standards purchased from C.D. Keeling in 1991, which are on the WMO-78 scale.

A custom program developed using LabView™ controls the system and graphically displays the CO_2 concentration as well as the temperatures, pressures and gas flow during the 10-min equilibration. The CO_2 in the gas phase changes greatly within the first minute of a new sample and then goes through nearly two more oscillations. The oscillations dampen quickly as the concentration asymptotically approaches equilibrium. The flows are stopped, and the program records an average of ten readings from the infrared analyzer along with other sensor readings. The data files from the discrete pCO_2 program are reformatted so that a Matlab program designed for processing data from the continuous pCO_2 systems can be used to calculate the fugacity of the discrete samples at 20°C. The details of the data reduction are described in Pierrot et.al. (2009).

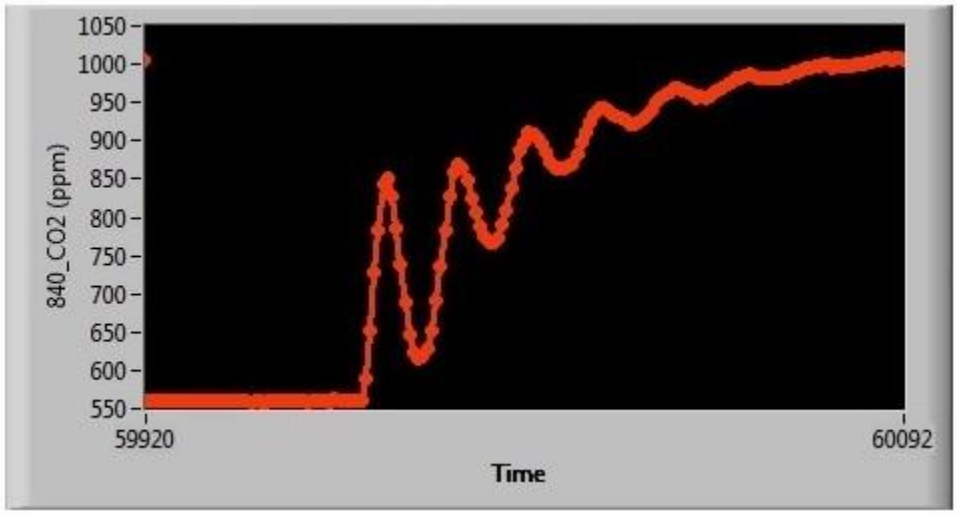

Figure 9. CO_2 oscillations during start of first sample in set of twelve.

The instrumental system was originally designed and built by Tim Newberger and was supported by C. Sweeney and T. Takahashi. Their skill and generosity has been essential to the successful use and modification of this instrumental system. Kieran Claassen (UCSD) and Alexandra Fine (AOML/CIMAS) assisted in collecting samples.

Standard Gas Cylinders:

Cylinder#	ppm CO_2
JB03282	288.55
JB03268	384.30

CB11243	591.87
CA05980	792.51
CA05984	1036.95
CA05940	1533.7

References

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6.7 Dissolved Inorganic Carbon (DIC)

Principal Investigators: Rik Wanninkhof (AOML), Richard Feely (PMEL)
Analysts: Charles Featherstone (AOML), Alison MacLeod (AOML/CIMAS)

Sample collection:
Samples for DIC measurements were drawn (according to procedures outlined in the PICES Publication, *Guide to Best Practices for Ocean CO_2 Measurements*) from Niskin bottles into 294 mL borosilicate glass bottles using silicone tubing. The flasks were rinsed once and filled from the bottom with care not to entrain any bubbles, overflowing by at least one-half volume. The sample tube was pinched off and withdrawn, creating a 6 mL headspace, followed by 0.12 mL of saturated $HgCl_2$ solution which was added as a preservative. The sample bottles were then sealed with glass stoppers lightly covered with Apiezon-L grease and were stored at room temperature for a maximum of 12 hours.

Equipment:
The analysis was done by coulometry with two analytical systems (AOML 3 and AOML 4) used simultaneously on the cruise. Each system consisted of a coulometer (CM5015 UIC Inc) coupled with a Dissolved Inorganic Carbon Extractor (DICE). The DICE system was developed by Esa Peltola and Denis Pierrot of AOML and Dana Greeley of PMEL to modernize a carbon extractor called SOMMA (Johnson et al. 1985, 1987, 1993, and 1999; Johnson 1992).

The two DICE systems (AOML 3 and AOML 4) were set up in a seagoing container modified for use as a shipboard laboratory on the aft main working deck of the *R/V Ronald H. Brown.*

DIC Analysis:
In coulometric analysis of DIC, all carbonate species are converted to CO_2 (gas) by addition of excess hydrogen ion (acid) to the seawater sample, and the evolved CO_2 gas is swept into the titration cell of the coulometer with pure air or compressed nitrogen, where it reacts quantitatively with a proprietary reagent based on ethanolamine to generate hydrogen ions. In this process, the solution changes from blue to colorless, triggering a current through the cell and causing coulometrical generation of OH^- ions at the anode. The OH^- ions react with the H^+, and the solution turns blue again. A beam of light is shone through the solution, and a photometric detector at the opposite side of the cell senses the change in transmission. Once the percent transmission reaches its original value, the coulometric titration is stopped, and the amount of CO_2 that enters the cell is determined by integrating the total change during the titration.

DIC Calculation:
Calculation of the amount of CO_2 injected was according to the CO_2 handbook (DOE 1994). The concentration of CO_2 *($[CO_2]$)* in the samples was determined according to:

$$[CO_2] = \text{Cal. Factor} * \frac{(\text{Counts} - \text{Blank} * \text{Run Time}) * K\ \mu\text{mol/count}}{\text{pipette volume} * \text{density of sample}}$$

where *Cal. Factor* is the calibration factor, *Counts* is the instrument reading at the end of the analysis, *Blank* is the counts/minute determined from blank runs performed at least once for each cell solution, *Run Time* is the length of coulometric titration (in minutes), and *K* is the conversion factor from counts to micromoles.
The instrument has a salinity sensor, but all DIC values were recalculated to a molar weight (µmol/kg) using density obtained from the CTD's salinity. The DIC values were corrected for dilution due to the addition of 0.12 ml of saturated $HgCl_2$ used for sample preservation. The total water volume of the sample bottles was 294 mL (calibrated by Esa Peltola, AOML). The correction factor used for dilution was 1.0004. A correction was also applied for the offset from the CRM. This additive correction was applied for each cell using the CRM value obtained at the beginning of the cell. The average correction was
2.57 µmol/kg for AOML 3 and 1.16 µmol/kg for AOML 4 (CRM Batch 202).

The coulometer cell solution was replaced after 25 – 28 mg of carbon was titrated, typically after 9 – 12 hours of continuous use. The blanks ranged from 12 to 35.

Calibration, Accuracy, and Precision:
The stability of each coulometer cell solution was confirmed three different ways.
1) Gas loops were run at the beginning of each cell
2) CRM's supplied by Dr. A. Dickson of SIO, were analyzed at the beginning of the cell before sample analysis.
3) Duplicate samples from the same niskin , were measured near the beginning; middle and end of each cell.

Each coulometer was calibrated by injecting aliquots of pure CO_2 (99.999%) by means of an 8-port valve (*Wilke et al., 1993*) outfitted with two calibrated sample loops of different sizes (~1mL and ~2mL). The instruments were each separately calibrated at the beginning of each cell with a minimum of two sets of these gas loop injections.

The accuracy of the DICE measurement is determined with the use of standards (Certified Reference Materials (CRMs), consisting of filtered and UV irradiated seawater) supplied by Dr. A. Dickson of Scripps Institution of Oceanography (SIO). The CRM accuracy is determined manometrically on land in San Diego and the DIC data reported to the database have been corrected to batch 2022 CRM values. The CRM certified value for batch 202 is 2043.33 µmol/k.

The precision of the two DICE systems can be demonstrated via the replicate samples. Approximately 11% of the niskins sampled were duplicates taken as a check of our precision. These replicate samples were interspersed throughout the station analysis for quality assurance and integrity of the coulometer cell solutions. The average absolute difference from the mean of these replicates is 1.61 µmol/kg for AOML 3 and 1.46 µmol/kg for AOML 4. No major systematic differences between the replicates were observed.

The pipette volume was determined by taking aliquots of distilled water from volumes at known temperatures. The weights with the appropriate densities were used to determine the volume of the pipettes.

Table 12. Calibration data during this cruise.

UNIT	Ave L Loop Cal Factor	Ave S Loop Cal Factor	Pipette	Ave CRM[1]	STDEV[1]	AVG Dupes[2]	STDEV Dupes[2]
AOML 3	1.004177	1.005102	26.845 ml	Batch 202: 2040.8, N= 40	1.23	1.61	1.13
AOML 4	1.003966	1.004118	29.391 ml	Batch 202: 2043.6, N = 37	0.63	1.46	1.05

Underway DIC Samples

Underway samples were collected from the flow thru system in the Hydro-Lab during transit. Discrete DIC samples were collected approximately every 4 hours with duplicates every fifth sample. A total of 38 discrete DIC samples including duplicates were collected while underway. The average difference for replicates of underway DIC samples was 1.24 µmol/kg and the average STDEV was 0.88.

Summary:

The overall performance of the analytical equipment was good during the cruise. At the beginning of the cruise the pipette and condenser on DICE 3 had to be replaced due to breakage while in Newport, RI from the cold temperatures. The condenser on DICE 4 also had to be replaced due to breakage. The volume for DICE 3 replacement pipette was estimated and will need to be measured once the DICE van returns to AOML. The data on DICE 3 will be recalculated and updated once the new pipette volume is determined. Valve 13 was replaced on DICE 4 due to pipette filling issues.

Including the duplicates, 1955 samples were analyzed from 75 CTD casts for dissolved inorganic carbon (DIC) which means there is a DIC value for approximately 97% of the niskins tripped. The DIC data reported to the database directly from the ship are to be considered preliminary until a more thorough quality assurance can be completed shore side.

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6.8 Carbon Isotopes ($\delta^{13}C$)

Principal Investigators: Wei-jun Cai (U. Delaware)
Analysts: Bo Dong (U. Delaware), Najid Hussain (U. Delaware)

Sampling
Samples for $\delta^{13}C$-DIC measurements were drawn according to procedures outlined in the PICES Special Publication, Guide to Best Practices for Ocean CO_2 Measurements, from the rosette sample bottles into cleaned 250 mL borosilicate glass bottles. Bottles were rinsed three times and filled from the bottom, with one bottle volume of overflow. After samples were carried back to the lab, 1 mL of water was drawn and thrown away to allow thermal expansion and 50 µL of saturated $HgCl_2$ solution was added to stop biological activities, usually within 1 hour after samples were taken. Sample bottles were then sealed with glass stoppers lightly covered with Apiezon-L grease and stoppers were fixed with rubber bands and clips. Samples were either stored in open boxes for at least 8 hours to attain ambient room temperature before immediate measurement or stored in coolers to take back. $\delta^{13}C$-DIC samples were collected from all niskin bottles corresponding to a variety of depths with two to three replicate samples. Typically the replicate samples were taken from the surface, at the oxygen minimal depth, and bottom rosette sample bottles and run at different times during the cell. No systematic difference between the replicates was observed.

Analysis
The $\delta^{13}C$-DIC analytical equipment was set up in the bio lab. The analysis was conducted with two analytical systems (unit#1 and unit#2) placed on two ends of the bio lab and used simultaneously on Leg 1. Each system is composed of a whole-water CO_2 extraction device with a 12-port sample valve (AS-D1, Apollo Scitech, Newark, DE, USA; www.apolloscitech.com) and a CRDS isotopic detector (G2131-i, Picarro, Santa Clara, CA, USA www.picarro.com). Both instruments were coupled and automated with a single software to simultaneously measure DIC concentrations and $\delta^{13}C$-DIC signals via quantifying the CO_2 extracted from acidified samples. Briefly, an aliquot of sample is acidified with 5% H_3PO_4 in the gas stripping reactor and the liberated CO_2 is brought by the carrier gas (CO_2-free compressed air) to the CRDS analyzer. The raw data for CO_2 ($^{12}CO_2$ + $^{13}CO_2$) and $\delta^{13}C$-CO_2 are read from CRDS and are recorded at ~ 1 Hz frequency for a period of ~600 s. When the CO_2 measurements drops below a preset threshold (i.e., 15 consecutive data points of CO_2 is < (baseline value + 5) ppm), or the change drops below a preset threshold (i.e., standard deviation of CO_2 for 15 consecutive data points is <0.16 ppm), the software will terminate the analysis, because there is only a small amount of CO_2 left in the reactor and further gas stripping would change the area integration value of CO_2 very slowly. Terminating the analysis at this point results in an uncertainty of duplicate analysis <0.1%. The area under the curve of the mole fraction CO_2 gas is integrated over time to derive a net area for quantifying DIC concentrations. The $\delta^{13}C$-DIC is derived as the CO_2 weighted mean of $\delta^{13}C$-CO_2 data with a cutoff point of 400 ppm to avoid high noise at low CO_2 signal.

Three batches of $\delta^{13}C$ standard solutions (GOSHIP-A, GOSHIP-B, GOSHIP-C) with different $\delta^{13}C$ values were prepared by dissolving pure $NaH^{13}CO_3$ and $NaHCO_3$ powder with known $\delta^{13}C$ value

in Milli-Q water. During Leg 1, we used three volumes of a CRM (5.5/6.5/7.5 mL. then 5/6/7.5 mL, then 5.2/6.6/8 mL) to create a working standard curve between the net area and DIC mole amounts, the latter of which is calculated as the product of the CRM's volume and known concentration. The DIC concentration of a sample or homemade standard is then derived from the working standard curve and the known injection sample volume. CRM calibration was conducted every 4 – 7 days, and homemade standard GOSHIP-A series were used for calibration everyday between CRM calibrations. GOSHIP-B and GOSHIP-C series along with opened CRM were added to the sample list every 8 samples to check the accuracy. Time-based linear corrections were made for the δ^{13}C-DIC value obtained by two adjacent measurements of homemade standards whose δ^{13}C values were obtained by the IRMS method from the UC Davis laboratory.

The DIC value of samples was determined according to:
where is the average area under the curve of the mole fraction CO_2 gas integrated over time of 2 measurements with an RSD no larger than 0.1%, slope and intercept are calibration factors obtained from the standard curve, sample volume is volume of sample drawn for measurement (6.5, 6.0, 6.6 mL were used on leg 1), sample density is calculated from the CTD salinity and bio lab room temperature measured with a thermometer of 0.1°C accuracy.

The δ^{13}C value of samples denoted in ‰ was determined according to:
where is the CO_2 weighted mean of δ^{13}C-CO2 data with a cutoff point of 400 ppm, is the δ^{13}C value of reference standard Vienna-PeeDee Belemnite (V-PDB).

1946 CTD samples were collected from 1787 niskin bottles, corresponding to 8.9% duplicate rate. 1475 samples (75.8%) were measured on leg 1 including those measured during the transit to Spain. Among 132 pairs of duplicate samples measured, the average difference is 0.16 µmol/kg for DIC and 0.00‰ for δ^{13}C, the average absolute difference is 1.89 µmol/kg for DIC and 0.07‰ for δ^{13}C, DIC of 109 pairs (82.6%) had a relative standard deviation no more than 0.1% and δ^{13}C of 99 pairs (75.0%) had a standard deviation no more than 0.07‰. Comparing the DIC values to the preliminary DIC values of AOML, the average difference is 1.31 µmol/kg and the average absolute difference is 4.68 µmol/kg among 1460 samples, 724 (49.6%) of them had a relative standard deviation no more than 0.1%. During Leg 1, opened CRM with roughly more than 60% left was measured 128 times on both units as checks, 60 (46.9%) of them were within ±0.1% of given values, 90 (70.3%) of them were within ±0.2%. Further QA/QC will be conducted to improve our data quality, like attempts to correct measurements during unit leaking.

6.9 Radiocarbon (^{14}C)

Principal Investigators: Rolf Sonnerup (CICOES), Roberta Hansman (WHOI)
Sampler: Victoria Dina (WHOI)

A total of 336 samples were collected from 14 stations. Samples were collected about every 2.5 degrees, generally collecting those stations that were sampled on the last occupation of this transect. At each station 21 unique depths were sampled and 3 duplicate samples were collected: one within the top 500 m, one between 500-1500 m, and one deeper than 1500 m. Samples were collected in 100 mL airtight glass bottles. Using silicone tubing, the flasks are rinsed 2 times with the water from the sample bottle. While keeping the tubing near the bottom of the flask, the flask is filled and flushed by allowing it to overflow one and a half times its full volume. Once the sample is taken, a small amount of water (~5 mL) is removed to create a head-space and 100 uL of 50% saturated mercuric chloride solution is added in the sampling bay. This is the same supply of mercuric chloride solution used for the other DIC samples collected. After all samples are collected from a station the glass stoppers are dried and greased using Apiezon M high vaccuum seal grease, banded, and electrical taped shut to keep the glass stoppers in place during shipping. The filled bottles are stored in NOSAMS crates inside the ship's main laboratory prior to being loaded into a container and shipped back to the United States for analysis.

6.10 Dissolved Organic Carbon

Principal Investigators: Dennis Hansell (RSMAS)
Sampler: Victoria Dina (WHOI)

DOC and TDN samples were taken from every sample bottle at approximately every other station, and surface samples collected at the remaining stations. 901 samples were taken from 75 stations in total. Samples from depths of 250 m and shallower were filtered through pre-combusted 47mm glass fiber filters. Samples from deeper depths were not filtered. Filter holders and silicone tubing were 10% HCl cleaned for 4 hours and DI water rinsed. Bottles were rinsed by sample for 3 times before filling. ~35 mL of water were taken for each sample. Samples were then treated with 100 uL of 4 N HCl and stored to be shipped back to UMiami for analysis.

6.11 Nutrients

Principal Investigators: Jia-Zhong Zhang (AOML) and Calvin Mordy (PMEL)
Analysts: Eric Wisegarver (PMEL) and Alex Fine (AOML/CIMAS)

Equipment and Techniques

Dissolved nutrients (phosphate, silicate, nitrate, and nitrite) were measured by using a Seal Analytical AA3 HR automated continuous flow analytical system with segmented flow and colormetric detection. Detailed methodologies are described by Gordon et al. (1992).

Silicic acid was analyzed using a modification of Armstrong et al. (1967). An acidic solution of ammonium molybdate was added to a seawater sample to produce silicomolybic acid. Oxalic acid was then added to inhibit a secondary reaction with phosphate. Finally, a reaction with ascorbic acid formed the blue compound silicomolybdous acid. The color formation was detected at 660 nm. The use of oxalic acid and ascorbic acid (instead of tartaric acid and stannous chloride by Gordon et al.) were employed to reduce the toxicity of our waste steam.

Nitrate and Nitrite analysis were also a modification of Armstrong et al. (1967). Nitrate was reduced to nitrite via a copperized cadmium column to form a red azo dye by complexing nitrite with sulfanilamide and N-1-naphthylethylenediamine (NED). Color formation of nitrate + nitrite was detected at 520 nm. The same technique was used to measure nitrite, (excluding the reduction step), and nitrate concentrations were determined by the difference of these two analyses.

Phosphate analysis was based on a technique by Bernhart and Wilhelms (1967). An acidic solution of ammonium molybdate was added to the sample to produce phosphomolybdate acid. This was reduced to the blue compound phosphomolybdous acid following the addition of hydrazine sulfate. The color formation was detected at 820 nm.

Sampling and Standards

Nutrient samples were drawn in 50 mL sample tubes that had been stored in 10% HCl. The bottles are rinsed 3-4 times with sample prior to filling. Samples were then brought to room temperature prior to analysis. Fresh mixed working standards were prepared before each analysis. In addition to the samples, each analysis consisted of a 4 point standard curve with each concentration run in duplicate at the beginning. Also, one mixed working standard from the previous analytical run was used at the beginning of the new run to determine differences between the two standards. Low Nutrient Seawater (LNSW) was used as a medium for the working standards.

The working standards were made by the addition of 3, 6, and 9 mL of a secondary nitrite standard and 3, 6, and 9 mL of a secondary mixed standard (containing silicic acid, nitrate, and phosphate) into a 250 mL calibrated volumetric flask of LNSW. Working standards were prepared daily.

Dry standards of a high purity were pre-weighed at PMEL. All standards were dissolved at sea. The secondary mixed standard was prepared by the addition of nitrate and phosphate primary standards to the silicic acid standard.

Nutrient concentrations were reported in micromoles per kilogram. Lab temperatures were recorded for each analytical run.

Approximately 1790 samples were analyzed.

References

Armstrong, F.A.J., Stearns, C.R. and Strickland, J.D.H. (1967) The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. Deep-Sea Res. 14:381-389.

Bernhard, H. and Wilhelms, A. (1967) The continuous determination of low level iron, soluble phosphate and total phosphate with AutoAnalyzer. Technicon Symposia, I. pp.385-389.

Gordon, L.I., Jennings Jr., J.C., Ross, A.A. and Krest, J.M. (1993) A suggested protocol for the continuous automated analysis of seawater nutrients (phosphate, nitrate, nitrite and silicic acid) in the WOCE Hydrographic program and the Joint Global Ocean Fluxes Study, WOCE Operations Manual, vol. 3: The Observational Programme, Section 3.2: WOCE Hydrograghic Programme, Part 3.1.3: WHP Operations and Methods. WHP Office Report WHPO 91-1; WOCE Report No. 68/91. November 1994, Revision 1, Woods Hole, MA., USA, 52 loose-leaf pages.

6.12 Salinity

Principal Investigators: Rick Lumpin (AOML), Zachary Erickson (PMEL)
Analysis: Jay Hooper (AOML/CIMAS), Christian Saiz (AOML/CIMAS)

A single Guildline Autosal, model 8400B salinometers (S/N 71464), located in the salinity analysis room, was used for all salinity measurements. The Autosal was calibrated January 2014. The salinometer readings were logged on a computer using Ocean Scientific International's logging hardware and software. The Autosal's water bath temperature was set to 24°C, which the Autosal is designed to automatically maintain. The laboratory's temperature was also set and maintained to just below 24°C, to help further stabilize reading values and improve accuracy. The room temperature was also monitored by a digital thermometer used to verify the stability of the Autosal room temperature. Salinity analyses were performed after samples had equilibrated to the Autosal room temperature at least 12 hours, typically 18 hours, after collection. The salinometer was standardized for each group of samples analyzed (usually 2 casts and up to 52 samples) using two bottles of standard seawater: one at the beginning and end of each set of measurements. The salinometer output was logged to a computer file. The software prompted the analyst to flush the instrument's cell and change samples when appropriate. Prior to each run a sub-standard flush, approximately 200 ml, of the conductivity cell was conducted to flush out the DI water used in between runs. For each calibration standard, the salinometer cell was initially flushed 6 times before several conductivity ratio reading was taken, usually 5-6 readings. For each sample, the salinometer cell was initially flushed at least 3 times before a set of conductivity ratio readings were taken. After each run the Autosal conductivity cell was flushed with approximately 200 ml of a triton-DI water solution and then rinsed and stored with DI water until the net run.

IAPSO Standard Seawater Batch P-166 was used to standardize all casts.

The salinity samples were collected in 200 ml Kimax high-alumina borosilicate bottles that had been rinsed at least three times with sample water prior to filling. The bottles were sealed with custom-made plastic insert thimbles and Nalgene screw caps. This assembly provides very low container dissolution and sample evaporation. Prior to sample collection, inserts were inspected for proper fit and loose inserts replaced to insure an airtight seal. PSS-78 salinity [UNES81] was calculated for each sample from the measured conductivity ratios. The offset between the initial standard seawater value and its reference value was applied to each sample. Then the difference (if any) between the initial and final vials of standard seawater was applied to each sample as a linear function of elapsed run time. The corrected salinity data was then incorporated into the cruise database. When duplicate measurements were deemed to have been collected and run properly, they were averaged and submitted with a quality flag of 6. On A16N, 1195 salinity measurements were taken, including 146 duplicates, and approximately 74 vials of standard seawater (SSW) were used. Up to two duplicate samples, one for shallow casts (if less than 1000 m), were drawn from each cast to determine total analytical precision.

The standard calibration values and duplicates are below in Figure 10 and Figure 11. The duplicates taken during the cruise showed a median precision of -0.00024+/- 0.006 psu.

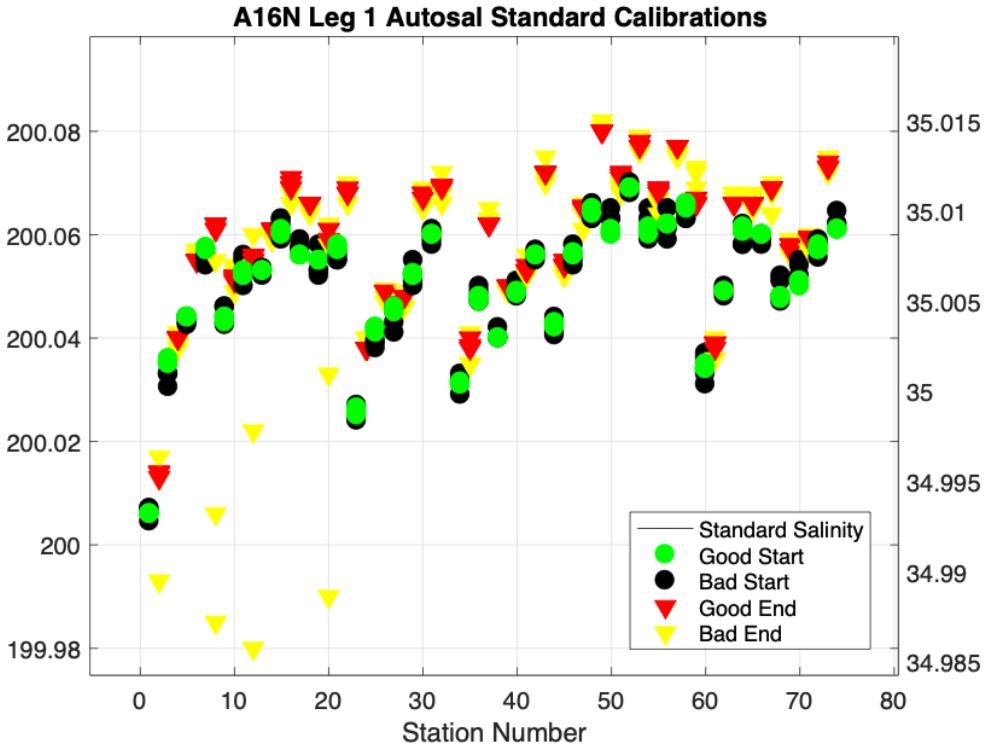

Figure 10. Standard vial calibrations throughout the cruise before and after each Autosal run. The green dots and red triangles are the good values used before and after each run to calculate salinity and drift corrections, respectively. The black dots and yellow triangles are the bad values not used. The left vertical axis is 200X the conductivity ration and the right axis is the corresponding salinity.

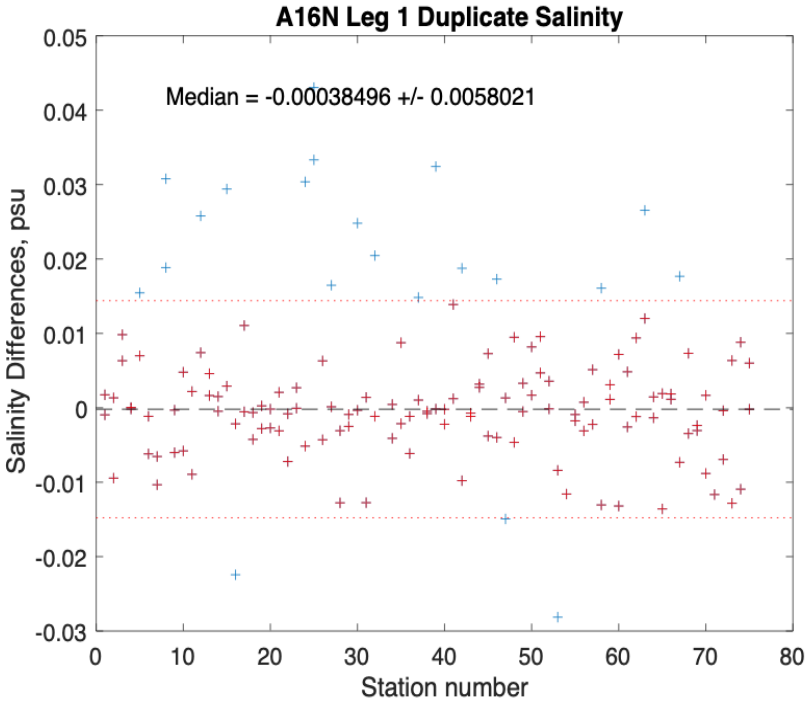

Figure 11. Salinity residuals of the duplicate samples.

6.13 Bio-GO-SHIP

PIs: Harriet Alexander (WHOI), Sophie Clayton (ODU), Jason Graff (OSU), Adam Martiny (UCI), Nicole Poulton (Bigelow), Luke Thompson (AOML)
Samplers: Star Dressler (U. Guam) and Tyler Christian (AOML/CIMAS)

6.13.1 Continuous Inline Sampling

An underway system utilizing a diaphragm pump provided continuous flow from surface waters for optical instrumentation (BB3, ACS), imaging Flow CytoBot (IFCB), and flow cytometer (FCM). Further details on FCM, IFCB, and optical sampling are outlined in sections below.

On March 11, it was determined that the ship's inline system used for the diaphragm pump was contaminated with rust. PI's determined that this rust would theoretically appear as noise in all continuous data collection, and daily cleaning protocols were set to mitigate rust buildup. Using the diaphragm pump is pertinent for these instruments as it minimizes damage caused to cells as they pass through the underway system. The alternative underway system on the *NOAA Ship Ronald H. Brown* utilizes an impeller pump, which is recognized to significantly alter particle size distribution and particle counts.

6.13.2 Underway Sampling

eDNA, RNA, large volume particulate organic matter (POM), high performance liquid chromatography (HPLC), and FCM samples were collected at approximately 0600, 1200, and 2000 local time via the underway tap. Local time "1200" varied according to time changes on board, but was set to correlate with approximate solar noon at the given sampling longitude. The other sampling times were set based on six hours prior to solar noon and eight hours following solar noon. The sampled parameters are outlined in further detail in sections 1.5 -1.8. Underway samplings were skipped if the CTD rosette Bio-cast was set to occur within a two to three hour window of an underway sampling time. There were 63 total underway sampling events throughout Leg 1, as well as 32 total eDNA samples, collected alongside the other core GO-ship samples taken in transit while sailing away from Brazil and heading into Spain.

From 08 March-11 March at 1200, the underway system utilized a diaphragm pump to minimize damage to organic particles. It was determined on 11 March that the ship's inline system used for the diaphragm pump was contaminated with rust and was problematic for the filtering protocols of underway sampling. PI's determined that the diaphragm pump water would continue to be used for continuous inline instrumentation; however, the underway sampling parameters would be gathered through the *Brown's* "uncontaminated scientific underway" system that utilizes an impeller pump. Underway sampling utilized this impeller pump system for all inline samplings starting 11 March at 2200.

6.13.3 eDNA and RNA

For underway sampling, 8-liter samples were gathered for eDNA at local time 0600, 1200, and 2000, and a 8-liter sample was gathered for RNA at 2000 (at all underway samples before March 11, RNA was being sampled for inline at 1200 local time). For bio-cast CTD stations, eDNA was sampled at 1000 m, 200 m, 100 m, and 5 m, and RNA was sampled at 5 m (eight liter samples each). Filtering took place immediately following Niskin or underway sampling, with first priority filtration set for RNA and the deepest eDNA sample. Nitrile gloves were worn for sample collection and processing. Prior to gathering sample water from Niskin or underway, each container was quickly rinsed three times with sample water. Following filtration protocols, each container was rinsed with tap water or DI water.

For filtration, clean tubing ran from each water sample, through a peristaltic pump with the ability to run two samples at a time, to separate measured containers situated in a sink to track volume filtered. Each sample line was first cleaned with sample water, and the end of each tubing was then secured with a Sterivex 0.22 µm filter cartridge. Approximately 8 liters of sample water ran through each filter. Following filtration, each filter was cleared of remaining liquid, and processed for either “Protocol A” or “Protocol B”.

Most eDNA and all RNA samples followed Protocol B, utilizing Sterivex filters prepped prior to filtering with pre-measured Zymo ZR BashingBeads and processed with 1000 µL of DNA/RNA Shield added to cartridge post filtration. Protocol A samples were gathered approximately every 3 days, where a duplicate inline DNA sample would be processed using a Sterivex filter without beads, with 1600 µL of lysis buffer (800 µl x 2 using two pipette tips) added to cartridge post filtration. Protocol A samples were taken to verify that data is comparable between the two methods, so perhaps future eDNA samples can follow only Protocol B procedure. All samples were labeled following protocol and stored at -80º C for later analysis. Sample lines were cleaned with 10% bleach solution and then DI water immediately following sampling at each depth. On A16N Leg 1, 63 total samples were processed for eDNA underway sampling, and 88 total samples were processed for eDNA bio-cast CTD stations; 26 total samples were processed for RNA underway sampling, and 22 total samples were processed for RNA bio-cast CTD stations. Additionally, 32 total eDNA samples were collected alongside the other core GO-ship samples taken in transit while sailing away from Brazil and heading into Spain.

6.13.4 Large Volume Particulate Organic Matter (POM)

POM was comprised of two sample parameters, particulate organic carbon/nitrogen (POC/N) and particulate organic phosphorus (POP). For both bio-cast CTD’s and underway sampling, eight liter triplicates were gathered for both POC and POP (24 total liters per parameter). CTD samples were all gathered from surface bottles fired at approximately 5 m. Nitrile gloves were worn for sample collection and processing. Prior to gathering sample water from Niskin or underway, each container was quickly rinsed three times with sample water. Following filtration protocols, each container was rinsed with tap water or DI water.

Filtering took place immediately following Niskin or underway sample collection. Each sample container, secured with a spigot at the bottom of the container, was filled to a pre-measured 8-liter mark. Hosing was connected to each spigot, which led to separate filter housings with pre-combusted, 25 mm GF/F filters. Tubing from the outflow of filter housings led to an aspirator pump that emptied into a sink. Following filtration, POP sample filters were rinsed with approximately 5 mL of Na_2SO_4 solution to remove traces of dissolved phosphorus from the filter. Each filter was removed with tweezers, folded into aluminum foil with the sample-side folded inwards, labeled according to protocol, and stored at -80º C for later analysis. Sample lines and filter housings were quickly rinsed with DI water. On A16N Leg 1, 184 POP samples and 187 POC samples were processed for underway sampling; 65 POP samples and 66 POC samples were processed for bio-cast CTD stations.

6.13.5 High Performance Liquid Chromatography (HPLC)

One two liter HPLC sample was gathered at each underway sampling, with approximately 10% of samples gathered as duplicates (2 L x 2). For bio-cast CTD stations, two liter HPLC samples were gathered at depths of 100 m, 40 m, and 5 m. Nitrile gloves were worn for sample collection and processing. Prior to gathering sample water from Niskin or underway, each container was quickly rinsed three times with sample water. Following filtration protocols, each container was rinsed with tap water or DI water.

Filtering took place immediately following Niskin or underway sampling. Water samples were filtered through pre-combusted, 25 mm GF/F filters secured on a filtration manifold attached to a vacuum pump. Filters were folded in half sample-side inwards, placed in a cryovial, labeled following protocol, and stored at -80º C for later analysis. On A16N Leg 1, 63 total samples were processed for underway sampling, and 57 total samples were processed for bio-cast CTD stations (not including BGC Argo Float HPLC samples).

6.13.6 Flow Cytometry (FCM)

FCM samples were collected with each underway sampling and at each bio-cast CTD station in 50 mL brown Falcon tubes and preserved for later analysis. For bio-casts, seawater for FCM samples was collected at depths of 1000 m, 500 m, 200 m, 150 m, 100 m, 75 m, 40 m, and 5 m. For the third BGC Argo float, additional FCM depths were added to correlate with depths associated with float sampling. Falcon tubes were rinsed quickly three times with sample water prior to sample collection. Nitrile gloves were worn for sample collection and processing. Sample processing took place immediately following sample collection, or if unable to process immediately, samples were stored in the walk-in refrigerator until the sampler was ready to process.

From the Falcon tubes, 1.8 mL of seawater was pipetted into a 2 mL cryovial. While under a fume hood, 18 µL of a preservation mixture (half 25% Glutaraldehyde and half 2% Kolliphor)

was added to each cryovial. The cryovial was inverted several times and placed on a vial stand in a refrigerator for approximately 10 minutes. The vials were labeled following protocol and then stored at -80 °C. On A16N Leg 1, 63 total samples were processed for underway sampling, and 180 total samples were processed for bio-cast CTD stations.

The shipboard Cytek Northern Lights FCM received continuous water from the diaphragm pump inline system throughout Leg 1, with sample water being processed following a SpectroFlo software template. This protocol was followed until 27 March, when issues with the FCM loader inhibited continuous sampling. New protocol was then implemented to run one tube of inline sample water on the FCM per hour.

6.13.7 Imaging FlowCytobot (IFCB)

The IFCB received continuous water from the diaphragm pump inline system throughout Leg 1. The software was managed remotely by Sophie Clayton. Particle concentrations as identified by the IFCB were low throughout the entirety of Leg 1, so the samplers utilized a particle concentrator to preserve one sample of seawater concentrated for plankton per day, to be analyzed at a later date.

To concentrate samples, a 20-liter carboy was filled with water supplied from the diaphragm pump inline system and passed through tubing to a PVC particle concentrator secured with 10 µm mesh fabric. This “reverse filtration” system allows particles smaller than 10 µm to pass through, while containing all larger particles within a 100 mL volume. This 100 mL sample was transferred to a brown 125-mL Nalgene bottle, preserved with 1 mL of Lugol’s preservation solution, labeled according to protocol, and stored at room temperature. On A16N Leg 1, 28 total samples were preserved. Ten of these samples were accidentally gathered from the impeller pump system rather than the diaphragm pump system.

6.13.8 Inline Optics

The optical instruments, including one BB3 backscatter detector, and one ACS attenuation and absorbance detector, received continuous water from the diaphragm pump inline system throughout Leg 1. At the first 10 minutes of every hour, the underway water would pass through a filter to remove most particles prior to passing through the instrumentation. The flow-through system was monitored through a flow meter connected to computer software that recorded a readout of flow rate and managed the filtration switching times. Inlinino software, connected to communication channels, recorded the BB3 and ACS data, as well as live tracking of latitude/longitude coordinates. Due to the large amounts of rust in the underway system, the optical instruments were cleaned with DI water and isopropyl alcohol wipes daily. The ACS was recognized as damaged and decommissioned on 25 March.

6.14 Sargassum Sampling

Principal Investigators: Dennis McGillicuddy (WHOI)
Shipboard Personnel: Ellen Park (MIT/WHOI), Kieran Claassen (SIO), Zach Erickson (PMEL)

On the first leg of A16N, the science party conducted opportunistic sampling of Sargassum from alongside the ship. A16N crossed the eastern portion of the Great Atlantic Sargassum Belt (GASB), which has become of particular interest to both the public and scientific community. Underway, we received weekly satellite updates of the GASB coverage (Figures 12-14).

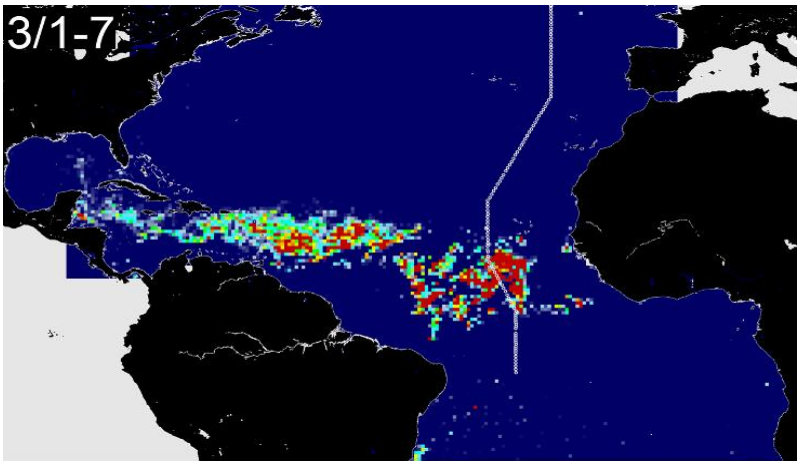

Figure 12. Great Atlantic Sargassum Belt coverage for compiled from March 1-7, 2023 with GO-SHIP A16N cruise track overlaid. (Optical Oceanography Lab at University of South Florida College of Marine Science (https://optics.marine.usf.edu/projects/saws.html)).

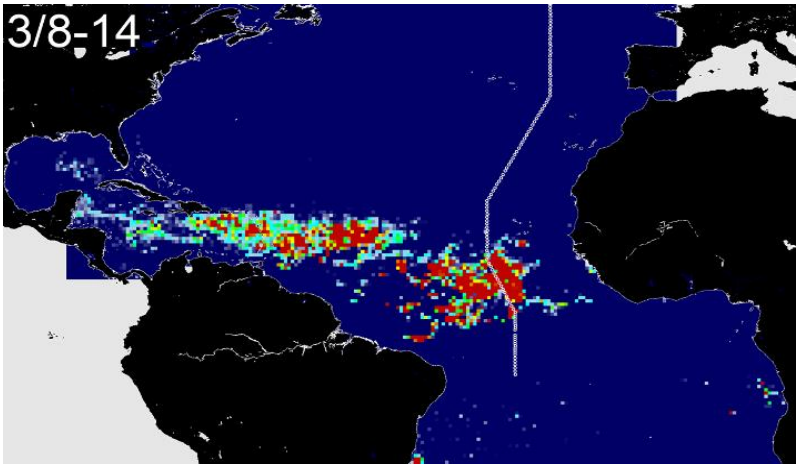

Figure 13. Great Atlantic Sargassum Belt coverage for compiled from March 8-14, 2023 with GO-SHIP A16N cruise track overlaid. (Optical Oceanography Lab at USF College of Marine Science (https://optics.marine.usf.edu/projects/saws.html)).

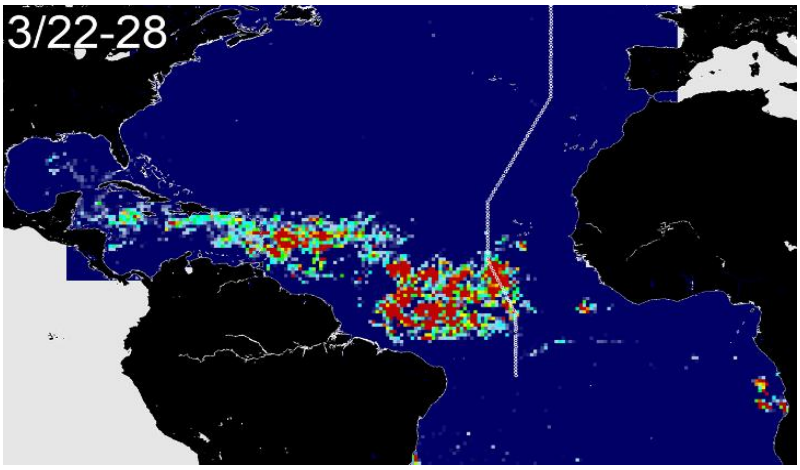

Figure 14. Great Atlantic Sargassum Belt coverage for compiled from March 22-28, 2023 with GO-SHIP A16N cruise track overlaid. (Optical Oceanography Lab at USF College of Marine Science (https://optics.marine.usf.edu/projects/saws.html)).

Sargassum samples were collected using a dip net fixed to a pole while the ship was on station either before or after a CTD cast because the net could not be deployed while the CTD was in the water. The samples were kept out of contact from metal, etc. to prevent contamination as much as possible (see Figure 12). Samples were handled using rubber gloves. After collection, they were sorted by species (*S. natans I, S. natans VIII, S.* fluitans), split into triplicate samples for each species if a sufficient amount was collected, rinsed with deionized water, dried in a salad spinner, and then placed in a drying oven for 10-20 hours to dry completely (see Figure 15). The oven was set to maintain temperatures between 55 and 65ºC. “Bone dried” samples were then placed in a quart-sized, labelled Ziploc bag.

Figure 15. (left) Ellen Park (MIT/WHOI) collecting Sargassum using a net on the starboard side of the ship (Photo by Jeffrey Greeley). (right) Taydra Low (U. Wisconsin) (front) drying Sargassum samples after they have been sorted and rinsed in a salad spinner. Mackenzie Blanusa (RSMAS) (back) sorting collected Sargassum by species (Photo by Ellen Park).

We received assistance with collecting and IDing/prepping samples from Mackenzie Blanusa (RSMAS), Tyler Christian (AOML/CIMAS), Taydra Low (U. Wisconsin), and Katelyn Schockman (AOML/CIMAS). During Leg 1, we collected a total of 68 samples from 11 stations (see Table 13). Sargassum collection operations were further detailed and explained in the GO-BGC cruise expedition log (https://www.go-bgc.org/expedition/atlantic-2023/sargassum-sampling) and a WHOI media alert (https://www.whoi.edu/press-room/news-release/scientists-collect-samples-from-great-atlantic-sargassum-belt-during-unprecedented-bloom).

Table 13. Time and location of Sargassum sample collections and additional information.

Collection #	Lat (°N)	Lon (°W)	Date and Time (GMT)	Station #	Species Observed (# of Replicates)
1	2.67	24.98	2023-03-15 22:17	24	Not enough Sargassum collected for sample
2	3.50	25.25	2023-03-16 11:43	26	*S. natans I* (3), *S. fluitans* (3), *S. natans VIII* (3)
3	3.98	25.50	2023-03-17 04:14	27	*S. natans I* (3), *S. fluitans* (3)
4	4.50	25.75	2023-03-17 16:03	28	*S. fluitans* (2)
5	6.97	26.98	2023-03-19 02:44	33	*S. natans I* (3), *S. fluitans* (3), *S. natans VIII* (2)
6	7.50	27.25	2023-03-19 09:27	34	*S. natans I* (3), *S. fluitans* (3), *S. natans VIII* (1)
7	8.98	28.00	2023-03-20 11:12	37	*S. natans I* (1), *S. fluitans* (3), *S. natans VIII* (1)
8	9.50	28.25	2023-03-20 20:22	38	*S. natans I* (3), *S. fluitans* (3)
9	10.00	28.50	2023-03-21 03:32	39	*S. natans I* (2), *S. fluitans* (3)
10	10.50	28.75	2023-03-21 10:49	40	*S. natans I* (3), *S. fluitans* (3), *S. natans VIII* (2)
11	10.98	28.98	2023-03-21 15:57	41	*S. natans I* (3), *S. fluitans* (3), *S. natans VIII* (2)
12	12.00	29.00	2023-03-22 10:15	43	*S. natans I* (1), *S. fluitans* (3)

6.15 Experimental Underway pH Measurement Package

Principal Investigator: Wei-jun Cai (U. Delaware)
Analyst: Bo Dong (U. Delaware)

Setup
Two sensors were set up to measure the pH of underway water flow.

One Honeywell Durafet pH sensor was set up in the bio lab connected to the underway outlet. The underway water ran through the sensor directly and then fill up the water jacket covering the sensor so that it can measure the ambient temperature. The sensor was connected to a Honeywell UDA 2142 data logger which was connected to a laptop. A python script was written to log the pH and temperature every 10 seconds. The sensor was calibrated before deployment with Fisher buffer solutions of pH 7.00 and 10.00.

One Seabird SeapHOx sensor was set up on the back deck. It was placed in a cooler fixed on the back deck. The pH sensor head was connected to the underway outlet in the main lab through an extended tube. A drainage hole was drilled on the side of the cooler allowing overflowing water flowing out of the ship. The sensor was factory pre calibrated and was set to log pH, salinity, density, temperature, dissolved oxygen every 60 seconds.

During Leg 1 when regular underway samples were not collected, two underway samples were collected every day for spectrophotometric pH and DIC measurement to calibrate the sensor in further data processing.

7. Stations

Table 14. Station information.

Station	Cast	LADCP #	Bio	Lat	Lon	Depth	Start Date & Time	End Date & Time	Duration
				(N)	(W)	(m)	GMT	GMT	hour:min
Start				-8.39	34.97			2023-03-06 12:26	
Test		1 & 2		-7.22	30.12	5416	2023-03-07 16:10	2023-03-07 17:16	1:06
1	1[a]	3		-6.00	25.00	5814	2023-03-08 22:29	2023-03-09 01:32	3:03
1	2	4		-6.00	25.00	5813	2023-03-09 07:22	2023-03-09 11:56	4:34
2	1	5	Y	-5.50	25.00	5655	2023-03-09 15:53	2023-03-09 17:01	1:08
2	2	6		-5.50	25.00	5657	2023-03-09 18:24	2023-03-09 22:54	4:30
3	1	7		-5.00	25.00	5683	2023-03-10 04:45	2023-03-10 08:56	4:11
4	1		Y	-4.50	25.00	5533	2023-03-10 12:06	2023-03-10 13:07	1:01
4	2	8		-4.50	25.00	5539	2023-03-10 13:55	2023-03-10 17:54	3:59
5	1	9		-4.00	25.00	5368	2023-03-10 21:09	2023-03-11 01:05	3:56
6	1	10		-3.50	25.00	5552	2023-03-11 04:07	2023-03-11 08:13	4:06
7	1	11		-3.00	25.00	5349	2023-03-11 11:08	2023-03-11 15:00	3:52
8	1	12	Y	-2.67	25.00	5368	2023-03-11 17:35	2023-03-11 18:32	0:57
8	2	13		-2.67	25.00	5354	2023-03-11 19:03	2023-03-11 22:49	3:46
9	1	14		-2.33	25.00	5029	2023-03-12 01:34	2023-03-12 05:13	3:39
10	1	15		-2.00	25.00	4945	2023-03-12 07:45	2023-03-12 11:27	3:42
11	1		Y	-1.67	25.00	4935	2023-03-12 13:53	2023-03-12 14:49	0:56
11	2	16		-1.67	25.00	4934	2023-03-12 15:11	2023-03-12 18:40	3:29
12	1	17		-1.33	25.00	4714	2023-03-12 21:10	2023-03-13 00:34	3:24
13	1	18		-1.00	25.00	3122	2023-03-13 03:28	2023-03-13 06:07	2:39
14	1	19	Y	-0.67	25.00	3089	2023-03-13 08:40	2023-03-13 09:42	1:02
14	2	20		-0.67	25.00	3218	2023-03-13 10:14	2023-03-13 12:50	2:36
15	1	21		-0.33	25.00	3191	2023-03-13 15:21	2023-03-13 17:56	2:35
16	1	22		0.00	25.00	3202	2023-03-13 20:26	2023-03-13 23:02	2:36
17	1	23		0.33	25.00	3668	2023-03-14 01:36	2023-03-14 04:29	2:53
18	1	24		0.67	25.00	4481	2023-03-14 07:03	2023-03-14 10:26	3:23
19	1	25	Y	1.00	25.00	3342	2023-03-14 12:52	2023-03-14 13:51	0:59
19	2	26		1.00	25.00	3332	2023-03-14 14:18	2023-03-14 17:02	2:44
20	1	27		1.33	25.00	3636	2023-03-14 19:38	2023-03-14 22:30	2:52
21	1	29		1.67	25.00	3821	2023-03-15 01:02	2023-03-15 04:06	3:04
22	1	30		2.00	25.00	3879	2023-03-15 06:33	2023-03-15 09:39	3:06
23	1	31	Y	2.33	25.00	3786	2023-03-15 12:04	2023-03-15 13:09	1:05
23	2	33		2.33	25.00	3756	2023-03-15 13:38	2023-03-15 16:36	2:58
24	1	34		2.67	25.00	4093	2023-03-15 19:05	2023-03-15 22:13	3:08
25	1	35		3.00	25.00	4403	2023-03-16 00:40	2023-03-16 04:06	3:26
26	1	36		3.50	25.25	4159	2023-03-16 07:19	2023-03-16 10:35	3:16

26	3[b]	40		3.50	25.25	4177	2023-03-16 19:31	2023-03-16 22:37	3:06
27	6[c]	47		4.00	25.50	4011	2023-03-17 08:41	2023-03-17 11:44	3:03
28	1	48	Y	4.50	25.75	4090	2023-03-17 15:02	2023-03-17 16:00	0:58
28	2	49		4.50	25.75	4089	2023-03-17 16:36	2023-03-17 19:36	3:00
29	1	50		5.00	26.00	4512	2023-03-17 23:06	2023-03-18 2:18	3:12
30	1	51		5.50	26.25	4258	2023-03-18 05:37	2023-03-18 08:51	3:14
31	1	52	Y	6.00	26.50	4298	2023-03-18 12:13	2023-03-18 13:11	0:58
31	2	53		6.00	26.50	4293	2023-03-18 13:44	2023-03-18 16:54	3:10
32	1	54		6.50	26.75	4640	2023-03-18 20:05	2023-03-18 23:23	3:18
33	1	55		6.97	26.99	4375	2023-03-19 02:43	2023-03-19 05:56	3:13
34	1	56	Y	7.50	27.25	4638	2023-03-19 09:31	2023-03-19 10:27	0:56
34	2	57		7.50	27.25	4626	2023-03-19 10:52	2023-03-19 14:10	3:18
35	1	58		8.00	27.50	5081	2023-03-19 17:27	2023-03-19 20:59	3:32
36	1	59		8.50	27.75	4930	2023-03-20 00:16	2023-03-20 03:49	3:33
37	1	60		9.00	28.00	5213	2023-03-20 07:16	2023-03-20 10:58	3:42
38	1	61	Y	9.50	28.25	5413	2023-03-20 15:22	2023-03-20 16:15	0:53
38	2	62		9.50	28.25	5398	2023-03-20 16:35	2023-03-20 20:17	3:42
39	1	63		10.00	28.50	5352	2023-03-20 23:35	2023-03-21 03:25	3:50
40	1	64		10.50	28.75	5394	2023-03-21 07:02	2023-03-21 10:43	3:41
41	1	65	Y	11.00	29.00	5980	2023-03-21 14:00	2023-03-21 14:52	0:52
41	2	66		11.00	29.00	5976	2023-03-21 15:31	2023-03-21 19:42	4:11
42	1	67		11.50	29.00	5932	2023-03-21 22:46	2023-03-22 03:04	4:18
43	1	68		12.00	29.00	5701	2023-03-22 06:13	2023-03-22 10:20	4:07
44	1	69	Y	12.50	29.00	5519	2023-03-22 13:18	2023-03-22 14:12	0:54
44	2	70		12.50	29.00	5519	2023-03-22 14:36	2023-03-22 18:43	4:07
45	1	71		13.00	29.00	5694	2023-03-22 21:39	2023-03-23 01:27	3:48
46	1	72		13.50	29.00	5535	2023-03-23 04:25	2023-03-23 08:21	3:56
47	1	73	Y	14.00	29.00	5440	2023-03-23 11:24	2023-03-23 12:14	0:50
47	2	74		14.00	29.00	5439	2023-03-23 12:39	2023-03-23 16:26	3:47
48	1	75		14.50	29.00	5377	2023-03-23 19:29	2023-03-23 23:07	3:38
49	1	76		15.00	29.00	5296	2023-03-24 02:01	2023-03-24 05:44	3:43
50	1	77		15.50	29.00	5258	2023-03-24 08:42	2023-03-24 12:29	3:47
51	1	78	Y	16.00	29.00	4499	2023-03-24 15:24	2023-03-24 16:13	0:49
51	2	79		16.00	29.00	4486	2023-03-24 16:38	2023-03-24 19.47	3:09
52	1	80		16.50	29.00	4940	2023-03-24 22:45	2023-03-25 02:14	3:29
53	1	81		17.00	29.00	4880	2023-03-25 05:04	2023-03-25 08:39	3:35
54	1	82	Y	17.50	29.00	4612	2023-03-25 11:39	2023-03-25 12:33	0:54
54	2	83		17.50	29.00	4617	2023-03-25 13:01	2023-03-25 16:13	3:12
55	1	85		18.00	29.00	4549	2023-03-25 19:11	2023-03-25 22:22	3:11
56	1	86		18.50	29.00	4688	2023-03-26 01:12	2023-03-26 04:39	3:27
57	1	87		19.00	29.00	4561	2023-03-26 07:39	2023-03-26 10:57	3:18
58	1	88	Y	19.50	29.00	4953	2023-03-26 13:52	2023-03-26 14:42	0:50

58	2	89		19.50	29.00	4950	2023-03-26 15:06	2023-03-26 18:30	3:24
59	1	90		20.00	29.00	4797	2023-03-26 21:26	2023-03-27 00:44	3:18
60	1	91		20.50	28.72	5135	2023-03-27 03:57	2023-03-27 07:37	3:40
61	1	92	Y	21.00	28.44	5038	2023-03-27 10:52	2023-03-27 11:49	0:57
61	2	93		21.00	28.44	5028	2023-03-27 12:11	2023-03-27 15:41	3:30
62	1	94		21.50	28.16	5352	2023-03-27 18:56	2023-03-27 22:31	3:35
63	1	9		22.00	27.87	5432	2023-03-28 01:39	2023-03-28 05:29	3:50
64	1	96		22.50	27.60	5485	2023-03-28 08:43	2023-03-28 12:35	3:52
65	1	97	Y	23.00	27.32	5518	2023-03-28 15:58	2023-03-28 16:50	0:52
65	2	98		23.00	27.33	5522	2023-03-28 17:13	2023-03-28 21:08	3:55
66	1	99		23.50	27.03	5502	2023-03-29 00:36	2023-03-29 04:31	3:55
67	1	100		24.00	26.75	5454	2023-03-29 07:47	2023-03-29 11:41	3:54
68	1	101	Y	24.50	26.47	5420	2023-03-29 15:03	2023-03-29 16:01	0:58
68	2	102		24.51	26.48	5421	2023-03-29 16:31	2023-03-29 20:13	3:42
69	1	103		25.00	26.19	5396	2023-03-29 23:36	2023-03-30 03:31	3:55
70	1	104		25.50	25.91	5350	2023-03-30 06:50	2023-03-30 10:40	3:50
71	1	105	Y	26.00	25.63	4386	2023-03-30 14:05	2023-03-30 14:59	0:54
71	2	106		26.00	25.63	4336	2023-03-30 15:21	2023-03-30 18:29	3:08
72	1	107		26.50	25.34	5244	2023-03-30 21:48	2023-03-31 01:31	3:43
73	1	108		27.00	25.06	5236	2023-03-31 04:57	2023-03-31 08:40	3:43
74	1	109	Y	27.50	24.78	5204	2023-03-31 13:39	2023-03-31 14:32	0:53
74	2	110		27.50	24.78	5204	2023-03-31 14:54	2023-03-31 18:28	3:34
75	1	111		28.00	24.50	5215	2023-03-31 23:39	2023-04-01 03:34	3:55
76				28.50	24.22	5197	2023-04-01 08:24		
END				36.62	6.33		2023-04-06 06:00		

[a]Station1, Cast 1 shorted out near the bottom and was aborted.

[b]Station 26, Cast 1 lost the CTD. Cast 2 was a test deployment.

[c]Station 27, Cast 1-5 had a variety of issues related to an errant CTD Deck Unit alarm.