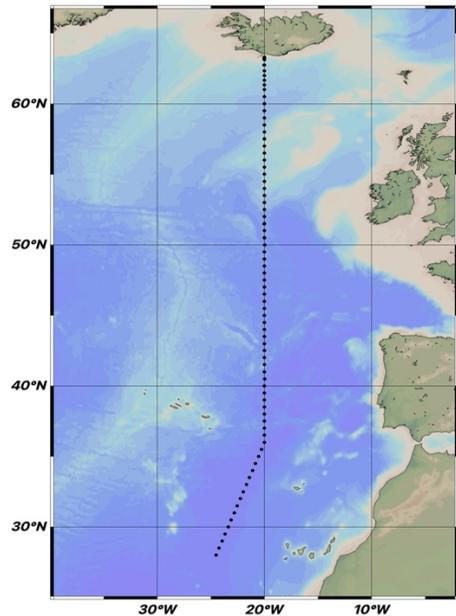




## Cruise Report: GO-SHIP A16N Leg 2 (last update November 2023)



### Cruise Summary Information

Section Designation	<b>GO-SHIP A16N Leg 2</b>
Expedition designation (expocode)	<b>33RO20230413</b>
Chief/Co-Chief scientist	<b>Leticia Barbero / Laura Cimoli</b>
Dates	<b>April 13<sup>th</sup> 2023 to May 9<sup>th</sup> 2023</b>
Vessel	<b>NOAA Ship Ronald H. Brown</b>
Ports of call	<b>Rota, Spain – Reykjavik, Iceland</b>
Geographic Boundaries	<b>28–63.3°N, 24.5–20°W</b>
Stations	<b>75, with 21 dedicated “Bio” casts</b>
Floats and drifters deployed	<b>1 core ARGO, 4 BGC-ARGO, 5 surface drifters</b>
EEZ consents	<b>Iceland:</b> consent # UTN22110189/34.R.611 <b>Portugal:</b> consent ref. 39984, Process DGPE/USEN-111/2022 <b>Spain:</b> ref CO/23/41



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# TABLE OF CONTENTS

<b>1.</b>	<b>A16N LEG 2 PROJECT.....</b>	<b>4</b>
1.1.	PARTICIPATING INSTITUTIONS .....	5
1.2.	PROGRAMS AND PRINCIPAL INVESTIGATORS.....	6
1.3.	SCIENCE TEAM AND RESPONSIBILITIES.....	7
<b>2.</b>	<b>PROGRAM AND A16N PROJECT OVERVIEW .....</b>	<b>8</b>
2.1.	BRIEF OVERVIEW OF THE A16N LINE .....	8
2.2.	A16N, LEG 2 2023 GOALS AND LOGISTIC PARTICULARITIES .....	9
2.3.	STATION LOCATIONS.....	11
2.4.	EXCLUSIVE ECONOMIC ZONE CLEARANCE REQUESTS .....	13
2.5.	ISSUES.....	13
2.6.	ACKNOWLEDGMENTS .....	15
<b>3.</b>	<b>UNDERWAY DATA ACQUISITION.....</b>	<b>16</b>
3.1.	THERMOSALINOGRAPH MEASUREMENTS.....	16
3.2.	SURFACE ACOUSTIC DOPPLER CURRENT PROFILER (sADCP) .....	17
3.3.	UNDERWAY SEAWATER pCO <sub>2</sub> MEASUREMENTS.....	17
3.4.	UNDERWAY PH .....	18
<b>4.</b>	<b>DESCRIPTION OF MEASUREMENTS FROM VERTICAL PROFILES.....</b>	<b>19</b>
4.1.	TYPE OF CTD CASTS .....	19
4.2.	CONDUCTIVITY, TEMPERATURE, DEPTH (CTD) HYDROGRAPHIC MEASUREMENTS .....	20
4.3.	LOWERED ACOUSTIC DOPPLER CURRENT PROFILER (LADCP) .....	23
4.4.	CHIPODS.....	26
4.5.	CHLOROFLUOROCARBON (CFC), SULFUR HEXAFLUORIDE (SF <sub>6</sub> ), AND NITROUS OXIDE (N <sub>2</sub> O).....	27
4.6.	DISSOLVED OXYGEN (O <sub>2</sub> ) .....	29
4.7.	DISSOLVED INORGANIC CARBON (DIC) .....	34
4.8.	DISCRETE PH ANALYSIS.....	38
4.9.	TOTAL ALKALINITY .....	39
4.10.	DISCRETE pCO <sub>2</sub> MEASUREMENTS .....	41
4.11.	Δ <sup>13</sup> C-DISSOLVED INORGANIC CARBON (DIC) .....	45
4.12.	DISSOLVED ORGANIC CARBON (DOC) .....	48
4.13.	RADIOCARBON 14C/13C .....	48
4.14.	NUTRIENTS.....	49
4.15.	DISCRETE SALINITY SAMPLING.....	50
<b>5.</b>	<b>BIO-GO-SHIP.....</b>	<b>52</b>
5.1.	CONTINUOUS INLINE SAMPLING .....	52
5.2.	UNDERWAY SAMPLING .....	53
5.3.	BIO-CAST CTD STATION SAMPLING .....	53
5.4.	BIOGEOCHEMICAL (BGC)-ARGO FLOAT STATION SAMPLING.....	53
5.5.	EDNA AND RNA.....	54
5.6.	LARGE VOLUME PARTICULATE ORGANIC MATTER (POM) .....	55
5.7.	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) .....	55
5.8.	FLOW CYTOMETRY (FCM) .....	56
5.9.	IMAGING FLOWCYTOBOT (IFCB).....	56
5.10.	INLINE OPTICS.....	57
<b>6.</b>	<b>OTHER ACTIVITIES: FLOAT AND DRIFTER DEPLOYMENTS.....</b>	<b>57</b>
6.1.	CORE AND BGC ARGO FLOATS .....	57
6.2.	SURFACE DRIFTERS.....	60
<b>7.</b>	<b>BIBLIOGRAPHY .....</b>	<b>60</b>



## 1. A16N leg 2 Project

This report details work performed in support of the 2023 re-occupation of the northern leg of the Global Ocean Ship-based Hydrographic Investigations Program ([GO-SHIP](#)) hydrographic section A16N. GO-SHIP brings together scientists with interests in physical oceanography, the carbon cycle, marine biogeochemistry and ecosystems, and other users and collectors of ocean interior data, and coordinates a network of globally sustained hydrographic sections as part of the global ocean/climate observing system including physical oceanography, the carbon cycle, marine biogeochemistry and ecosystems.

The research expedition took place aboard the NOAA Ship *Ronald H. Brown* between April 13 and May 9, 2023. The cruise departed from Rota, Spain instead of the initially requested port of Madeira, Portugal. This change of port imposed by the ship operator added several days of transit and caused the break between the last station in leg 1 and its reoccupation in leg 2 to be 2 weeks apart. The goal of the expedition was to re-occupy the stations sampled on previous A16N expeditions in 1993, 2003 and 2013 and was largely successful, with all planned level 1 stations re-occupied.

The survey consisted of CTD/DO rosette, LADCP, water samples, and underway measurements. A total of 75 stations were occupied with a CTD/DO/rosette/LADCP package. At 21 of those stations, a second CTD cast was performed to provide sufficient water for biological samples in support of the BIO-GO-SHIP pilot project.

Four core Argo, 3 BGC-Argo floats and 5 surface drifters were deployed along the route, in international waters.

CTD/DO data and water samples were collected on each cast, from surface (2-5 m) to usually within 10 m of the bottom. Water samples were measured on board for salinity, dissolved oxygen, nutrients, dissolved inorganic carbon (DIC), pH, total alkalinity (TA),  $p\text{CO}_2$  and other parameters. Additional water samples were collected and stored for shore analyses of DOC,  $^{14}\text{C}$ , chlorophyll concentration, HPLC analysis, and DNA/RNA composition.

A seagoing science team assembled from 20 different institutions from the USA participated in the collection and analysis of the samples. The programs, principal investigators, science team, responsibilities, instrumentation, analyses, and analytical methods are outlined in the following cruise document.



## 1.1. Participating Institutions

<b>Abbreviation</b>	<b>Institution</b>
AOML	NOAA - Atlantic Ocean and Meteorological Laboratory
Bigelow	Bigelow Laboratory for Ocean Sciences
CICOES	Cooperative Institute for Climate, Ocean, and Ecosystem Sciences / University of Washington
CIMAS	Cooperative Institute for Marine and Atmospheric Studies / University of Miami
LDEO	Lamont-Doherty Earth Observatory/Columbia University
NGI	Northern Gulf Institute
ODU	Old Dominion University
OSU	Oregon State University
PMEL	NOAA - Pacific Marine Environmental Laboratory
Rosenstiel	Rosenstiel School of Marine, Atmospheric & Earth Science / University of Miami
SIO	Scripps Institution of Oceanography/University of California at San Diego
SJSU	San José State university
UDel	University of Delaware
U. Guam	University of Guam
U. Hawaii	University of Hawaii
U. Washington	University of Washington
UCI	University of California Irvine
UCSB	University of California Santa Barbara
UCSD	University of California San Diego
WHOI	Woods Hole Oceanographic Institution

Table 1: A16N leg 2 participating institutions.

## 1.2. Programs and Principal Investigators

Program	Principal Investigator	Affiliation	Email Address
14C	Rolf Sonnerup Roberta Hansman	CICOES WHOI	rolf@uw.edu rhansman@whoi.edu
Alkalinity	Chris Langdon	Rosenstiel	clangdon@earth.miami.edu
Argo floats	Steve Jayne Pelle Robbins Susan Wijffels	WHOI WHOI WHOI	sjayne@whoi.edu probbins@whoi.edu swijffels@whoi.edu
CFCs/SF <sub>6</sub> /N <sub>2</sub> O	Rolf Sonnerup	PMEL/CICOES	rolf@uw.edu
Chipods	Jonathan Nash	OSU	nash.coas@gmail.com
CTD	Rick Lumpkin Zachary Erickson	AOML PMEL	rick.lumpkin@noaa.gov zachary.k.erickson@noaa.gov
DIC	Rik Wanninkhof Richard Feely	AOML PMEL	rik.wanninkhof@noaa.gov richard.a.feely@noaa.gov
Dissolved O <sub>2</sub>	Rik Wanninkhof Chris Langdon	AOML Rosenstiel	Rik.Wanninkhof@noaa.gov clangdon@earth.miami.edu
DOC	Dennis Hansell	Rosenstiel	dhansell@miami.edu
eDNA	Luke Thompson	NGI/AOML	luke.thompson@noaa.gov
GO-BGC floats	David Nicholson Susan Wijffels Lynne Talley	WHOI WHOI SIO	dnicholson@whoi.edu swijffels@whoi.edu ltalley@ucsd.edu
HPLC	Adam Martiny	UCI	amartiny@uci.edu
LADCP	Andreas Thurnherr	LDEO	ant@ldeo.columbia.edu
Nutrients	Calvin Mordy Jia-Zhong Zhang	PMEL/CICOES AOML	calvin.w.mordy@noaa.gov jia-zhong.zhang@noaa.gov
pCO <sub>2</sub> (discrete)	Rik Wanninkhof	AOML	rik.wanninkhof@noaa.gov
pCO <sub>2</sub> (underway)	Denis Pierrot Rik Wanninkhof	AOML AOML	denis.pierrot@noaa.gov rik.wanninkhof@noaa.gov
pH	Chris Langdon	Rosenstiel	clangdon@earth.miami.edu
POC/PON	Adam Martiny	UCI	amartiny@uci.edu
SADCP	Jules Hummon Eric Firing	U. Hawaii U. Hawaii	hummon@hawaii.edu efiring@hawaii.edu
Salinity (discrete)	Rick Lumpkin Zachary Erickson	AOML PMEL	rick.lumpkin@noaa.gov zachary.k.erickson@noaa.gov
Surface Drifters (NOAA)	Rick Lumpkin Shaun Dolk	AOML AOML	Rick.Lumpkin@noaa.gov shaun.dolk@noaa.gov
IFCB	Sophie Clayton	ODU	sclayton@odu.edu
Flow cytometry	Nicole Poulton	Bigelow	npoulton@bigelow.org
RNA	Harriet Alexander	WHOI	halexander@whoi.edu
Transmissometer	Jason Graff	OSU	jason.graff@oregonstate.edu
δ13C	Wei-Jun Cai	U. Delaware	wcai@udel.edu

Table 2: A16N leg 2 Programs and PIs.

### 1.3. Science Team and Responsibilities

Duty	Name	Affiliation
Chief Scientist /data manager	Leticia Barbero	CIMAS/AOML
Co-Chief Scientist	Laura Cimoli	UCSD
CTD processing	Kristy McTaggart	PMEL
Salinity/CTD/LADCP	Jay Hooper	CIMAS/AOML
Salinity/LADCP	Pedro Peña	AOML
CTD Watch stander	Shannon McClish	U. Hawaii
CTD Watch stander	Audria Denner	UCSD
LADCP	Michael Cappola	UDel
Nutrients	Eric Wisegarver	PMEL
Nutrients	Ian Smith	CIMAS/AOML
Dissolved oxygen	Riley Palmer	Rosenstiel
Dissolved oxygen	Marina Ruddick	Rosenstiel
DIC	Chuck Featherstone	AOML
DIC	Dana Greeley	PMEL
Total Alkalinity/pH	Bo Yang	Rosenstiel
Total Alkalinity/pH	Jessica Leonard	Rosenstiel
Total Alkalinity/pH	Laura Stieghorst	Rosenstiel
Total Alkalinity/pH	Seamus Jameson	Rosenstiel/SJSU
CFCs/SF <sub>6</sub> /N <sub>2</sub> O	David Cooper	CICOES
CFCs/SF <sub>6</sub> /N <sub>2</sub> O	Carol Gonzalez	CICOES
CFCs/SF <sub>6</sub> /N <sub>2</sub> O	Isabel Schaal	WHOI
pCO <sub>2</sub> discrete	Patrick Mears	CIMAS/AOML
pCO <sub>2</sub> discrete	Leah Chomiak	CIMAS/AOML
<sup>14</sup> C/DOC	Victoria Dina	Rosenstiel
<sup>13</sup> DIC	Bo Dong	UDel
<sup>13</sup> DIC	Zhentao Sun	UDel
Bio-GO-SHIP	Star Dressler	U. Guam
Bio-GO-SHIP	Rachel Cohn	CIMAS/AOML

Table 3: Science team and responsibilities





and 2013). A quick google scholar search of “Atlantic” and “A16N” brings up hundreds of references, an indication of the critical importance of this line for oceanographic studies.

Since the CLIVAR era, and on to GO-SHIP, the National Atlantic Oceanographic and Atmospheric Administration (NOAA) has taken leadership for the occupation of this line, often on board the NOAA Ship *Ronald H. Brown*, and splitting the A16N line into 2 legs. Although previous occupations have run the A16N line North to South, in this case, due to logistical limitations and the time frame assigned for executing the line (March-May), the section was occupied South to North. This decision was made to increase the chances of being able to successfully collect the data in the northern section of the line. The drawback to this strategy, is that the connection with the A16S line which starts at the southernmost station of A16N to head south into the South Atlantic will be farther apart in time.

## 2.2. A16N, leg 2 2023 goals and logistic particularities

The specific goals of this cruise were as follows:

1.- Re-occupation of the historic stations in this line, including up to the coast of Iceland. Based on the number of days assigned for this leg, an initial estimate of 70-72 stations were planned.

2.- A second cast dedicated to biological measurements at select stations throughout the line as time allowed, and nominally once per day while stations were being performed (not during transit days).

3.- Deployment of up to 4 core Argo floats, and up to 3 BGC-Argo floats

4.- Deployment of up to 10 drifters in support of NOAA’s Global Drifter Program

5.- Collection of near surface seawater (temperature, salinity,  $p\text{CO}_2$ , ADCP) and atmospheric measurements.

6.- Surface seawater from the seawater line collected throughout the cruise to conduct phytoplankton and eDNA studies.

7.- Sargassum samples to be collected on stations if present.

Logistic complications:

In previous A16N occupations, the port requested for the mid-point break has been Madeira. We requested the same port this time, or, alternatively, the Canary Islands. Both options are a short distance away from the cruise track (Figure 2). However, the ship operator selected the Spanish/US Navy Base of Rota (Spain). This added a substantial amount of transit time to the cruise and increased the break between leg 1 and leg 2. The reason for selecting this port was for cheaper fuel cost for the NOAA ship. However, in addition to the extra transit time for both legs 1 and 2, access to the base was very restricted. Any scientist who was not either a Spanish or US citizen was not allowed on the base and had to be taken via small boat to a location outside the base that was only determined at the last minute, generating unnecessary uncertainty for several cruise participants. For the next re-occupation of A16N we strongly recommend pushing for



Madeira or the Canary Islands. The Canary Islands usually have lower fuel costs than many other ports in Europe due to an advantageous tax system.



*Figure 2. Map showing the station locations of the A16N leg 2 cruise. Also indicated in the map are the locations of Madeira, the Canary Islands, and Rota.*

### 2.3. Station locations

The table below details the locations of the stations occupied during the cruise as well as whether there was a dedicated bio-cast. Figure 3 shows the depths sampled at each station.

Station Number	Latitude (Deg. North)	Longitude (Deg. East)	Depth (m)	Date	Time (UTZ)	Bio-cast
76	28.000	-24.518	5214	4/18/2023	01:04	
77	28.504	-24.236	5195	4/18/2023	09:04	
78	28.992	-23.942	5278	4/18/2023	15:37	X
79	29.499	-23.667	5213	4/19/2023	02:08	
80	29.999	-23.378	5331	4/19/2023	08:36	X
81	30.498	-23.099	5284	4/19/2023	19:21	
82	30.998	-22.809	5247	4/20/2023	02:59	
83	31.497	-22.530	5306	4/20/2023	11:53	X
84	32.001	-22.253	5170	4/20/2023	19:17	
85	32.492	-21.967	5212	4/21/2023	02:50	X
86	33.000	-21.688	5247	4/21/2023	10:16	
87	33.494	-21.406	5336	4/21/2023	20:21	
88	33.996	-21.118	5229	4/22/2023	04:00	
89	34.502	-20.846	5250	4/22/2023	13:30	X
90	35.002	-20.554	5100	4/22/2023	16:38	
91	35.491	-20.275	5280	4/23/2023	03:59	
92	35.994	-19.992	5345	4/23/2023	13:20	Cancelled
93	36.503	-20.005	5170	4/23/2023	20:28	
94	37.001	-19.990	3797	4/24/2023	03:14	
95	37.498	-19.996	4849	4/24/2023	10:45	
96	37.995	-19.996	5107	4/24/2023	21:54	Partial
97	38.499	-19.994	4044	4/25/2023	04:53	
98	38.999	-19.993	4716	4/25/2023	14:52	X
99	39.501	-19.995	4657	4/25/2023	22:17	
100	39.999	-19.992	4759	4/26/2023	04:59	
101	40.501	-19.981	4915	4/26/2023	11:52	Partial
102	40.999	-19.996	4708	4/26/2023	18:23	
103	41.499	-19.995	2559	4/27/2023	01:00	
104	41.999	-19.993	2445	4/27/2023	06:24	
105	42.497	-19.995	4164	4/27/2023	12:43	Partial
106	42.991	-19.992	5120	4/27/2023	19:17	
107	43.502	-19.994	3994	4/28/2023	01:57	
108	43.999	-19.991	4003	4/28/2023	09:51	X
109	44.500	-19.997	4246	4/28/2023	16:08	
110	45.000	-19.989	4308	4/28/2023	22:28	
111	45.499	-19.997	4530	4/29/2023	04:53	
112	45.997	-19.998	4900	4/29/2023	12:57	X
113	46.499	-19.994	4800	4/29/2023	19:40	
114	46.998	-19.995	4535	4/30/2023	02:11	
115	47.501	-19.992	4552	4/30/2023	08:41	Partial
116	48.002	-19.994	4360	4/30/2023	15:00	
117	48.503	-19.998	4044	4/30/2023	20:56	



118	49.003	-19.995	4415	5/1/2023	04:06	
119	49.501	-20.003	3869	5/1/2023	10:51	X
120	49.998	-19.984	4402	5/1/2023	19:14	
121	50.500	-20.000	3916	5/2/2023	01:28	
122	51.001	-20.004	3658	5/2/2023	07:31	
123	51.500	-20.000	3640	5/2/2023	15:02	X
124	52.000	-20.000	3762	5/2/2023	20:57	
125	52.498	-20.001	2780	5/3/2023	02:37	
126	53.003	-20.001	2675	5/3/2023	09:56	X
127	53.497	-20.005	2289	5/3/2023	15:05	
128	54.000	-20.000	1417	5/3/2023	19:54	
129	54.496	-20.001	1384	5/4/2023	00:31	
130	54.999	-20.000	1611	5/4/2023	05:03	
131	55.016	-20.002	1090	5/4/2023	10:33	Partial
132	56.000	-20.000	1450	5/4/2023	15:27	
133	56.500	-20.000	1362	5/4/2023	19:49	
134	56.999	-20.000	970	5/5/2023	00:24	
135	57.500	-20.000	1168	5/5/2023	04:44	X
136	58.000	-20.000	1636	5/5/2023	09:25	
137	58.498	-20.007	2570	5/5/2023	14:44	
138	59.000	-20.000	2842	5/5/2023	22:08	X
139	59.002	-20.000	2768	5/6/2023	03:41	
140	60.000	-20.000	2750	5/6/2023	10:53	X
141	60.500	-20.000	2530	5/6/2023	16:31	
142	61.000	-20.000	2401	5/6/2023	21:50	
143	61.334	-20.000	2347	5/7/2023	02:03	
144	61.667	-20.001	1060	5/7/2023	06:14	
145	62.000	-20.000	1711	5/7/2023	11:07	X
146	62.333	-20.000	1800	5/7/2023	15:05	
147	62.749	-20.000	1405	5/7/2023	19:16	
148	63.000	-20.000	1010	5/7/2023	23:03	
149	63.214	-20.000	550	5/8/2023	01:21	
150	63.301	-20.000	260	5/8/2023	03:33	X

Table 4: Station locations



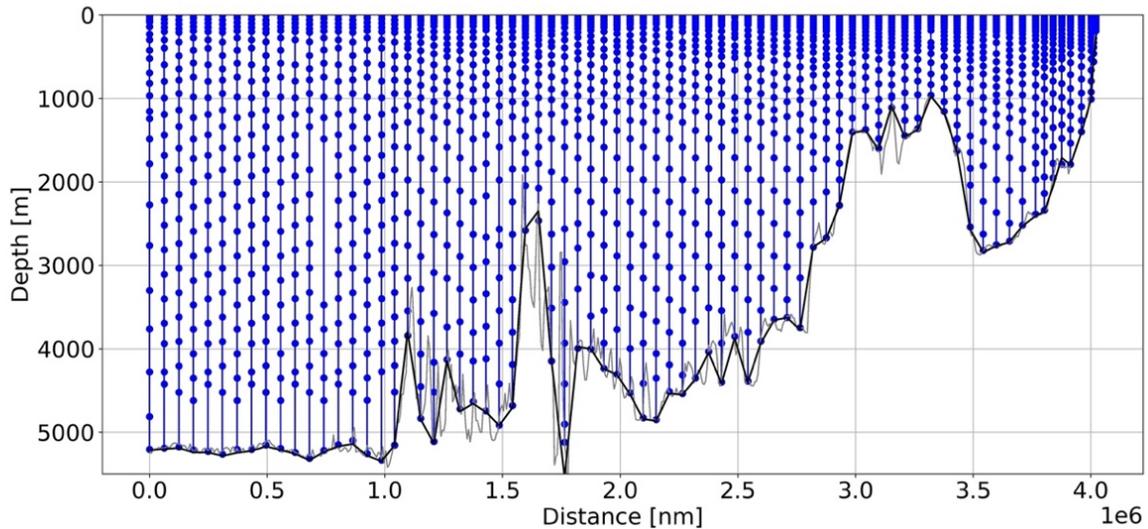


Figure 3. Bathymetry of A16N leg 2. Each line corresponds to a station. The dots indicate the depths at which a bottle was tripped. Figure courtesy of Laura Cimoli.

## 2.4. Exclusive Economic Zone Clearance requests

Clearance was requested from the sovereign nations of Spain, Portugal and Iceland. Consents were provided by all three nations via notes 111/18.2, 39984/2023, and UTN22110189/34.R.611, respectively. However, when A16N leg 1 was cut short and leg 2 had to start stations further south than initially planned, the new route crossed through Canary Islands EEZ waters, which had not been in the initial request. Although we requested a last minute amendment to the area of transit, due to the timing of the request (Easter week and a weekend) we were not able to receive the updated consent in time. As a result, our underway systems had to be shut down while we transited through the Canary Islands region. This causes issues in particular for the processing of the SADCP data. For the next cruise, we suggest including Canary Islands in the initial EEZ request.

The northernmost station in the line is now located in an Icelandic area that has some level of nature protection measures in place. We had to ask for permission to sample, which thankfully was granted in a very short notice. For the next re-occupation this should be addressed at the time of requesting clearance.

## 2.5. Issues

### 1.- Decreased speeds:

Engine issues were at the root of the early stop to leg 1. During the port call some troubleshooting was done by the ship's engineers, but upon departure our transiting speeds were slower than usual. That added almost 2 days to our transit time to the first station. This, in addition to having to occupy several stations that leg 1 did not get to, meant we started the cruise already behind schedule and with no weather window available. Our transit speed remained 9-9.5kn for

the first 20 stations of the cruise. At that point, the engineers and CO felt confident enough on the engine's behavior to increase our transit speeds to ~11kn for the remainder of the cruise.

## 2.- Time saving measures

The following time saving measures were implemented at different points throughout the cruise:

1.- In the next re-occupation of A16N, if spacing out stations should become necessary, it would be best to do so in the stations located around 30-40deg N, before entering the region of dense shelf water production.

2.- The deck crew had ABs ready at each station to help with deployment and recovery operations. Having trained personnel who were always on time and were well coordinated with the survey tech and the winch operator made operations quicker and safer.

3.- Several station spacing options were studied, with a thought of where along the line the impact would be less. We fortunately did not need to implement them, but for future reference, the subtropical region was found to be the one that would suffer less from increased station spacing.

4.- bio-casts were combined with the main cast, adapting some of the main cast bottle depths to serve certain bio samples. By tripping a bottle specifically at 1000 and 500 m, the dedicated bio-cast only had to go to a 200m depth, saving about 30 minutes. This measure came at the expense of having a DNA sample at 1000m (eDNA sample volume was a full 10L). On two occasions, the bio-cast was completely cancelled (saving an estimated 1.3 hours).

5.- Multiple cruise participants volunteered their time to help the Bio scientist more quickly sample their 17 bottles. A recommendation was made moving forward to make sure that if more than one person is sailing for bio sampling, they all be available at the time of the dedicated cast rather than relying on other scientists.

We had unexpectedly good weather given the region we were in and we were never forced to halt operations due to weather conditions. This, combined with a very motivated crew who worked tirelessly to find efficiencies and increase speeds where they could, allowed us to complete all our planned stations.

## 3.- Winch:

The cruise started with the CTD hooked to the aft winch, because the forward one had vibration issues that were limiting the winch speed and that had impacted leg 1. Around station 125, the aft winch developed an oil leak, forcing us to switch to the forward winch for a few stations, and having to use reduced speeds, particularly on the descent. The engineers and chief bosun worked to figure out a way to make the aft winch usable again. We switched back to the aft winch for another few stations, but then glitches in the profiles started to show up around station 135. The frequency of the glitches increased slightly over the next 2-3 stations, so we decided to switch back to the forward winch (which did not need to be re-terminated). The swap happened during transit, so no time was lost. It turned out the aft winch had a serious engine issue and was out of use for the rest of the cruise. The remainder of the casts was done at slower speeds and with the winch operators paying close attention to the vibration on the winch.



#### 4.- "Bloomy" waters:

We crossed the mid-Atlantic in the middle of the spring bloom, and sometimes things would be sucked up by the CTD pumps either during the soak or during the first 0-300m of descent. Thankfully, this always affected one pump only. If it happened right after deployment of the CTD, we often chose to bring the CTD back onboard to clean the pumps up, but with the caveat that the clogging might happen again upon re-deployment given the high productivity at the site. Sometimes we just let the CTD continue downward, and the affected pump would eventually clean itself out. If it affects both pumps and the CTD is already quite (a few hundred meters) deep, the decision will need to consider the time lost on a second cast versus the risk of the pump remaining clogged.

#### 5.- Planned energy black-out:

We were asked to switch off the whole ship to solve an issue with one of the generators. Everything went smoothly and many groups had PSU (power support unit) to carry on working. The black-out was not more than 10-15 minutes. The only issue was the scheduled black-out had been scheduled for 1pm in consultation with the chief and co-chief scientist and the science crew had planned accordingly. At about 8:30am, while in the middle of sampling, the bridge informed us that it had been pushed forward to 10am. We had little time to prepare/back-up the data, and had to wake up off-duty lead analysts.

#### 6.- Drills:

Unlike on other GO-SHIP cruises in the past, we were not allowed to sample or do a cast during drills. We were told we would be allowed to do this only once for the whole cruise, so we had to decide when to use the "card". The bridge had a strong preference to schedule the drills around noon. There was some flexibility in moving the day of the drill, but without necessarily much reason [e.g. moved one day because it was going to happen around 12:30pm, which was considered "late"; another time not moved "no matter what" and ended up being at 2pm]. It was a bit stressful to try to schedule them in a way to minimize science time lost, considering the time restrictions the cruise was dealing with.

#### 7.- Station issues:

- Station 88: eight bottles misfired. This was noticed immediately by the CTD-watchstander because the number of the "fired" bottle did not change. Firing bottles from the CTD station button directly (rather than from the software), did not work. The firing system started again on its own after a while. The troubleshooting indicated the issues was with the carousel, which was subsequently replaced.
- Station 92: issues with the pump impacted sensors. After troubleshooting, and due to time restrictions, the scheduled bio-cast had to be cancelled.

## 2.6. Acknowledgments

The successful completion of the cruise relied on dedicated contributions from many individuals on shore and on the NOAA R/V *Ronald H. Brown*. A team on land worked quickly to put together and air-ship a full spare CTD/O rosette system after the loss of a rosette during leg 1. This allowed leg 2 to sail with a full set of CTD gear plus a spare. The NOAA OAR Office of



International Affairs and the communications teams at AOML and GOMO coordinated and helped disseminate news and outreach pieces about our cruise through multiple social media sites. NOAA OMAO worked to coordinate an official tour of the ship by the US Ambassador to the U.S. in Iceland (<https://globalocean.noaa.gov/u-s-ambassador-to-iceland-visits-noaa-ship-ronald-h-brown-after-55-day-go-ship-cruise-docks-in-reykjavik/>).

On the ship, we are grateful for the dedication from CDR Marc Moser, the officers, engineers, and crew. Good communication with all parties was essential for dealing with problems and helping us anticipate issues and mitigate impacts. Their dedication and motivation to making this cruise a success was noted and very much appreciated.

The GO-SHIP cruises are sponsored by NOAA's Global Ocean Monitoring and Observing Program and the National Science Foundation.

We gratefully acknowledge the consents provided by the sovereign nations of Portugal, Spain and Iceland to conduct research in their territorial waters.

### **3. Underway Data Acquisition**

While the main focus of GO-SHIP cruises is the acquisition of top quality measurements in the water column through use of a CTD rosette package (see section below), underway measurements are continuously collected and several of the underway measurements are considered level 1, i.e. essential to GO-SHIP.

During the transit to the first station, discrete samples were collected from the seawater line for various chemical analyses (DIC, TA, pH, O<sub>2</sub>, pCO<sub>2</sub>, nutrients). A separate dataset of surface measurements is compiled from these samples.

The ship maintains a number of sensors continuously collecting data. These sensors include thermosalinographs, multibeam mapping systems, meteorological sensor suite, surface acoustic doppler current profiler and underway pCO<sub>2</sub>. Navigation data was acquired at 1-second intervals from the ship's Furuno Marine Touch Screen navigational radar. Centerbeam depth data, with a correction for hull depth included in each data line, was 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 was recorded on the CTD Cast Logs.

#### **3.1. Thermosalinograph Measurements**

The ship has two thermosalinographs that continuously measure sea surface temperature and salinity from the seawater line. However, these data have not been traditionally quality controlled. During A16N, the AOML group led by Dr. Rik Wanninkhof undertook the task of quality controlling the data by comparing them to TSG data collected by the group's TSG connected to the underway pCO<sub>2</sub> system and the surface bottle salinity samples collected at each CTD station. The quality-controlled TSG data were incorporated into the underway dataset.



### 3.2. Surface Acoustic Doppler Current Profiler (sADCP)

*PI:* 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 Spanish EEZ region of the Canary Islands.

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.

### 3.3. Underway seawater $p\text{CO}_2$ measurements

*PI:* Denis Pierrot (AOML/NOAA)

*Shipboard personnel:* Patrick Mears (CIMAS/AOML)

The NOAA Ship *Ronald H. Brown* has a semi-permanent automated underway  $p\text{CO}_2$  system maintained by AOML situated in the hydrolab. The design of the instrumental system is based on Wanninkhof and Thoning (1993) and Feely et al. (1998), while details of the instrument and its data processing are described in Pierrot et al. (2009).

The repeating cycle of the system includes four gas standards, five ambient air samples, and 100 headspace samples from its equilibrator within 4.8 hours. The concentrations of the standards range from 232 to 541 ppm  $\text{CO}_2$  in compressed natural air. They were purchased from NOAA/ESRL in Boulder 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 1.8-2.5 liters/min.

The equilibrator headspace is circulated through a non-dispersive infrared analyzer (IR) (LI-COR™ model 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 (= 3/8") 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.

The automated  $p\text{CO}_2$  analytical system operated well throughout the cruise.

Standard Gas Cylinders

Cylinder Number	ppm $\text{CO}_2$
CB09731	232.26
CA08234	399.36
CC720367	430.75
CA06355	541.86

### 3.4. Underway pH

*PI:* Wei-Jun Cai (UDel)

*Shipboard personnel:* Bo Dong (UDel)

Two sensors were set up to measure the pH of underway water flow. One Honeywell Durafet pH sensor was set up in 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 2 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.

## 4. Description of Measurements from Vertical Profiles

### 4.1. Type of CTD casts

Following the previous occupations of the A16N line, stations were nominally spread out every 30 nm, or approximately every half degree in latitude. At the tail end of the section, near the coast of Iceland, stations were more closely spaced to better capture features of the coastal region.

#### *i. Main CTD cast*

At each station, one cast was performed from “surface” (approximately 1.5m depth) to bottom, stopping at about 10m from the seafloor. Bottles were tripped at depths following a rotating scheme of alternating depths, to facilitate interpolation efforts to aid in data interpretation post-cruise.

Samples were drawn in the following order: CFCs (which includes also SF<sub>6</sub> and N<sub>2</sub>O), O<sub>2</sub>, pCO<sub>2</sub>, pH/TA, DIC, C<sup>13</sup>, DOC, nutrients, salinity. If bio samples were collected from the main cast they often were collected after DOC. Duplicates were spread out so that no more than 2 parameters were drawing duplicates from the same Bullister bottle. Due to equipment output limitation, certain parameters did not collect all samples from all depths (e.g. discrete CFCs, pCO<sub>2</sub>, DIC). The regular scheme was for these parameters to have samples collected from all 24 depths at the full degree stations (e.g. at 37N, 38N, etc), and collect from a subset of depths at the half degree stations (e.g. at 37.5N, 38.5N, etc). Attention was paid that all scientists drawing samples for measurements that can be altered by gas exchange were no more than 6 bottles apart. In plain words, the CFCs analyst and the DIC analyst could not be more than 6 Bullister bottles apart in their sampling. This ensures that bottles do not remain open a long time before the DIC analyst collected their sample. A cruise participant, often the CTD-watch stander but sometimes also the chief or co-chief scientist, was assigned “sample cop” duties during sampling. Their role is to ensure that scientists draw their samples from the right bottles, that they don’t jump ahead of other colleagues to keep the sampling order, and to write down any issues with the CTD e.g. leaking bottles, caught lanyards, or other issues surrounding the sampling process.

#### *ii. Bio-casts*

Time-permitting, once per day, at a station taking place between 10 am and 3 pm local time, a second cast was performed to collect biologically relevant water samples. The cast was performed to 1000 m, tripping 17 bottles at the following depths: 1000 m (eDNA, FCM), 500 m (FCM), 200 m (eDNA, FCM), 150 m (FCM), 100 m (eDNA, FCM, HPLC), 75 m (FCM), 40 m (FCM, HPLC), 5m (eDNA, FCM, POC, POP, RNA).

When time did not allow, the bio-cast was performed to 200 m, with the 1000 and 500m bottles being tripped on the main cast and some water allocated for bio.

On three of these stations, where BGC-ARGO floats were deployed, additional samples were collected for validation of the float measurements.

## 4.2. Conductivity, Temperature, Depth (CTD) Hydrographic measurements

*PIs:* Zachary Erickson (PMEL/NOAA), Rick Lumpkin (AOML/NOAA)

*Shipboard personnel:* Kristy McTaggart (PMEL/NOAA), Jay Hooper (CIMAS/AOML)

The CTD/rosette system was deployed off the starboard side. The ship's personnel were responsible for the deployment and recovery of the CTD/rosette. During recovery, the CTD/rosette package was lowered onto a cart and rail system, maintained by the ship, allowing the CTD/rosette package to be safely brought into the staging bay for sampling. A PMEL 24-position rosette system with 12-liter Bullister bottles was used for CTD/rosette stations 1-26/1 on leg 1. An AOML 24-position rosette system with 12-liter Bullister bottles was used for CTD/rosette casts 26/2-150 (the remainder of leg 1 and all of leg 2).

Two altimeters were mounted on the rosette system and used during casts to monitor distance from the bottom.

### 4.2.1. CTD Data Acquisition

The CTD data acquisition system consisted of the ship's SBE-11*plus* (V2) deck unit s/n 11P111660 and a networked Dell Optiplex 7040 Windows 10 workstation running SBE Seasave V7 version 7.26.7.107 software. NMEA GPS data were received through the deck unit. The workstation was used for data acquisition and to close bottles on the rosette. Raw data files were archived immediately after each cast on Survey and Science networked PCs. No real-time data were lost during this cruise.

CTD deployments were initiated by Survey after the Bridge advised that the ship was on station. The transmissometer employed on leg 2 was uncapped and cleaned prior to each deployment using a very dilute Triton-X deionized water solution. 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 lowered it to 20 meters. After a 60-second startup delay, the pumps turned on. The console operator watched the CTD data for reasonable values, waited as long as three minutes at the soak depth for sensors to stabilize, instructed the winch operator to bring the package to the surface, paused for 30 seconds, and began the descent to a target depth approximately 10-20 meters above the sea floor. The descent 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.

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 scientist created a sample log for the cast that would be used to record the water samples taken from each Bullister 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.

Bottles were closed on the upcast through the software. Each bottle was tripped 30 seconds after the winch stopped at each sample 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 a bottle 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, and turned off the deck unit.

At the end of each cast, primary and secondary CTD/O<sub>2</sub> sensors were flushed with a very dilute Triton-X and de-ionized water solution 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 rinse solution to guard against airborne contaminants. The rosette carousel was rinsed with warm freshwater. The transmissometer windows were rinsed and capped after each cast.

Manufacturer / Model	Serial Number	Calibration Date	Station/Casts Used
Sea-Bird 9plus CTD	1401	4-Sep-19	0012-0261
Sea-Bird 3Plus primary temperature	4335	02-Nov-22	0012-0261
Sea-Bird 4C primary conductivity	3157	20-Oct-22	0012-0261
Sea-Bird 43 primary oxygen	3420	29-Oct-22	0012-0261
Sea-Bird 5T primary pump	8794	n/a	0012-0261
Sea-Bird 3Plus secondary temperature	4569	22-Oct-22	0012-0261
Sea-Bird 4C secondary conductivity	2887	21-Oct-22	0012-0171
Sea-Bird 4C secondary conductivity	2973	27-Apr-22	0181-0261
Sea-Bird 43 secondary oxygen	3420	29-Oct-22	0012-0261
Sea-Bird 5T secondary pump	8774	n/a	0012-0261
Sea-Bird 35 reference temperature	76	19-Jul-19	0012-0261
Sea-Bird 32 24-position carousel	500	n/a	0012-0261
Valeport altimeter	56635	n/a	0012-0042
Valeport altimeter	56634	n/a	0051-0261
Benthos altimeter (RHB)	74954	n/a	0031-0042
LADCP			

Table 5: *PMEL yellow frame components and calibration data*

Manufacturer / Model	Serial Number	Calibration Date	Stations Used
Sea-Bird 9plus CTD	1207	06-Jun-22	0263-1501
Sea-Bird 3Plus primary temperature	4341	22-Oct-22	0263-1501
Sea-Bird 4C primary conductivity	4600	01-Nov-22	0263-1501
Sea-Bird 43 primary oxygen	313	04-Jul-19	0263-1501
Sea-Bird 5T primary pump	5885	n/a	0263-1501
Sea-Bird 3Plus secondary temperature	4211	25-Oct-22	0263-1501
Sea-Bird 4C secondary conductivity	3068	21-Oct-22	0263



Sea-Bird 4C secondary conductivity	1374	27-Apr-22	0271-1501
Sea-Bird 43 secondary oxygen	312	04-Jul-19	0263-1501
Sea-Bird 5T secondary pump	8793	n/a	0263-1501
Sea-Bird 35 reference temperature	72	19-Jul-19	0381-1501
Sea-Bird 32 24-position carousel	500	n/a	0263-1501
C-Star transmissometer	1636DR	12-Jun-21	0761-1501
Benthos altimeter (RHB)	74954	n/a	0263-1501
Valeport altimeter (RHB)	73372	n/a	0263-0371
Benthos altimeter (RHB)	73376	n/a	0761-
LADCP			
Chipods			

Table 6: *AOML black frame components and calibration data*

#### 4.2.2. CTD Data Processing

The reduction of profile data began with a standard suite of processing modules using Sea-Bird Data Processing Version 7.26.7 software 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, t0, t1, c0, c1, oxvo1, oxvo2, ox1 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 (within  $\pm 4$  seconds of the confirm bit) and derives both primary and secondary salinity, potential temperature ( $\theta$ ), and potential density anomaly ( $\sigma_\theta$ ). Primary and secondary oxygen (in  $\mu\text{mol/kg}$ ) were derived in DATCNV and averaged in BOTTLESUM, as recommended recently by Sea-Bird.

WILDEDIT makes two passes through the data in 100 scan bins. The first pass flags points greater than 2 standard deviations; the second pass removes points greater than 20 standard deviations from the mean with the flagged points excluded. Data were kept within 0.005 of the mean.

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 ( $\alpha$ ) and the thermal anomaly time constant ( $\beta^{-1}$ ), respectively, as suggested by Sea-Bird.

LOOPEDIT removes scans associated with pressure slowdowns and reversals. If the CTD velocity is less than  $0.25 \text{ m s}^{-1}$  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,  $N^2$ , and linearly interpolates temperature, conductivity, and oxygen voltage over those records where  $N^2$  is less than or equal to  $-1 \times 10^{-5} \text{ s}^{-2}$ . Some profiles failed the criteria near the surface. These data were retained and will be flagged in the final CCHDO formatted .CSV files.

Program calctd.m reads the delooped data files and applies calibrations to pressure, temperature, conductivity, and oxygen; and computes calibrated salinity. Calibrations will be finalized and applied post-cruise.

### **4.3. Lowered Acoustic Doppler Current Profiler (LADCP)**

*PI:* Andreas Thurnherr (LDEO)

*Shipboard personnel:* Michael Cappola (UDEL), Jay Hooper (CIMAS/AOML), Pedro Peña (AOML/NOAA)

#### ***4.3.1 Data Acquisition system***

The Lowered Acoustic Doppler Current Profiler (LADCP) used on GOSHIP A16N consists of two Acoustic Doppler Current Profilers (ADCP) attached to the CTD Rosette. While the CTD moves through the water, the ADCPs continuously transmit acoustic pings to obtain short velocity profiles which are combined with post processing software to create a continuous full depth velocity profile. One ADCP is installed at the top of the rosette (uplooker) and one is

installed at the bottom of the rosette (downlooker). The velocity profiles are constrained at the surface and the bottom, using the ship's ADCP and bathymetry tracking software, respectively. ADCPs were provided by Lamont-Doherty Earth Observatory and the deck cable and battery system was provided by NOAA/AOML. Michael Cappola (University of Delaware) was responsible for all hardware setup, data acquisition, and initial data processing at sea, with shore support from Andreas Thurnherr (Lamont-Doherty Earth Observatory). LADCP operational assistance was provided by Jay Hooper (NOAA/AOML) and Pedro Pena (NOAA/AOML).

### *Hardware*

Following the lost CTD from A16N leg 1, two additional ADCPs were sent out for leg 2, which brought the total available ADCPs back to three. We installed one Teledyne/RDI 300 kHz Workhorse ADCP (WHM300 SN754) as the downlooker, one Teledyne/RDI 300 kHz Workhorse ADCP (WHM300 SN24497) as the uplooker, and retained one Teledyne/RDI 600 kHz Workhorse ADCP (WHM600 SN20031) as a backup. The two operational ADCPs were powered by a battery bank which was installed at the base of the rosette. These components were cabled together using a 5 way "star cable" which had two additional leads for charging and data acquisition. While the CTD was deployed, these leads would be dummy plugged. While the CTD was onboard, these leads were connected to a deck cable which was routed into the Hydro Lab. In the Hydro Lab, power to charge the battery bank was provided by a 48v 1.5A charger. Data was downloaded and commands were sent via a Mac Mini data acquisition computer. This computer was used for all user interface with the instruments and the initial processing. Data was automatically backed up using "time machine" software and via storage on the ship's network science drive.

### *Software*

All data acquisition and instrument interface was done using TRDI terminal commands which were organized into several functions as a part of the Aquire2 software suite. The downlooker ADCP had additional "wm15" firmware to support the bottom tracking velocity constraint. All vertical velocity profiles were processed with LADCP w Software V2.0. All horizontal velocity profiles were processed with LDEO LADCP software: Version IX\_14. All software was provided and maintained by Andreas Thurnherr (Lamont-Doherty Earth Observatory).

### *Nominal Operations*

About 15 minutes before each CTD station, the ADCPs were communicated with to ensure functionality. 5 minutes before each cast, the ADCP was started and the battery charger was secured. The deck cable was disconnected and the cables were dummy plugged. These loose cable ends were secured to the CTD rosette frame. At this point the CTD and LADCP system was given control to the survey technicians. The CTD was deployed. Upon recovery, the cable ends were rinsed with freshwater, the deck cable was connected, the battery was placed on the charger, and the downloading commenced. Once download was complete, the raw data was automatically backed up. Finally, the vertical and horizontal velocity was processed. This processed data was then backed up to the science drive, as well as transmitted to Lamont-Doherty Earth Observatory for quality control and analysis.

The LADCP system was successfully deployed for all A16N stations, as well as the 14 bio casts that extended to at least 1000 meters.



### 4.3.2 Data Quality

The ADCPs both performed exceptionally well, with no fatal errors throughout the cruise.

The data quality in the beginning of the cruise had higher errors due to the lack of particles in the water while the ship sampled the gyre. The low signal strength from the lack of backscatter reduced the ability to obtain reliable velocity profiles. This metric was observed by looking at the root mean square difference of the LADCP vs the SADCPC (Figure 4). We saw a marked improvement in this metric once leaving the gyre and the signal quality continued to improve for the duration of the cruise.

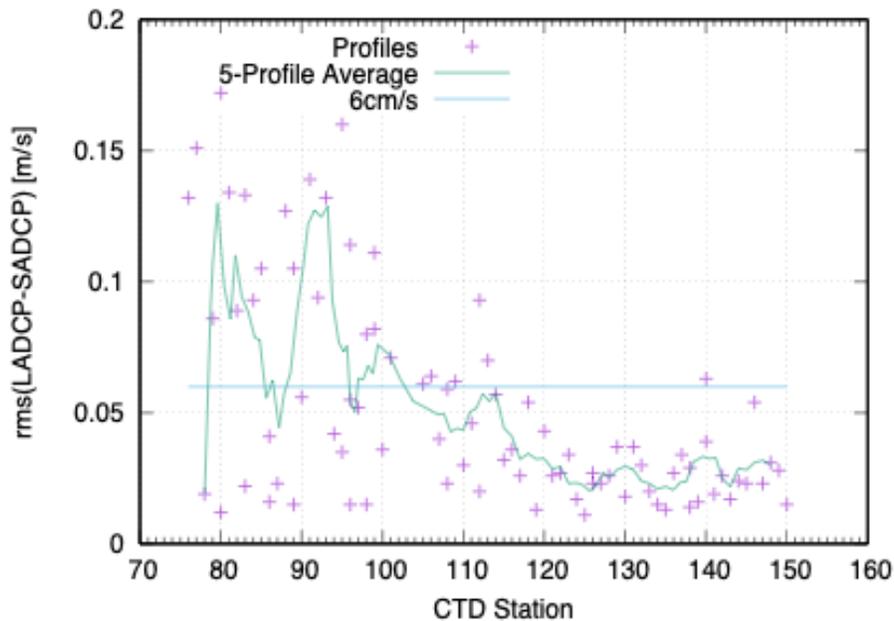


Figure 4. Root mean square difference of the LADCP-SADCP. A metric of data quality near the surface.

### 4.3.3 Data Issues

Our battery bank failed early in the beginning of the cruise. It stopped charging and eventually failed during station 078.02. It failed during the upcast, so a complete downcast profile was still completed. That being said, the lower voltage caused the transmission strength to be very low for stations 077.01, 078.01, and 078.02. This likely had a negative impact on the data quality for those casts.

On station 103.02, there was excessive biofouling on the CTD intake pump. Only the primary sensors were affected. For this cast, the LADCP data was processed manually using the secondary sensors instead of the primary sensors.

On station 141.01, the vertical velocity failed to process with the nominal settings. Solving this issue required post processing and a software patch. This cast is not processable with the software version available on the cruise.

#### 4.4. Chipods

*PI:* Jonathan Nash (OSU)

*Shipboard personnel:* Jay Hooper (CIMAS/AOML), Pedro Peña (AOML/NOAA)

##### 4.4.1 Overview

Chipods are instrument packages that measure turbulence in the ocean. Specifically, they are used to compute turbulent diffusivity of heat ( $K$ ) which is inferred from measuring dissipation rate of temperature variance ( $\chi$ ) combined with a shipboard CTD. Chipods are self-contained, robust and record temperature and derivative signals from FP07 thermistors at 100 Hz; they also record sensor motion at the same sampling rate. Details of the measurement and methods for processing  $\chi$  can be found in Moum\_and\_Nash (2009). In an effort to expand the global coverage of deep ocean turbulence measurements, the ocean mixing group at Oregon State University has supported chipod measurements on many global repeat hydrography cruises since December 2013.

##### 4.4.2 System Configuration and Sampling

Two chipods were mounted on the rosette to measure temperature ( $T$ ), its time derivative ( $dT/dt$ ), and  $x$  and  $z$  (horizontal and vertical) accelerations at a sampling rate of 100 Hz. One chipod was oriented such that its sensors pointed upward. The other one was pointed downward. The up-looking sensor (SN2014, was positioned higher than the Bullister bottles on the rosette in order to avoid measuring turbulence generated by flow around the rosette and/or its wake while its profiling speed oscillates as a result of swell-induced ship-heave. The down-looking sensor was positioned as far from the frame as possible and as close to the leading edge of the rosette during descent as possible to avoid measuring turbulence generated by the rosette frame and lowered ADCP.

The chipods continuously recorded data at all stations, from 76 to 150, without interruptions.

##### 4.4.3 Issues

One downward facing chipod was installed on Leg1 after the loss of the first rosette (i.e. from station 27 onwards). No upward facing chipod was installed on Leg1 due to lack of proper mounting brackets/frame.

Data is saved on SD cards but it was not possible to check the data from chipods on board at the end of Leg 2 due to lack of proper software availability. The software had been shared with the scientists but it was not working as expected and we troubleshooting remotely was not possible. We tried all options: reading the SD cards directly, and reading them via usb connections. None worked. We ended up making a raw copy of the SD cards to back-up the data, just in case. The data was eventually collected and readable.

For future cruises we recommend:

- making sure the PI ships also all the frames/brackets needed for mounting the chipods, especially the upward facing one.

- either the PI sends a windows laptop with a working software or makes sure the software does work on someone's laptop before departure or there is no way to check the data while onboard (e.g. in between cruise legs or if anything happened to the chipods).

#### 4.5. Chlorofluorocarbon (CFC), Sulfur Hexafluoride (SF<sub>6</sub>), and Nitrous Oxide (N<sub>2</sub>O)

*PI:* Rolf Sonnerup (PMEL/CICOES)

*Shipboard personnel:* David Cooper (CICOES), Carol Gonzalez (CICOES), and Isabel Schaal (WHOI)

Samples for the chlorofluorocarbons (CFCs, freons) F<sub>11</sub> and F<sub>12</sub>, sulfur hexafluoride (SF<sub>6</sub>) and nitrous oxide (N<sub>2</sub>O) were collected and analyzed during A16N leg 2. 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 11-liter Bullister bottles. Samples for CFC and SF<sub>6</sub> 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 Bullister 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 to approximately 30 degrees 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<sup>-1</sup>) 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<sup>-1</sup>) 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<sub>6</sub> and N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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  $\sim 170^{\circ}\text{C}$ . The sample gases held in the trap were then injected onto a precolumn ( $\sim 60$  cm of 1/8" O.D. stainless steel tubing packed with 80-100 mesh Porasil B, held at  $80^{\circ}\text{C}$ ) 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 ( $\sim 170$  cm of 1/8" OD stainless steel tubing packed with MS5A and held at  $80^{\circ}\text{C}$ ) the outflow from the first precolumn was diverted to the second analytical column ( $\sim 150$  cm 1/8" OD stainless steel tubing packed with Carbograph 1AC, 80-100 mesh, held at  $80^{\circ}\text{C}$ ). 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  $\text{N}_2\text{O}$  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 ( $250^{\circ}\text{C}$ ). The third analytical column and second pre-column were held at  $160^{\circ}\text{C}$  in a Shimadzu GC-8A gas chromatogram, with the detector held at  $250^{\circ}\text{C}$ .

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  $\sim 12$  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 ( $\text{pmol kg}^{-1}$ ),  $\text{SF}_6$  concentrations are given in femtomoles per kilogram of seawater ( $\text{fmol kg}^{-1}$ ).  $\text{N}_2\text{O}$  concentrations are given in nanomoles per kilogram of seawater ( $\text{nmol kg}^{-1}$ ). 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.

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  $\text{SF}_6$  and F12 was greater than 99% and the efficiency for F11 was about 99%. The purging efficiency for  $\text{N}_2\text{O}$  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 1683 seawater samples will be submitted from the 75 stations of Leg 2. Duplicates were taken from 60 stations to estimate precision and variability. These duplicates are divided between lower level CFC/ $\text{SF}_6$  samples from deeper water ( $\text{F11} < 0.4 \text{ pmol/kg}$ ) and higher level samples taken from the upper water column ( $\text{F11} > 0.4 \text{ pmol/kg}$ ).  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  measurements, and 3% from the mean for  $\text{SF}_6$  measurements. Deviation from the mean of

pairs from the lower concentration CFC/SF<sub>6</sub> samples averaged less than 4% from the mean for F12, less than 1% from the mean for F11, and is not calculable for SF<sub>6</sub> 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<sub>6</sub> data were unresolved from baseline noise. It is anticipated that some of the flagged data will be replaced with more accurate values.

A small number of water samples had anomalous SF<sub>6</sub> 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.

#### 4.6. Dissolved oxygen (O<sub>2</sub>)

*PI:* Chris Langdon (Rosenstiel)

*Shipboard personnel:* Riley Palmer and Marina Ruddick (Rosenstiel)

##### 4.6.1 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 17.7-21.9°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 = 0.89uL) 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.

##### 4.6.2 Sampling and Data Processing

Dissolved oxygen samples were drawn from Bullister 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 Bullister bottle. 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 Bullister bottle integrity. 1 ml of MnCl<sub>2</sub> 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, if the station had a depth greater than 2000



meters. Fewer Bullisters were fired for stations with depths fewer than 2000 meters and the lowest number of Bullisters fired was 13. Two duplicates were drawn at each station. The total number of samples collected from the rosette was 1879. 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 17.7 and 21.9 C. Reagent blanks were run at the beginning ( $1.5 \pm 1.0$  uL) of leg one.

#### 4.6.3 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.

#### 4.6.4 Duplicate Samples

For the entirety of the cruise, two duplicates were drawn at each station: the first from Bullister 1 (deepest depth of that station) and the second from a different depth. The bottles selected for the second duplicate, and hence the oxygen flasks, were changed for each cast. A total of 149 sets of duplicates were run (Figure 5). One set of duplicates were removed from analysis (Station 117, Bullister 1) and coded with quality flag #3 due to sampling error.

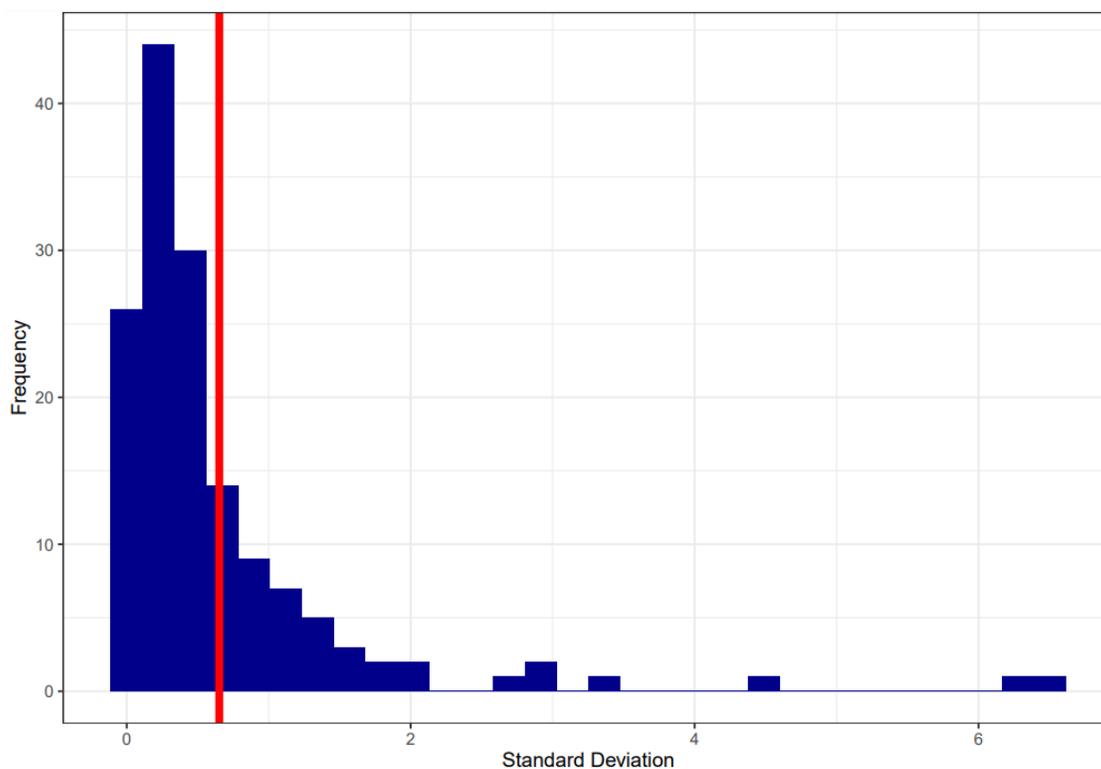


Figure 5. Standard deviation of duplicate oxygen analyses performed during A16N 2023. Average is 0.65 umol/kg (red line), median is 0.36 umol/kg, IQR is 0.71- 0.14 = 0.57 umol/kg, and  $n = 149$ .

#### 4.6.5 Quality Coding

Preliminary quality code flags have been assigned to the oxygen data. A summary of the quality coding can be found in Table 7.

Quality Flag	No.	Description
2	3351	good
3	44	sample value high or low compared to CTD value, potential sampling error
4	6	known sampling error
6	298	duplicates
9	17	not sampled or problem with Bullister bottle

Table 7: Discrete oxygen sampling statistics A16N 2023

#### 4.6.5 Problems

##### *NaI/NaOH dispenser*

The NaI/NaOH dispenser was found to be sticking during Leg one at stations 34 and 35. To prevent this issue from occurring on Leg two, the NaI/NaOH dispenser was cleared with DIW every two to three days.

##### *Titration Speed*

During the first half of leg two, the run time for samples was significantly longer than they had been on leg one. The average run time on leg one for a 24 Bullister cast was approximately two hours and 45 minutes. The run time on leg two was approximately four hours. In order to increase the run time, the slope was increased (see Slope Increase section) and the wait time was lowered from 800 to 600. The probe was also allowed to soak in sulfuric acid in order to clean it. The changes in slope and wait time as well as the cleaning of the probe helped to increase the speed of the sample run times.

##### *Slope increase*

A total of 7 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<sub>2</sub>  $\mu\text{mol/kg}$  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 78 (Bullister 8). At Station 87, after 5 samples had exceeded the number of data points, the slope was increased from 6 to 6.2. The run time on the samples was significantly longer for the first half of leg two than they were on leg one. To try to increase the speed at which the samples were run, the slope was increased several times. It was increased from 6.2 to 7.7, then 8 and then it was decreased to 7.8 because one of the endpoints did not reach 15 (Station 107, Bullister 21). The slope had to be changed again when the titrator was replaced (see New Titrator section); the slope was lowered to 3.5. After the titrator was replaced,

2 samples exceeded the number of data points and the slope was raised again from 3.5 to 4, and when it happened a second time, the slope was increased from 4 to 4.5.

### *New Titrator*

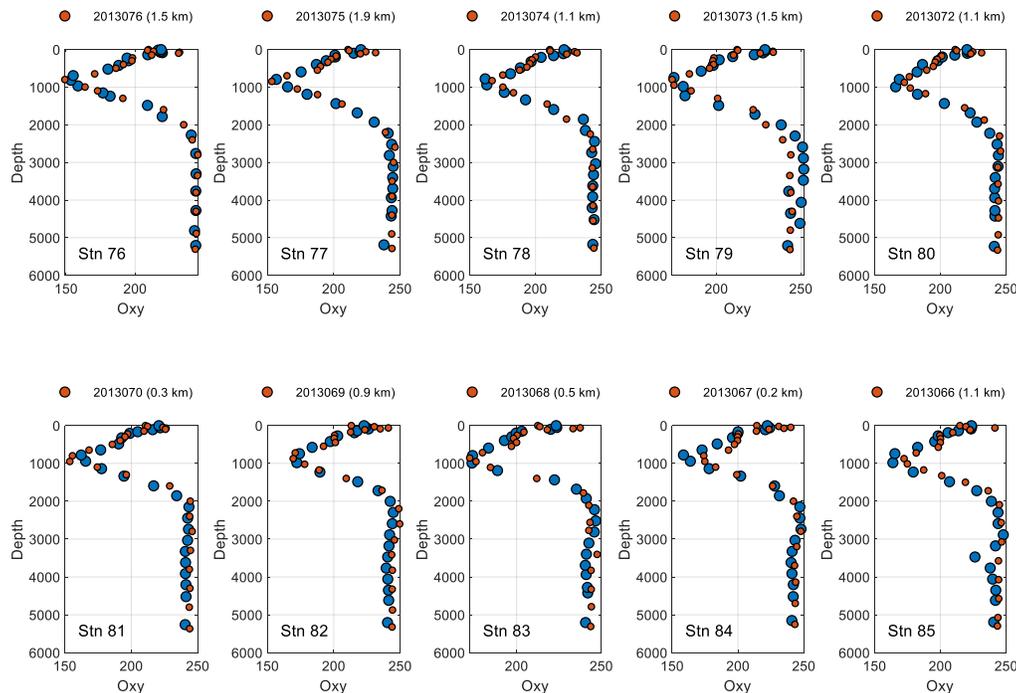
The Titrator was replaced at Station 137 due to mechanical malfunctioning. Standards were run after replacing the titrator to make sure it was running properly. A slope of 4 chosen as it allowed for the titration to be completed within the range of 30 data points.

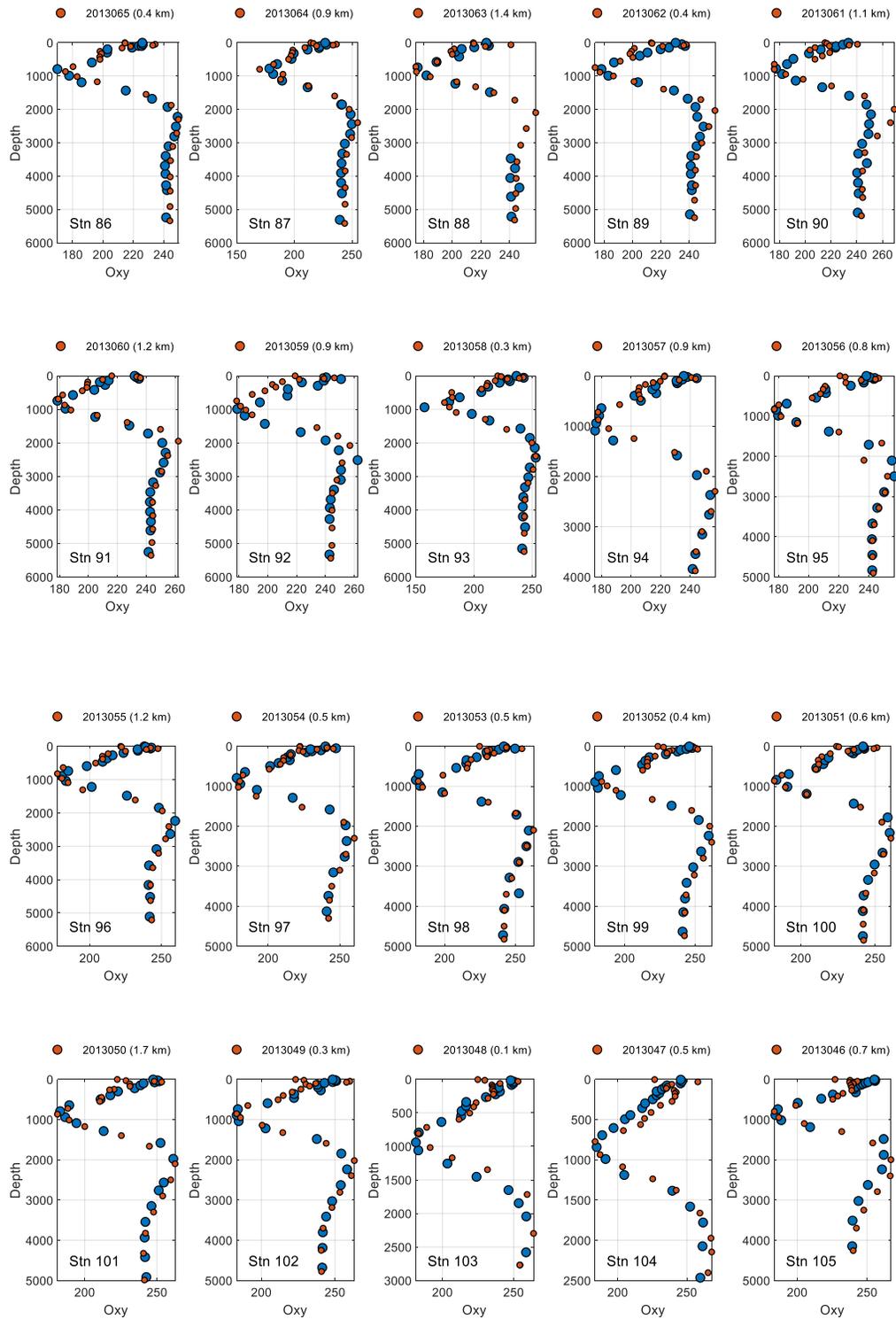
### *Broken flasks*

Flask 7: broken at station 140; The Bullister was resampled. Replaced with flask 57 on Station 141  
Flask 67: broken at station 144; The flask was dropped after the sample had been run.

## **Comparison of A16N 2013 vs. 2023**

A preliminary analysis was performed by Bo Yang (pH and TA; Rosenstiel) to compare oxygen values from A16N 2013 to A16N 2023. Only Stations 76 through 120 are included in the analysis due to the fact that stations 121-150 were not available at the time of analysis. The data are visualized in Figure 6 below.





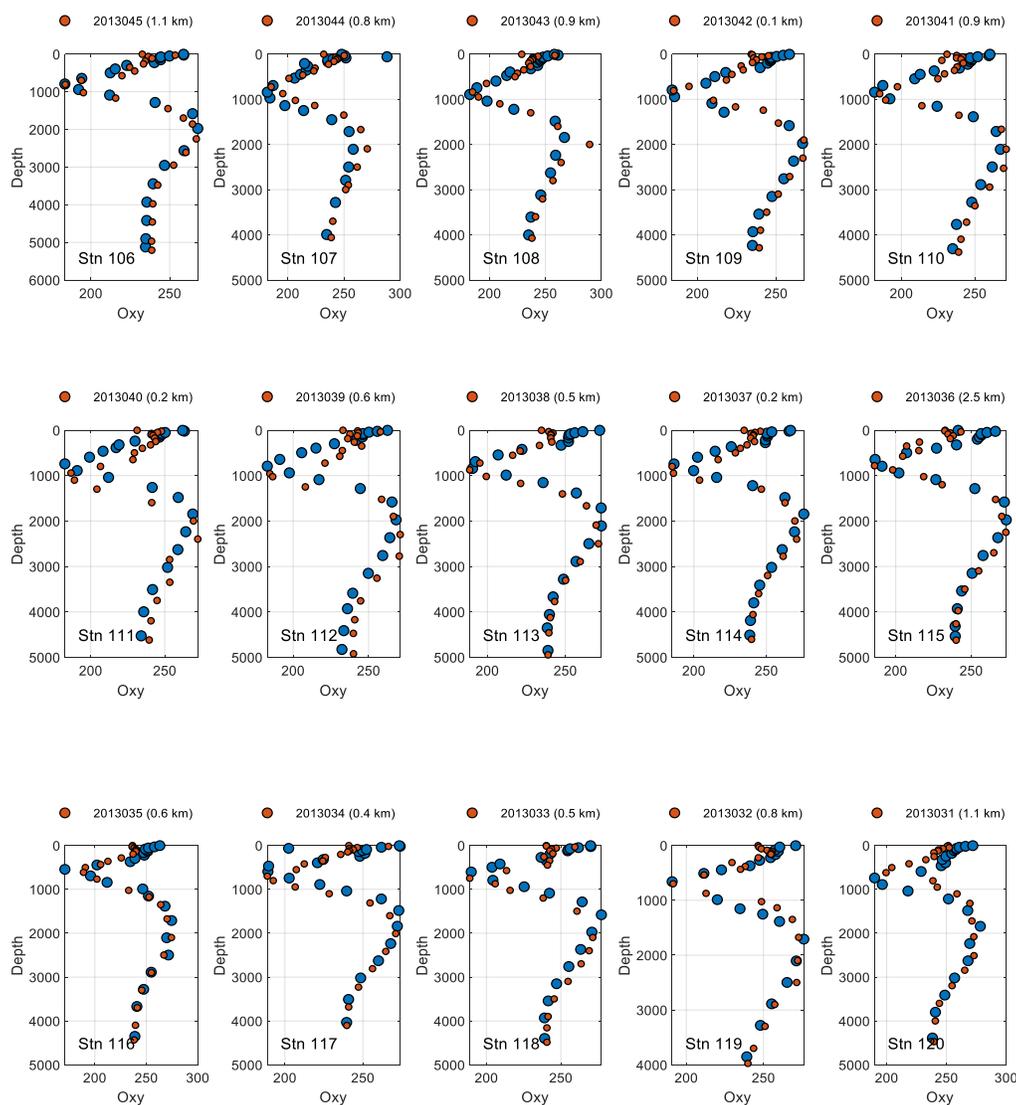


Figure 6: A16N 2013 to 2023 O<sub>2</sub> comparison

#### 4.7. Dissolved Inorganic Carbon (DIC)

*PIs:* Rik Wanninkhof (NOAA/AOML) and Richard Feely (NOAA/PMEL)

*Shipboard personnel:* Charles Featherstone (NOAA/AOML) and Dana Greeley (NOAA/PMEL)

##### 4.7.1 Sample collection:

Samples for DIC measurements were drawn (according to procedures outlined in the PICES Publication, *Guide to Best Practices for Ocean CO<sub>2</sub> Measurements*) from Bullister 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<sub>2</sub> 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.

#### **4.7.2 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 NOAA/AOML and Dana Greeley of NOAA/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*.

#### **4.7.3 DIC Analysis:**

In coulometric analysis of DIC, all carbonate species are converted to CO<sub>2</sub> (gas) by addition of excess hydrogen ion (acid) to the seawater sample, and the evolved CO<sub>2</sub> 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<sup>-</sup> ions at the anode. The OH<sup>-</sup> ions react with the H<sup>+</sup>, 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<sub>2</sub> that enters the cell is determined by integrating the total change during the titration.

#### **4.7.4 DIC Calculation:**

Calculation of the amount of CO<sub>2</sub> injected was according to the CO<sub>2</sub> handbook (DOE 1994). The concentration of CO<sub>2</sub> ( $[CO_2]$ ) in the samples was determined according to:

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

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 ( $\mu mol/kg$ ) 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<sub>2</sub> 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 5.82  $\mu\text{mol/kg}$  for AOML 3 and 0.94  $\mu\text{mol/kg}$  for AOML 4 (CRM Batch 201).

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 37.

#### ***4.7.5 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 Bullister bottle were measured near the beginning; middle and end of each cell.

Each coulometer was calibrated by injecting aliquots of pure  $\text{CO}_2$  (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 data base have been corrected to batch 201 CRM values. The CRM certified value for batch 201 is 2048.19  $\mu\text{mol/kg}^1$ .

The precision of the two DICE systems can be demonstrated via the replicate samples. Approximately 11% of the bullisters 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 1.46  $\mu\text{mol/kg}$  for AOML 3 and 1.49  $\mu\text{mol/kg}$  for AOML 4 - No major systematic differences between the replicates were observed (Table 8).

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.

Calibration data during this cruise:

UNIT	Ave L Loop Cal Factor	Ave S Loop Cal Factor	Pipette	Ave CRM <sup>1</sup>	STDEV <sup>1</sup>	AVG Dupes <sup>2</sup>	STDEV Dupes <sup>2</sup>
AOML 3	1.003985	1.004526	26.845 ml	Batch 201: 2042.4, N= 33	1.27	1.46	0.98
AOML 4	1.003820	1.004491	29.391 ml	Batch 201: 2048.1, N = 30	0.42	1.49	1.05

Table 8: DIC system calibration information

#### 4.7.6 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 15 discrete DIC samples including duplicates were collected while underway. The average difference for replicates of underway DIC samples was 1.44  $\mu\text{mol/kg}$  and the average STDEV was 1.16.

#### 4.7.7 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. Valve 4 on DICE 3 had to be replaced.

Including the duplicates, 1783 samples were analyzed from 75 CTD casts for dissolved inorganic carbon (DIC) which means there is a DIC value for approximately 96% 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.

#### 4.8. Discrete pH Analysis

*PIs:* Chris Langdon (Rosenstiel)

*Shipboard personnel:* Laura Stieghorst (Rosenstiel) and Seamus Jameson (Rosenstiel/SJSU)

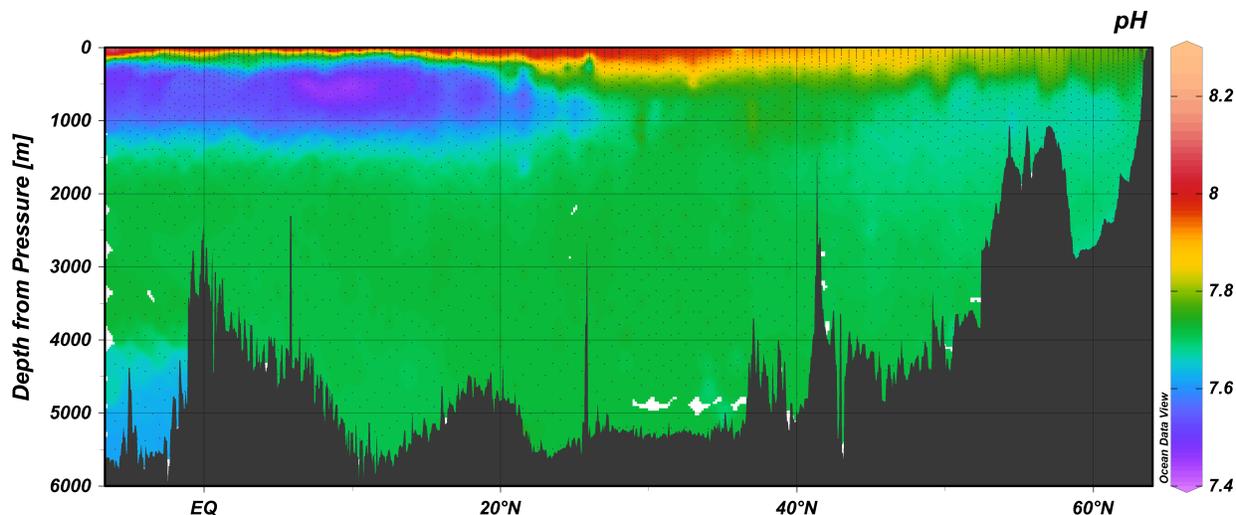


Figure 7: A16N\_2023 pH

##### 4.8.1 Sampling:

Samples were collected in 250 mL narrow mouth borosilicate glass bottles using silicone tubing. Bottles were rinsed with sample and overflowed by the bottle's volume. Samples were sealed using a glass stopper with no headspace left. A plastic over-cap was used to secure the glass stopper. Samples were warmed to 25.0°C before the measurement. From station 39 onwards, two duplicates were collected from most stations. The same bottle of sample was used for total alkalinity measurement after pH was measured.

##### 4.8.2 Analysis:

pH (total scale) was measured spectrophotometrically using a semi-automatic analyzer with purified meta-cresol purple (mCP). The semi-automatic analyzer (similar to the instrument reported by Carter et. al, 2013) consists of a HP8453 spectrophotometer, a Kloehn 6-port syringe pump with 10 ml burette, a Starna 10 cm flow cell (type 585.3) with water jacket, and a Hart Scientific FLUKE 1523 reference thermometer. A Thermo Scientific Haake SC150 water bath maintained the spectrophotometric cell temperature at 25.0°C. The absorbance of light ( $A$ ) was measured at four different wavelengths (434 nm, 578 nm, 730 nm, and 488 nm). The absorbance ratio (R-ratio) of  $A_{578}$  and  $A_{434}$  was used for pH calculation (together with temperature and salinity), with  $A_{730}$  as a reference to correct any disturbances ( $R = (A_{578} - A_{730}) / (A_{434} - A_{730})$ ). Details of the calculation can be found in Liu et. al, (2011). The absorbance at 488 nm ( $A_{488}$ ) was used to ensure that a constant amount of dye was added to the sample. Salinity data were obtained from the conductivity sensor on the CTD. Perturbation from mCP addition was corrected using the 'double dye' method (Clayton and Byrne, 1993; Dickson et. al, 2007).

#### 4.8.2 Reagents:

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).

#### 4.8.3 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 (Clayton and Byrne, 1993; Dickson et. al, 2007). Briefly, for each station we chose 1-2 samples with different pH and measured R-ratio twice (with the dye addition doubled at the 2<sup>nd</sup> time) to get the difference in R-ratio ( $\Delta R$ ). And then the mCP perturbation can be corrected with a linear regression between R-ratio and  $\Delta R$ .

Overall, 3461 pH samples were analyzed, with only 0.3% marked as questionable (Table 9). Repeat measurements on duplicate samples showed a difference between pH duplicates (sample 2 – sample 1) of  $7.6 \times 10^{-6} \pm 0.0021$  ( $n = 213$ ).

Flag	2 (Good)	3 (Questionable)	4 (Bad)	6 (Duplicate)
Number of Samples	3238	10	0	213
Percentage (%)	93.6	0.3	-	6.2

Table 9: A16N\_2023 pH quality flag assignment

#### 4.9. Total Alkalinity

PI: Chris Langdon (Rosenstiel)

Shipboard personnel: Bo Yang and Jessica Leonard (Rosenstiel)

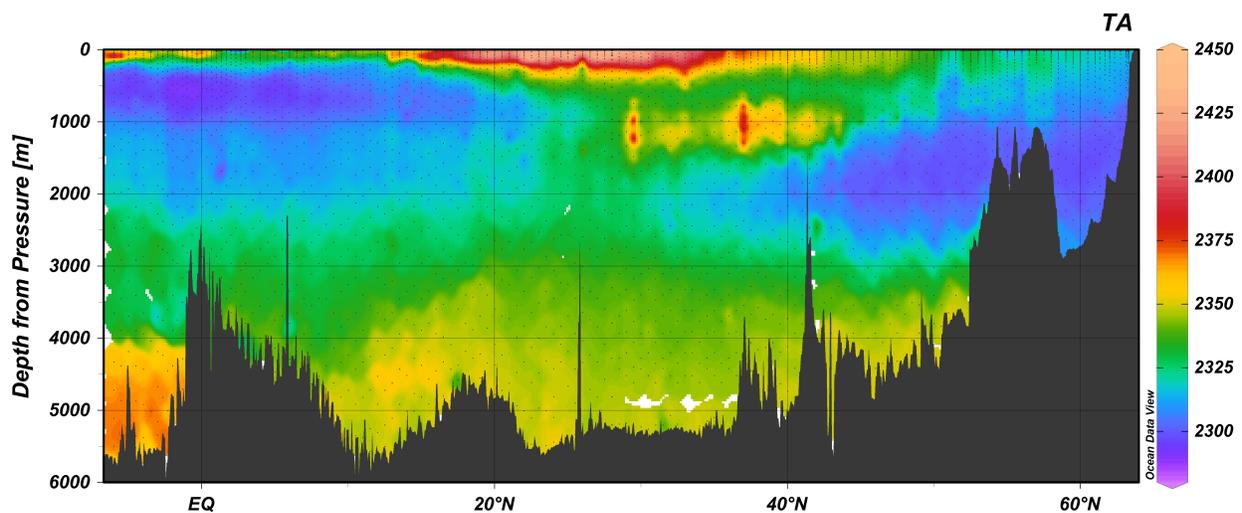


Figure 8: A16N\_2023 Total Alkalinity (TA,  $\mu\text{mol kg}^{-1}$ )

#### **4.9.1 Sampling:**

We used the leftover from pH measurements for TA analysis. A custom-made sample dispenser with a glass pipette was used to volumetrically measure out an accurate amount (96.083 ml at 25°C) of sample for titration.

#### **4.9.2 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 a 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 (powered 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 solution (~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<sub>2</sub> generated during this process. After that, a series of aliquots of 0.05 ml HCl solution were added and the pH was measured after each addition 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.

#### **4.9.3 Reagents:**

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

#### **4.9.4 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 UCSD. During the cruise, the acid concentration was checked several times by measuring CRM (leftover from DIC measurements).

#### **4.9.5 Data Processing:**

A custom-made LabView software was used for system control and TA calculation, which automatically calculated the TA. Briefly, 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 (see the details in section 7.3 of SOP 3b from Dickson et al., 2007). Overall, 3404 TA samples were analyzed, with only 0.4% marked as questionable or bad (Table 10). Repeat measurements on duplicate samples showed a difference between TA duplicates (sample 2 – sample 1) of  $0.02 \pm 1.32 \mu\text{mol kg}^{-1}$  ( $n = 192$ ).

Flag	2 (Good)	3 (Questionable)	4 (Bad)	6 (Duplicate)
Number of Samples	3199	12	1	192
Percentage (%)	94.0	0.4	-	5.6

Table 10: A16N\_2023 TA quality flag assignment

An internal consistency check was performed using measured TA, pH, and preliminary dissolved inorganic carbon (DIC) data (only good data points with quality flag 2 or 6 were used,  $n = 3209$ ). The mean difference between measured TA and TA calculated from the pH-DIC pair is  $0.50 \pm 4.11 \mu\text{mol kg}^{-1}$  (Figure 9).

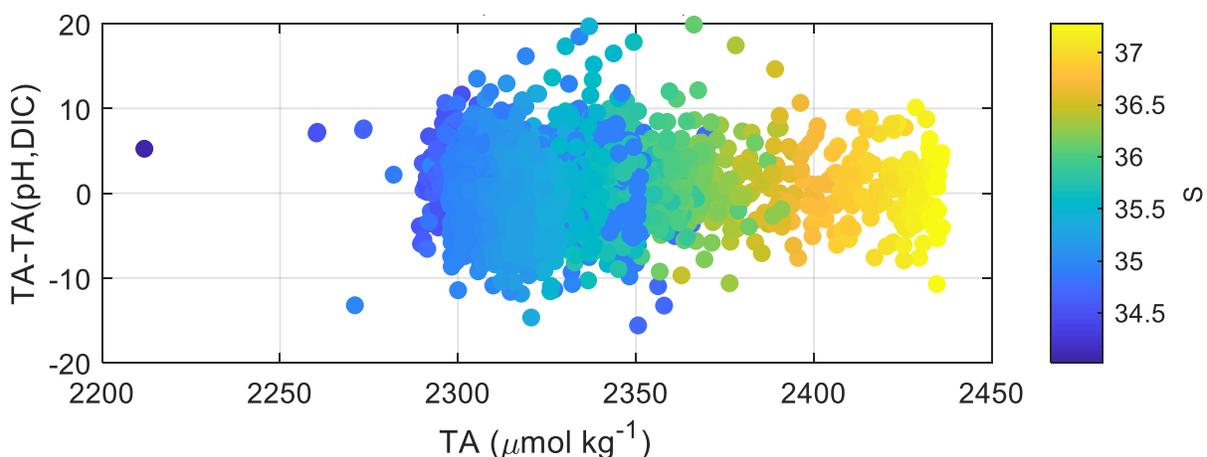


Figure 9: Difference between measured TA and TA calculated from pH-DIC pair, as a function of measured TA (with salinity presented in color).

#### 4.10. Discrete pCO<sub>2</sub> Measurements

PI: Rik Wanninkhof (AOML/NOAA)

Shipboard personnel: N. Patrick Mears and Leah Chomiak (CIMAS/AOML)

##### 4.10.1 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<sub>2</sub> 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.

One-thousand six hundred and thirty-nine unique samples were drawn from 75 CTD casts covering 94% of all unique depths. Seventy-five sets of duplicate bottles were drawn at numerous depths. The average relative standard error was 0.04%, while the median relative error was 0.03%.

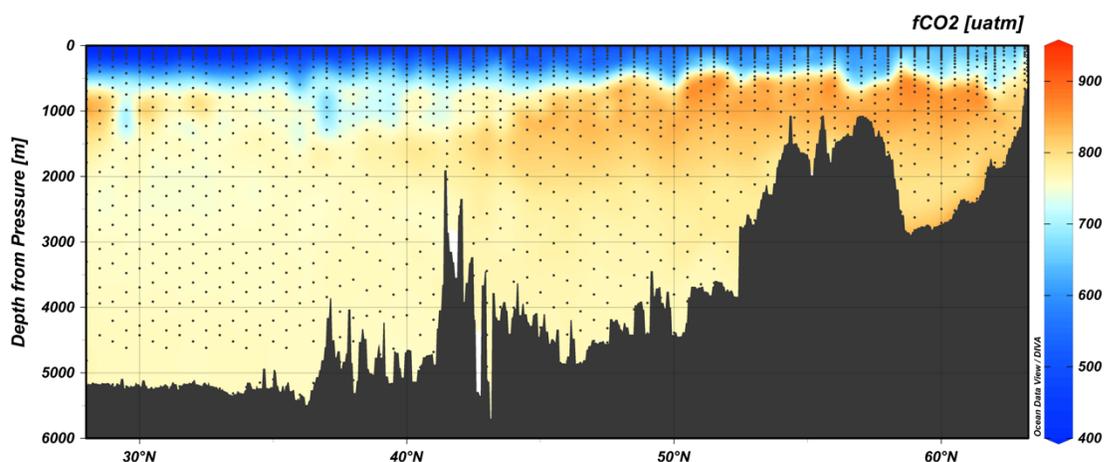


Figure 10: Measured  $f\text{CO}_2$  from bottle data collected along GO-SHIP A16N line from 28°N to 63.3°N. Scales are from 400 uatm to 950 uatm.

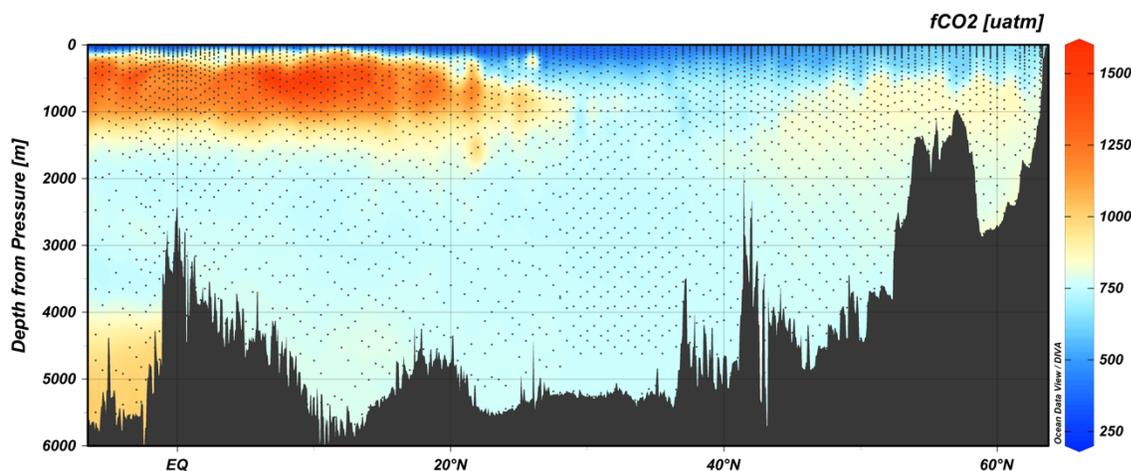


Figure 11: Measured  $f\text{CO}_2$  from bottle data collected along GO-SHIP A16N line from 6°S to 63.3°N. Scales are from 250 uatm to 1550 uatm.

#### ***4.10.2 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<sub>2</sub> 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 20 underway stations were collected with duplicate samples collected every 4 stations during the transit from Rota, Spain to the reoccupation of Station 75. A blockage in the gas line from the instrument chest to the dry box prevented one underway sample from being analyzed.

#### ***4.10.3 Analyzer Description:***

The principles of the discrete pCO<sub>2</sub> 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 27 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<sub>2</sub> concentration as well as the temperatures, pressures and gas flow during the 10-minute equilibration. The CO<sub>2</sub> 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<sub>2</sub> program are reformatted so that a Matlab program designed for processing data from the continuous pCO<sub>2</sub> 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).

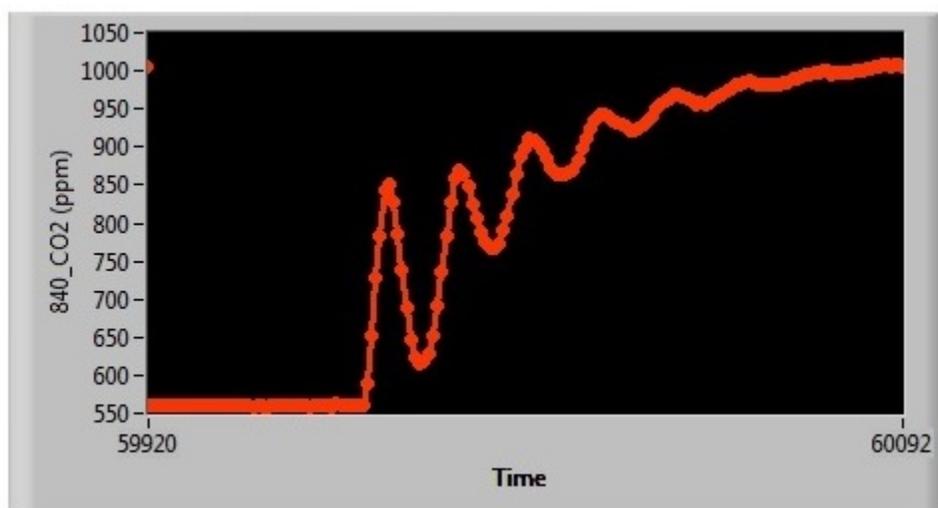


Figure 12. CO<sub>2</sub> oscillations during start of the first sample analysis in a set of twelve

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 assisted in collecting samples.

Standard Gas Cylinders:

<u>Cylinder#</u>	<u>ppm CO<sub>2</sub></u>
JB03282	288.55
JB03268	384.30
CB11243	591.87
CA05980	792.51
CA05984	1036.95
CA05940	1533.7

## 4.11. $\delta^{13}\text{C}$ -Dissolved Inorganic Carbon (DIC)

*PI:* Wei-Jun Cai (UDel)

*Shipboard personnel:* Bo Dong and Zhentao Sun (UDel)

### 4.11.1 Sampling:

Samples for  $\delta^{13}\text{C}$ -DIC measurements were drawn according to procedures outlined in the PICES Special Publication, Guide to Best Practices for Ocean  $\text{CO}_2$  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  $\mu\text{L}$  of saturated  $\text{HgCl}_2$  solution was added to stop biological activities, usually 1~2 hours after samples were taken to allow thermal expansion. 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 in open boxes for at least 48 hours to attain ambient room temperature before immediate measurement or stored in coolers to take back.  $\delta^{13}\text{C}$ -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.

### 4.11.2 Analysis:

The  $\delta^{13}\text{C}$ -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 bio lab and used simultaneously on leg 1. Each system is composed of a whole-water  $\text{CO}_2$  extraction device with a 12-port sample valve (AS-D1, Apollo Scitech, Newark, DE, USA; [www.apollostech.com](http://www.apollostech.com)) and a CRDS isotopic detector (G2131-i, Picarro, Santa Clara, CA, USA [www.picarro.com](http://www.picarro.com)). Both instruments were coupled and automated with a single software to simultaneously measure DIC concentrations and  $\delta^{13}\text{C}$ -DIC signals via quantifying the  $\text{CO}_2$  extracted from acidified samples. Briefly, an aliquot of sample is acidified with 5%  $\text{H}_3\text{PO}_4$  in the gas stripping reactor and the liberated  $\text{CO}_2$  is brought by the carrier gas ( $\text{CO}_2$ -free compressed air) to the CRDS analyzer. The raw data for  $\text{CO}_2$  ( $^{12}\text{CO}_2 + ^{13}\text{CO}_2$ ) and  $\delta^{13}\text{C}$ - $\text{CO}_2$  are read from CRDS and are recorded at  $\sim 1$  Hz frequency for a period of  $\sim 600$  s. When the  $\text{CO}_2$  measurements drops below a preset threshold (i.e., 15 consecutive data points of  $\text{CO}_2$  is  $<$  (baseline value + 5) ppm), or the change drops below a preset threshold (i.e., standard deviation of  $\text{CO}_2$  for 15 consecutive data points is  $< 0.16$  ppm), the software will terminate the analysis, because there is only a small amount of  $\text{CO}_2$  left in the reactor and further gas stripping would change the area integration value of  $\text{CO}_2$  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  $\text{CO}_2$  gas is integrated over time to derive a net area for quantifying DIC concentrations. The  $\delta^{13}\text{C}$ -DIC is derived as the  $\text{CO}_2$  weighted mean of  $\delta^{13}\text{C}$ - $\text{CO}_2$  data with a cutoff point of 400 ppm to avoid high noise at low  $\text{CO}_2$  signal.

Three batches of  $\delta^{13}\text{C}$  standard solutions (GOSHIP-A, GOSHIP-B, GOSHIP-C) with different  $\delta^{13}\text{C}$  values were prepared by dissolving pure  $\text{NaH}^{13}\text{CO}_3$  and  $\text{NaHCO}_3$  powder with known  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$ -DIC value obtained by two adjacent measurements of homemade standards whose  $\delta^{13}\text{C}$  values were obtained by the IRMS method from the UC Davis laboratory.

The DIC value of samples was determined according to:

$$[DIC] = \frac{(\overline{area} \times slope + intercept)}{sample\ volume \times sample\ density}$$

where  $\overline{area}$  is the average area under the curve of the mole fraction  $\text{CO}_2$  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  $\delta^{13}\text{C}$  value of samples denoted in ‰ was determined according to:

$$\delta^{13}\text{C} = \left( \frac{(\delta^{13}\text{C}/\delta^{12}\text{C})_{sample}}{(\delta^{13}\text{C}/\delta^{12}\text{C})_{V-PDB}} - 1 \right) \times 1000$$

where  $(\delta^{13}\text{C}/\delta^{12}\text{C})_{sample}$  is the  $\text{CO}_2$  weighted mean of  $\delta^{13}\text{C}$ - $\text{CO}_2$  data with a cutoff point of 400 ppm,  $(\delta^{13}\text{C}/\delta^{12}\text{C})_{V-PDB}$  is the  $\delta^{13}\text{C}$  value of reference standard Vienna-PeeDee Belemnite (V-PDB).

1944 CTD samples were collected from 1731 niskin bottles on leg 2, corresponding to 12.3 % duplicate rate. Overall for A16N, a total of 3890 samples were collected from 3518 niskin bottles, corresponding to 10.6 % duplicate rate. 1400 samples were measured on leg 2, and a total of 2875 samples were measured onboard (73.9 %).

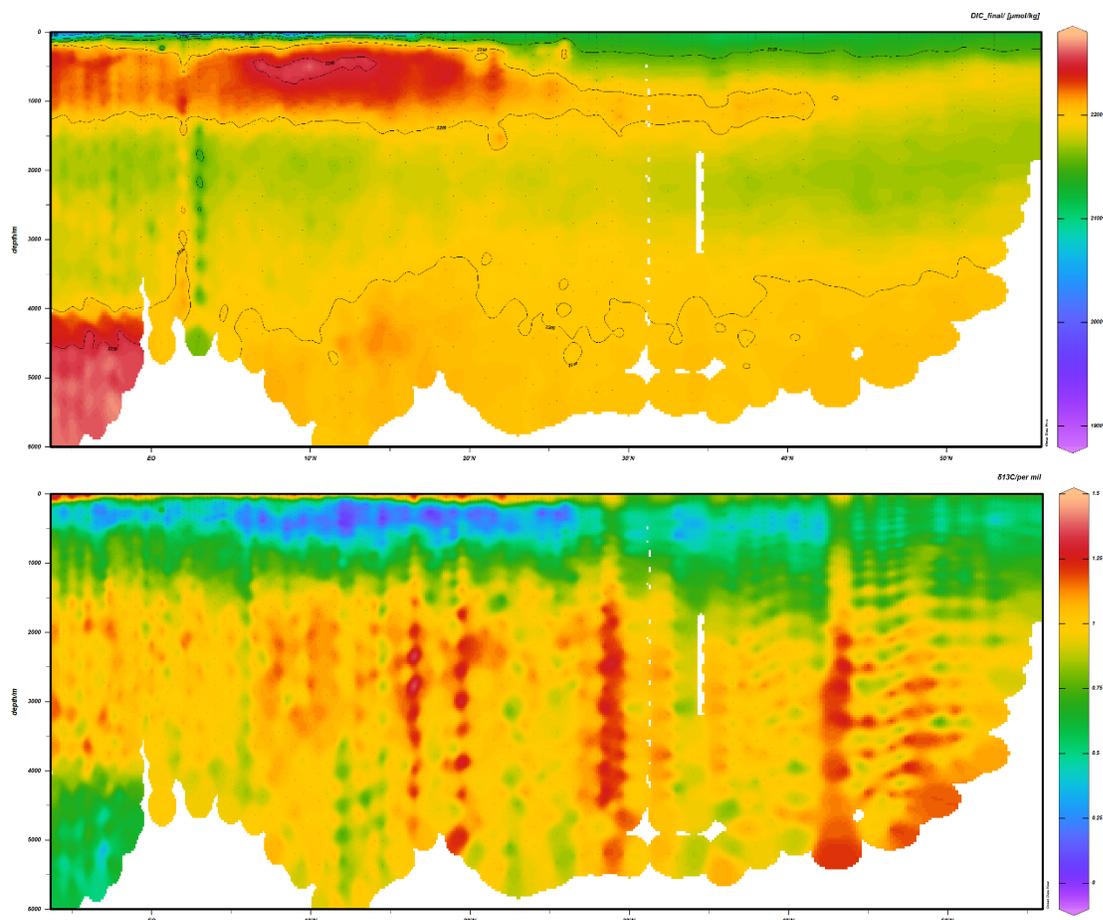


Figure 13: DIC ( $\mu\text{mol/kg}$ ), upper panel, and  $\delta^{13}\text{C}$  (per mil) measured on A16N 2023 (legs 1 and 2)

A total of 232 pairs of duplicate samples were measured on both legs, the average differences are  $0.03 \mu\text{mol/kg}$  for DIC and  $0.00 \text{‰}$  for  $\delta^{13}\text{C}$ , the standard deviation for DIC and  $\delta^{13}\text{C}$  are  $2.67 \mu\text{mol/kg}$  and  $0.08 \text{‰}$  respectively (Figure 14).

During the whole cruise, 16 bottles of new CRM and 64 bottles of opened CRM from the AOML DIC team were measured throughout the entire cruise, most of which were within  $\pm 5 \mu\text{mol/kg}$  of the certified values of CRM. Further QA/QC will be conducted after the rest of samples are measured in lab.

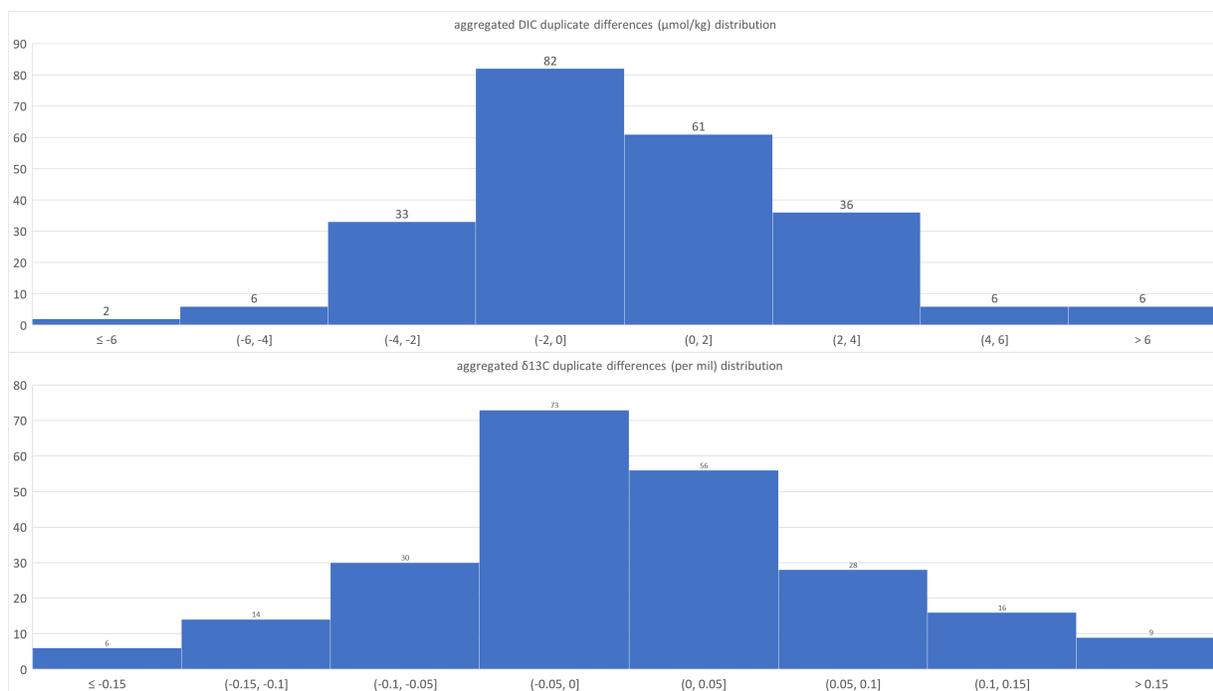


Figure 14: Distribution of aggregated DIC duplicate differences in  $\mu\text{mol/kg}$  (upper panel) and aggregated  $\delta^{13}\text{C}$  duplicate differences in per mil (lower panel)

#### 4.12. Dissolved Organic Carbon (DOC)

*PI:* Dennis Hansell (Rosenstiel)

*Shipboard personnel:* Victoria Dina (Rosenstiel)

DOC and TDN samples were taken from every sample bottle at approximately every other station, and surface samples collected at the remaining stations. 854 samples were taken from 74 stations in total. Samples from depths of 250m 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  $\mu\text{l}$  of 4 N HCl and stored to be shipped back to Rosenstiel for analysis.

#### 4.13. Radiocarbon $^{14}\text{C}/^{13}\text{C}$

*PI:* Rolf Sonnerup ((PMEL/CICOES) and Roberta Hansman (WHOI)

*Shipboard personnel:* Victoria Dina (Rosenstiel)

A total of 336 samples were collected from 14 stations.  $^{14}\text{C}/^{13}\text{C}$  samples were collected at every 2.5 degrees ( $\pm 1$  degree), in an approximate resampling of the stations analyzed during the 2013 occupation of this transect. 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. Three duplicate samples were collected at every station at <500 m, 500-1500 m, and >1,500 m respectively. Once the samples were taken, a small amount of water (~5 ml) was removed to create a head-space and 100 ul of 50% saturated mercuric chloride solution was 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 vacuum seal grease, and rubber banded 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.

#### **4.14. Nutrients**

*PI:* Calvin Mordy (PMEL/CICOES) and Jia-Zhong Zhang (AOML/NOAA)

*Shipboard personnel:* Eric Wisegarver (PMEL/NOAA) and Ian Smith (CIMAS/AOML)

##### ***4.14.1 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 colorimetric 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 silicomolybdic 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 stream.

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.

##### ***4.14.2 Sampling and Standards:***

Nutrient samples were drawn in 50ml 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 250ml 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 1730 samples were analyzed.

#### **4.15. Discrete Salinity Sampling**

*PI:* Rick Lumpkin (AOML/NOAA)

*Shipboard personnel:* James Hooper (CIMAS/AOML) and Pedro Peña (AOML/NOAA)

A single Guildline Autosol, model 8400B salinometers (S/N 71464), located in the salinity analysis room, was used for all salinity measurements. The Autosol was calibrated January 2014. The salinometer readings were logged on a computer using Ocean Scientific International's logging hardware and software. The Autosol's water bath temperature was set to 24°C, which the Autosol 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 Autosol room temperature. Salinity analyses were performed after samples had equilibrated to the Autosol room temperature at least 18 hours after collection. For the colder high latitude waters the cases were placed in a warm water bath for approximately 2 hours and then were let to come to temperature for at least 12 hours. 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 Autosol 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 leg 2, 1627 salinity measurements were taken, including 124 duplicates, and approximately 66 vials of standard seawater (SSW) were used. Up to two duplicate samples, one for shallow casts, were drawn from each cast to determine total analytical precision.

The standard calibration values and duplicates are below in Figure 15 and Figure 16. The duplicates taken during the cruise showed a median precision of  $-0.0004 \pm 0.006$  psu.

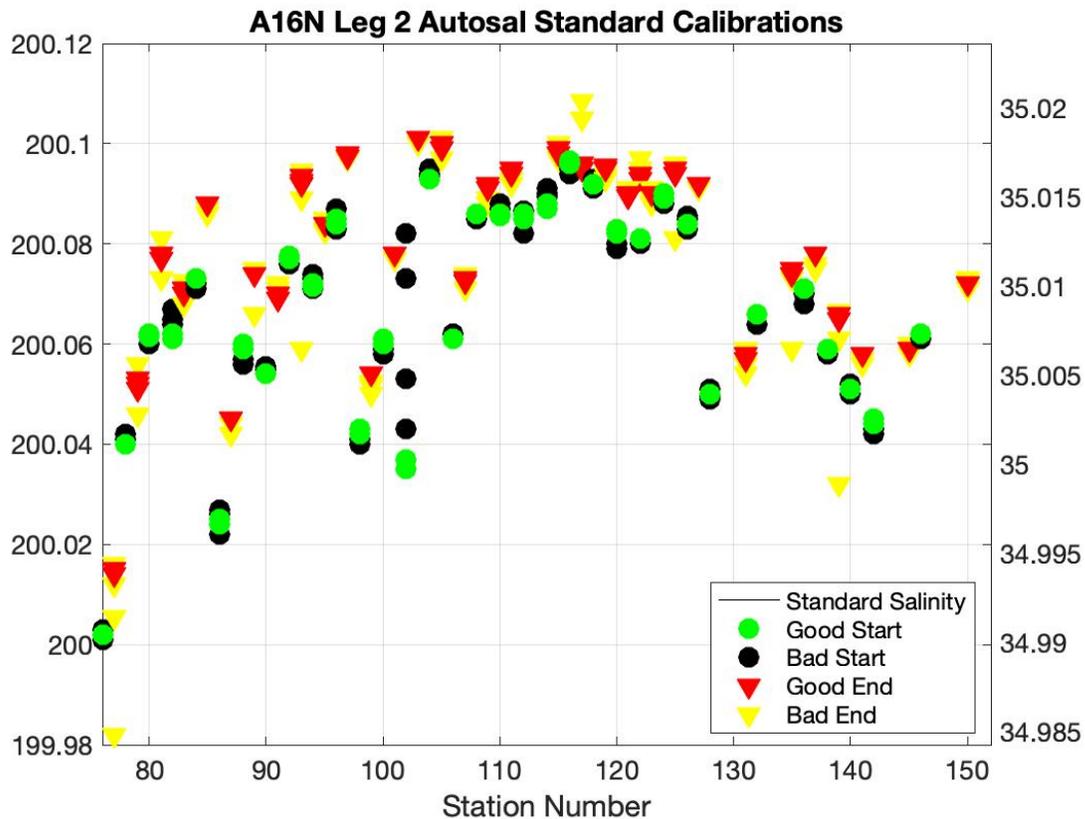


Figure 15: Standard vial calibrations throughout the cruise before and after each Autosal run. The green dots and red triangles are the good values used before and after each run to calculate salinity and drift corrections, respectively. The black dots and yellow triangles are the bad values not used. The left vertical axis is 200 X the conductivity ration and the right axis is the corresponding salinity.

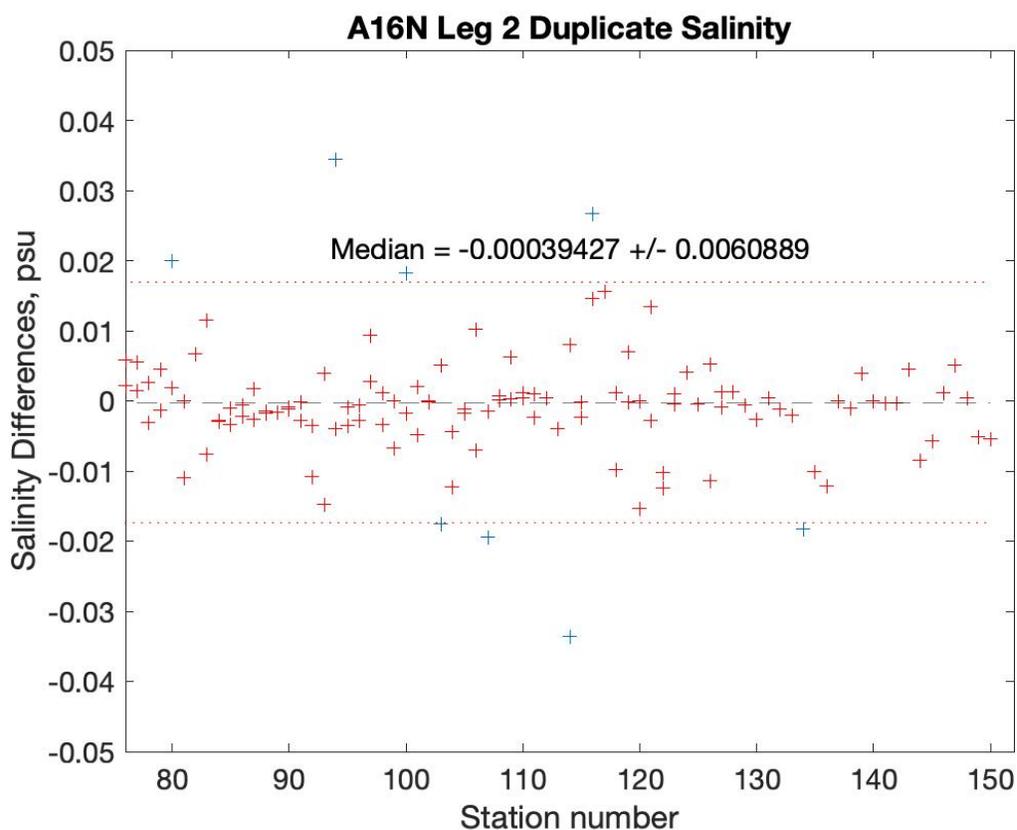


Figure 16: Duplicates throughout the A16N leg 2 cruise.

## 5. BIO-GO-SHIP

*PI:* Harriet Alexander (WHOI), Sophie Clayton (ODU), Jason Graff (OSU), Adam Martiny (UCI), Nicole Poulton (Bigelow), Luke Thompson (NGI/AOML)

*Shipboard personnel:* Rachel Cohn (CIMAS/AOML), Star Dressler (UGuam)

### 5.1. Continuous Inline Sampling

An underway system utilizing a diaphragm pump provided continuous flow from surface waters for optical instrumentation (BB3), Imaging Flow CytoBot (IFCB), and flow cytometer (FCM). Further details on FCM, IFCB, and optical sampling are outlined in sections 5.8, 5.9, and 5.10 respectively.

On March 11 (Leg 1), it was determined that the ship’s inline system used for the diaphragm pump was contaminated with rust. The PIs 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 NOAA Ship *Ronald H. Brown* utilizes an impeller pump, which is recognized to significantly alter particle size

distribution and particle counts. Nevertheless, the impeller system was utilized to gather all underway samples during Leg 2.

## 5.2. Underway Sampling

eDNA, RNA, large volume particulate organic matter (POM), high performance liquid chromatography (HPLC), and flow cytometry (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 62 total underway sampling events during Leg 2. Additionally, 22 eDNA samples were collected in conjunction with core GO-SHIP underway sampling during the transit from Spain back to the main sampling line.

## 5.3. Bio-Cast CTD Station Sampling

During a once-daily “bio-cast” station, the CTD rosette was deployed twice (21 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 fired one at a time at the surface, as compared to Leg 1 which “rapid fired” all remaining bottles at once. Water was divided for appropriate sampling of eDNA, RNA, POM, HPLC, and FCM. Further details on bio-cast sampling protocols are detailed in the sections below.

Due to time constraints on Leg 2, the daily bio-cast was occasionally skipped. Creative measures were implemented to assist in the collection of CTD water in these instances. The bio team would gather water from the main cast, where the main cast sample depths were altered to match the bio-cast standard depths when possible. If the bio team gathered water from the main cast, they would gather surface water from the underway impeller pump system to complement the CTD samples from depth. At other times, the Bio team would sample from the main cast, and a second shallow-depth CTD would be deployed to gather additional water for Bio team. Often in these cases, less samples were gathered for Bio-GO-Ship compared to what would be gathered during a standard bio-cast. There were 3 stations where the underway and CTD samplings overlapped/were combined. In these irregular sampling schemes, the sourced location of sampling water was always clearly noted on Bio-GO-Ships sampling logs. HPLC, eDNA, RNA, and POM samples were always collected at standard Bio-cast depths, but FCM samples were sometimes gathered at depths that differed from standard Bio-cast depths.

## 5.4. Biogeochemical (BGC)-Argo Float Station Sampling

At each bio-cast station correlating with a BGC-Argo float deployment (4 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 on the CTD, the deep chlorophyll maximum was often estimated at 15 m below the base of the mixed layer. Occasionally, the transmissometer would give some indication to a deep chlorophyll maximum. At each depth, 1-2 liters of water was gathered for POC and 1-2 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  $-80^{\circ}\text{C}$  for later analysis. Wet and dry blanks were included for both POC and HPLC. Twelve samples were processed for each float on Leg 2 (48 total samples), not including one dry blank and one wet blank filter for each parameter at each deployment station.

## 5.5. 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. 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 (8-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 two-three times with tap water and once with 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 rinsed with sample water, and the end of each tubing was then secured with a Sterivex 0.22- $\mu\text{m}$  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  $\mu\text{l}$  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  $\mu\text{l}$  of lysis buffer (800  $\mu\text{l}$  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, in which case future eDNA samples can follow only the Protocol B procedure. All samples were labeled following protocol and stored at  $-80^{\circ}\text{C}$  for later analysis. Sample lines were cleaned with 5% bleach solution and then DI water immediately following sampling at each depth. On A16N Leg 2, 70 total samples were processed for eDNA underway sampling, and 69 total samples were processed for eDNA bio-cast CTD stations; 27 total samples were processed for RNA underway sampling, and 17 total samples were processed for RNA bio-cast CTD stations. There were 3 stations where the underway and CTD samplings overlapped/were combined due to



time constraints. Additionally, 22 eDNA samples were collected alongside the other core GO-SHIP samples taken in transit from Spain back to the main sampling line.

## 5.6. 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 CTDs and underway sampling, 8-liter triplicates were gathered for both POC/N and POP (24 total liters per parameter). CTD samples were all gathered from surface bottles fired at approximately 5 m. All sampling water for POM was gathered through plastic tubing secured with 30  $\mu\text{m}$  mesh at the outflow to filter out larger particles. Once the ship arrived in more productive North Atlantic waters, a smaller volume was often collected (2-4 liters per 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 two-three times with tap water and once with 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  $\text{Na}_2\text{SO}_4$  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  $-80\text{ }^\circ\text{C}$  for later analysis. Sample lines and filter housings were quickly rinsed with DI water. On A16N Leg 2, 174 POP samples and 178 POC/N samples were processed from underway sampling; 45 POP samples and 47 POC/N samples were processed from bio-cast CTD stations. There were 3 stations where the underway and CTD samplings overlapped/were combined due to time constraints.

## 5.7. High Performance Liquid Chromatography (HPLC)

One 2-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, 2-liter HPLC samples were gathered at depths of 100 m, 40 m, and 5 m. Once the ship arrived in more productive North Atlantic waters, a smaller volume was often collected (~1 liter per 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 two-three times with tap water and once with 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  $-80\text{ }^\circ\text{C}$  for later analysis. On A16N Leg 2, 72 total samples were processed for underway sampling, and 63 total samples were processed for bio-cast CTD stations

(not including BGC Argo Float HPLC samples). There were 3 stations where the underway and CTD samplings overlapped/were combined due to time constraints.

### **5.8. 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  $\mu$ L 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 2, 62 total samples were processed for underway sampling, and 177 total samples were processed for bio-cast CTD stations. There were 3 stations where the underway and CTD samplings overlapped/were combined due to time constraints.

Throughout Leg 2, the shipboard Cytek Northern Lights FCM was operated on a SpectraFlo software template where a 1-mL inline water sample (diaphragm pump) was processed hourly over each 24-hour period.

### **5.9. Imaging FlowCytobot (IFCB)**

The IFCB received continuous water from the diaphragm pump inline system throughout Leg 2. The software was managed remotely by Sophie Clayton. Samplers onboard 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- $\mu$ m mesh fabric. This “reverse filtration” system allows particles smaller than 10  $\mu$ m to pass through, while containing all larger particles within a 100-mL volume. Once the ship arrived in more productive North Atlantic waters, the initial volume collected was lowered to 5-10 liters. The 100-mL concentrated 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 2, 21 total samples were preserved.

## 5.10. Inline Optics

The BB3 backscatter detector received continuous water from the diaphragm pump inline system throughout Leg 2. 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 data, as well as live tracking of latitude/longitude coordinates. Due to the large amounts of rust in the underway system, the BB3 was cleaned with DI water and isopropyl alcohol wipes daily.

## 6. Other activities: float and drifter deployments

Three types of floats were deployed during leg 2 of A16N: one core ARGO float, four biogeochemical (BGC) Argo floats, and 5 surface drifters.

### 6.1. Core and BGC Argo floats

*PI:* Kenneth Johnson (MBARI), Lynne Talley (UCSD/SIO), Susan Wijffels (WHOI), David Nicholson (WHOI), Pelle Robbins (WHOI)

*Shipboard personnel:* None assigned, multiple people contributed

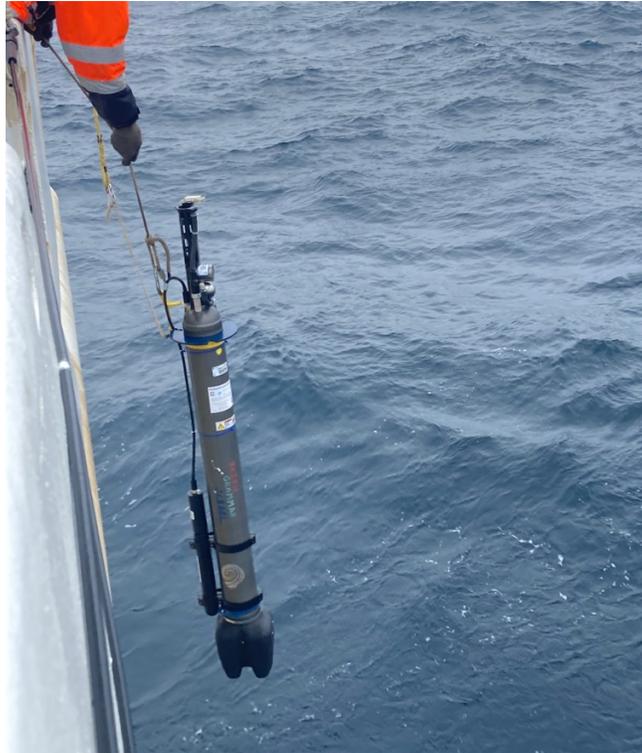
Argo is an international program that began in 1999 to measure the temperature and salinity of the upper 2,000 meters of the global ocean. The Argo program consists of an array of free drifting floats that move following the ocean currents. The floats are distributed over the global ocean and rely on ships providing a platform for deployment of new floats to keep the array with the number of active floats required for global coverage. Once deployed, the floats have a protocol where they park themselves at 1000 m drifting with currents, and every 10 days they conduct a profile from 2000 m to the surface.

The Global Ocean Biogeochemistry Array (GO-BGC, <https://go-bgc.org>) Argo program uses floats that, in addition to temperature and salinity sensors, include equipment to measure dissolved oxygen, nitrate, pH, fluorescence (Chl), backscatter, and irradiance. The BGC floats follow the same protocol as core floats, collecting one profile (2000 m to the surface) every 10 days.

By deploying the BGC float at the same location where GO-SHIP is conducting a station, the float has a reference profile of high quality temperature, salinity, dissolved oxygen, nutrients and carbon measurements to validate the initial sensor data from the float. Additional samples for measurement of HPLC and POC were collected during the bio-cast. Section 5.4 provides details on these additional samples.

The BGC floats were set to self-activate upon deployment and therefore, the only pre-deployment requirement was to carefully clean the bio-optical sensors. The lenses were cleaned with DI water and dabbed with lens wipes.

Both the core and the BGC floats were deployed from the aft stern, as the ship sailed away from the CTD station at 1kn speed. Floats were lowered using a slip line, with help from the deck crew (Figure 17).



*Figure 17: Deployment of BGC float 1476 on May 6<sup>th</sup>.*

The location of the float deployments was pre-determined by the float PIs and shared with the chief scientists prior to the cruise. Communication with the team during the cruise was great. Figure 18 shows a schematic of the suggested deployment sites for A16N.

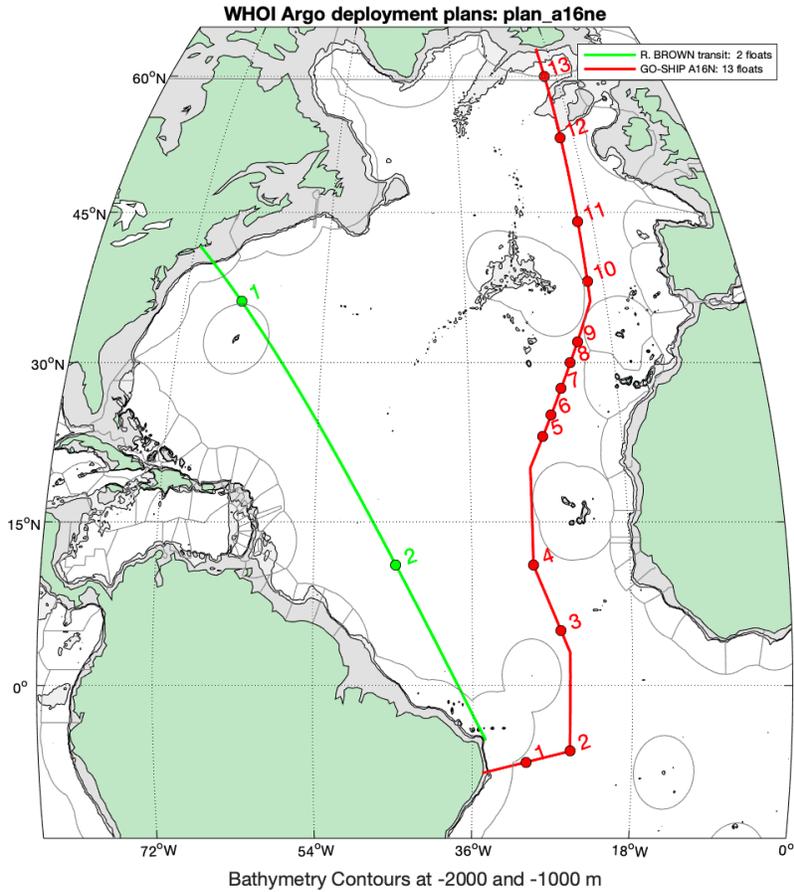


Figure 18: Core and BGC Argo float deployment locations proposed for the A16N cruise (transit, leg 1 and leg 2). Plot provided by P. Robbins (WHOI)

Table 11 shows the details of the Argo floats deployed during the cruise.

Date	Time (UTC)	Station	Latitude	Longitude	Argo type	Argo#	WMO ID
4/19/23	14:05:41	80	29° 59.927N	23° 22.656W	BGC	1354	4903485
4/22/23	23:00:25	90	35° 00.363N	20° 33.096W	CORE	7803	4903540
4/28/23	11:49:33	108	44° 00.006N	19° 59.482W	BGC	1477	4903533
5/03/23	11:34:42	126	53° 01.261N	20° 00.259W	BGC	1474	4903488
5/06/23	12:33:36	140	60° 00.164N	19° 59.995W	BGC	1476	4903532

Table 11: Core and BGC Argo float deployments during A16N leg 2

## 6.2. Surface drifters

*PIs:* Rick Lumpkin and Shawn Dolk (AOML/NOAA)

*Shipboard personnel:* Ship's deck crew and science crew

The NOAA Global drifter program aims to maintain a global 5° x 5° gridded array of satellite-tracked surface drifting buoys to provide in-situ observations of mixed layer currents, sea surface temperature, atmospheric pressure, winds, waves, and salinity. The data is used for weather predictions.

The floats come in cardboard boxes and do not require any activation pre-deployment. More information about the Global Drifter Program can be found here: <https://www.aoml.noaa.gov/phod/gdp/index.php>. Table 12 details the information about the drifter deployments.

Date	Time (UTC)	Station	GPS Lat	GPS Lon	Drifter#	WMO ID
4/19/23	14:10:47	80	30° 00.177N	23° 22.473W	61458890	1301799
4/22/23	23:02:37	90	35° 00.417N	20° 33.119W	61594090	1301791
4/26/23	7:17:37	100	40° 00.068N	19° 59.706W	61458870	1301798
4/29/23	7:00:22	111	45° 29.994N	19° 59.903W	61595110	1301794
5/2/23	3:23:55	121	50° 30.069N	19° 59.660W	61595100	1301793

Table 12: Deployment information for the NOAA Global Drifter Program floats deployed on A16N leg 2

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