Cruise Report

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Fourth Gulf of Mexico Ecosystems and Carbon Cycle (GOMECC-4) Cruise

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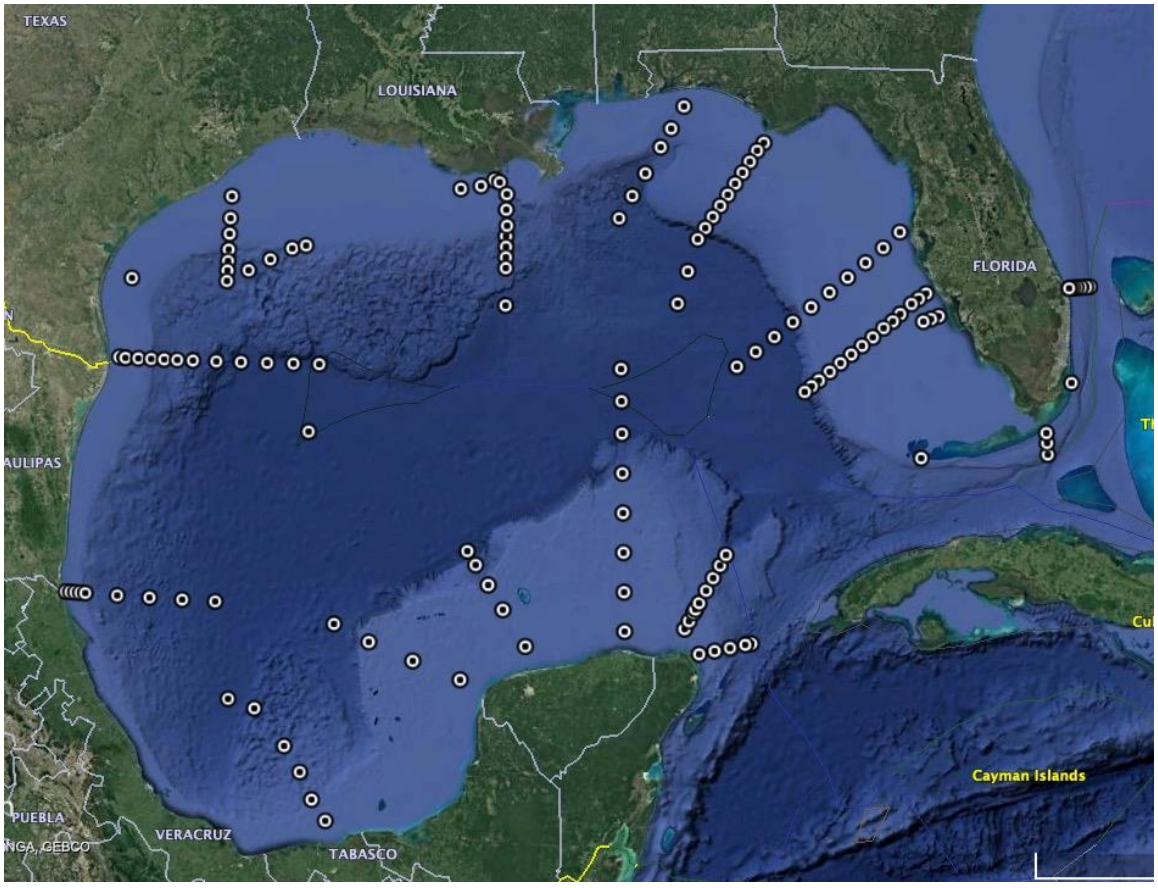

Cruise Summary Information

Cruise designation	**GOMECC-4**
Expedition designation and expocode	**RB21-03 , 33RO20210913**
Chief scientist	**Leticia Barbero**
Dates	**13 September-21 October 2021**
Ship	**R/V Ronald H. Brown**
Ports of call	**Key West, FL, USA – St. Petersburg, FL, USA**
Geographic Boundaries	**18.2–30°N, 79–98°W**
Stations	**141**
Floats and drifters deployed	**4 BGC-ARGO floats deployed**

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1. GOMECC-4 Project

The fourth Gulf of Mexico Ecosystems and Carbon Cycle (GOMECC-4) cruise on board the NOAA Ship *Ronald H. Brown* took place from September 13 to October 21, 2021. The survey of GOMECC-4 consisted of CTD/DO, rosette, LADCP, water samples, bongo-style net tows, surface net tows, underway measurements, and sediment cores. The ship departed from Key West, FL and went around the Gulf of Mexico in a counterclockwise direction, ending in St. Petersburg, FL.

A total of 141 stations were occupied with a CTD/DO/rosette/LADCP package. Of these stations, 138 were divided into 16 lines, and an additional three stations were occupied as part of a collaboration with the National Park Service. Four of the 16 lines were reoccupations of previous GOMECC cruises; the remaining lines were new stations occupied for the first time during this cruise.

Four BGC-Argo floats were deployed in U.S./international waters of the GOM, at depths 2000m or deeper. Up to four bongo net tows were conducted at each of the 16 lines to collect zooplankton samples. Sediment cores were collected for the first time in the GOMECC program at offshore sites along the Tampa, Pensacola, Louisiana, and Galveston Lines. 24- hour grazing incubation experiments were performed at 11 stations throughout the cruise.

CTD/DO data and water samples were collected on each cast, from surface (2-5 m) to usually within 5-8 m of the bottom. Water samples were measured on board for salinity, dissolved oxygen, nutrients, dissolved inorganic carbon (DIC), pH, carbonate concentration, total alkalinity (TA), and pCO_2. Additional water samples were collected and stored for shore analyses of chlorophyll concentration, HPLC analysis, and DNA/RNA composition of eukaryote plankton communities (<200 µm).

A seagoing science team assembled from 13 different institutions from the USA and Mexico participated in the collection and analysis of this data set. The programs, principal investigators, science team, responsibilities, instrumentation, analyses, and analytical methods are outlined in the following cruise document.

1.1. Programs and Principal Investigators

Program	Affiliation	Principal Investigator	Email Address
CTD/DO data, salinity	NOAA	Molly Baringer	Molly.Baringer@noaa.gov
Dissolved inorganic carbon (DIC), pCO_2	NOAA/UM CIMAS	Rik Wanninkhof Leticia Barbero	Rik.Wanninkhof@noaa.gov, Leticia.Barbero@noaa.gov
Total alkalinity	UM CIMAS	Leticia Barbero	Leticia.Barbero@noaa.gov
Dissolved oxygen	NOAA/ UM	Molly Baringer Chris Langdon	Molly.Baringer@noaa.gov, clangdon@rsmas.miami.edu
Nutrients	NOAA	Jia-Zhong Zhang	jia-zhong.zhang@noaa.gov
pH, carbonates	USF	Robert Byrne	rhbyrne@usf.edu
Carbon Isotopes	UDel	Wei-Jun Cai	wcai@udel.edu
Phytoplankton Taxonomy	ECOSUR	Daniel Pech	dpech@ecosur.mx
Zooplankton and Ichthyoplankton	CICESE/USM/ NOAA	Sharon Herzka Frank Hernandez Glenn Zapfe	sherzka@cicese.mx, frank.hernandez@usm.edu glenn.zapfe@noaa.gov
Plankton ecology	NCSU ULL	Astrid Schnetzer Beth Stauffer	aschnet@ncsu.edu, bas1301@louisiana.edu
eDNA	NGI	Luke Thompson	Luke.Thompson@noaa.gov
Sediment cores	NOAA	Emily Osborne	Emily.Osborne@noaa.gov
BGC Argo floats	NOAA	Emily Osborne	Emily.Osborne@noaa.gov
Transmissometry	TAMU	Wilford Gardner	wgardner@tamu.edu
LADCP	UM CIMAS	Leticia Barbero	Leticia.Barbero@noaa.gov
SADCP	NOAA/UH	Ryan Smith Julia Hummon	Ryan.Smith@noaa.gov, hummon@hawaii.edu

Table 1: : GOMECC-4 principal investigators.

1.2. Participating Institutions

CICESE – Ensenada Center for Scientific Research and Higher Education
ECOSUR – Colegio de Frontera Sur
FAMU – Florida A&M University
NCSU – North Carolina State University
NGI – Northern Gulf Institute
NOAA – National Oceanic and Atmospheric Administration
TAMUCC – Texas A&M University Corpus Christi
UABC – Universidad Autónoma de Baja California
UDel – University of Delaware
UH – University of Hawaii
ULL – University of Louisiana at Lafayette
UM – University of Miami
USF – University of South Florida
USM – University of Southern Mississippi

1.3. Science Team and Responsibilities

Table 2: GOMECC-4 cruise participants		
Duty	**Name**	**Affiliation**
Chief Scientist /data manager	Leticia Barbero	UM CIMAS
Co-Chief Scientist CTD/LADCP/salinity	Andrew Stefanick	NOAA
CTD/LADCP/O_2	Leah Chomiak	UM CIMAS
CTD Watchstander	Grace Owen	UM
CTD Watchstander/Sediments	Benjamin Ross	FAMU
Salinity	Ed Hunt	UM CIMAS
O_2	Mia Andrew-Nandlall	UM
O_2	Willem Weinberg	UM
Nutrients	Ian Smith	UM CIMAS
DIC	Charles Featherstone	NOAA
DIC	Eva Jundt	TAMUCC
pCO_2 discrete	Alicia Uribe	UABC
Total Alkalinity	Gabriela Cervantes	UABC
Total Alkalinity	Mariana Cupul	UABC
pH/carbonate	Macarena Martin-Mayor	USF
pH/carbonate	Loraine Martell-Bonet	USF
pH/carbonate	Juan Millan	USF
Plankton ecology/HABs	Miranda Irby	NCSU
Plankton ecology/HABs	Hans Prevost	ULL
Zooplankton/ Ichthyoplankton	Gonzalo Daudén-Bengoa	CICESE
Zooplankton/ Ichthyoplankton	Alexis Wilson	USM
13DIC	Qian Li	UDel
13DIC	Elliott Roberts	UDel
BGC Argo/Sediments	Emily Osborne	NOAA
eDNA	Sean Anderson	NGI

2. Cruise Narrative

2.1. Summary

This report describes the fourth Gulf of Mexico Ecosystems and Carbon Cycle (GOMECC-4) cruise on board the R/V *Ronald H. Brown* departing from Key West, FL into the Gulf of Mexico and then around the coastal waters of the Gulf of Mexico in a counter-clockwise direction. The cruise took place from September 13–October 21, 2021. The effort was in support of the coastal monitoring and research objectives of NOAA'S Ocean Acidification Program. The cruise was designed to obtain a snapshot of key carbon, physical, and biogeochemical parameters as they relate to ocean acidification (OA) in the coastal realm. This was the fourth occupation of the Gulf of Mexico as part of the Ocean Acidification Program's monitoring efforts, with the first three occurring in 2007, 2012 and 2017.

The cruise included a series of 15 transects approximately orthogonal to the coast of the Gulf of Mexico and a 16th partial transect along the 27°N line, between Florida and the Bahamas, as well as a comprehensive set of underway measurements along the entire cruise track (Figure 1). In addition to these transects, three more stations were sampled as part of a collaboration with the National Parks Service to monitor ocean acidification at national parks. Four parks participated in this effort: Padre Island (in Texas), Dry Tortugas, Everglades, and Biscayne (these three in Florida).

CTD/DO/LADCP/transmissometer/fluorometer/rosette stations were occupied at 141 specified locations. Underway measurements of shipboard surface acoustic Doppler current profiler (SADCP), partial pressure of carbon dioxide (pCO_2), dissolved oxygen (DO), temperature, and salinity were performed. During the transit times from line to line, underway discrete samples for DIC, pCO_2, TA, pH, carbonates, nutrients, and dissolved oxygen (DO) were taken every 2 hours.

A total of 26 scientists, from NOAA's AOML and multiple other universities and institutions, participated in the 40-day cruise. Water samples were collected from the 24-bottle rosette at each station and analyzed for salinity, oxygen, nutrients, DIC, TA, pCO_2, pH, carbonates, chlorophyll, eukaryote plankton (<200 µm), and eDNA. Automated underway systems were in operation for measuring atmospheric CO_2 and near-surface water pCO_2, and oxygen.

The general GOMECC-4 cruise track followed the coast of the Gulf of Mexico going in a counter-clockwise fashion. Each of the lines (transects) started as close as possible to the shore and ended at a deep station considered representative of oceanic conditions ("open ocean") (Figure 1).

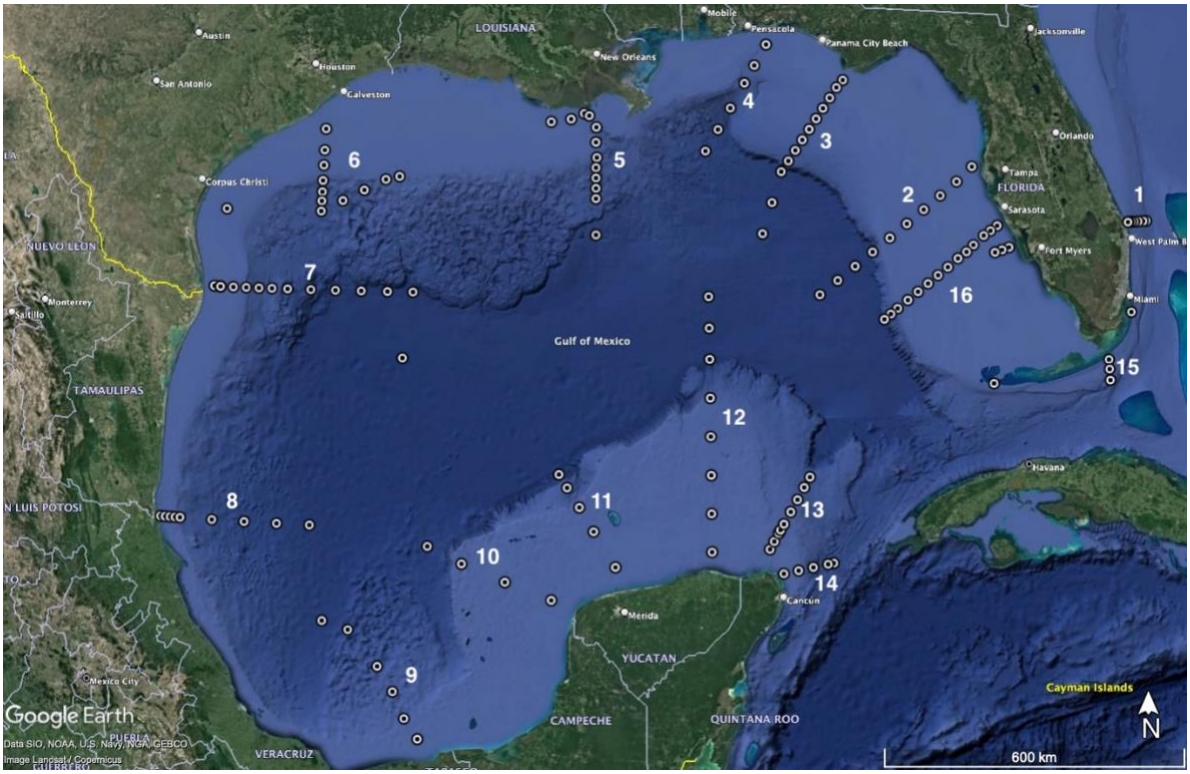

Figure 1: CTD station locations visited during the GOMECC-4 cruise. The numbers identify the different transects: 1) 27°N Line, 2) Tampa Line 3) Panama City Line, 4) Pensacola Line 5) Louisiana Line, 6) Galveston Line, 7) Brownsville Line, 8) Tampico Line, 9) Veracruz Line, 10) Campeche Line, 11) Merida Line, 12) Yucatan Line, 13) Cabo Catoche Line, 14) Cancun Line, 15) Florida Straits Line, and 16) Venice Line.

On all shallow stations, and depending on the strength of the local currents, the CTD/rosette was deployed to within 5-8 m of the bottom. On deep stations (1500 m depths or more), the CTD was deployed to within 10 m of the bottom. On multiple stations, several Niskin-style Bullister bottles were tripped at the chlorophyll maximum depth and at the surface in order to accommodate the water needs of the phytoplankton and eDNA sampling. Water samples from the rosette/CTD package were collected in up to 24 11 L-Bullister bottles at all stations, providing water samples for DO, total DIC, pH, pCO_2, TA, nutrients, salinity, chlorophyll-a, eDNA, and plankton community composition. Underway surface pCO_2, temperature, salinity, DO, multi-beam bathymetry, and meteorological measurements were collected, as well as a suite of biochemical samples for subsequent analysis.

The cruise track had to be adjusted several times to adapt to changes induced by COVID-19-related logistics. Two of our scientists had a false positive test prior to departure and were required to re-start their quarantine period. In order to be able to pick them up at a location within driving distance of their place of quarantine (Miami, FL) we started the cruise by doing a partial re-occupation of line 27N and then headed for the Tampa line to pick up our scientists there. The ship requested a stop half-way through the cruise on US waters to exchange personal. This provided an opportunity for science participants to obtain a Mexican visa at that same port stop, allowing us to meet the requirements for accessing Mexican waters.

Issues with the winch cable caused issues in the rosette. Troubleshooting and multiple re-terminations and winch switches impacted line 6. Bad weather and a strict deadline to be in Brownsville at a specific date for the consular visits and the ship's crew exchange meant we did not have time to troubleshoot the cable issues, resulting in a lost station and one station with CTD issues and mistrips that could not be repeated.

The main cruise objectives, as described in the project instructions (downloadable from http://www.aoml.noaa.gov/ocd/gcc/GOMECC4/) and detailed below, were achieved.

2.2. Issues/Goals not Achieved

The following issues impacted operations during the cruise:

1. Cuban clearance. We requested, and obtained, clearance to work in Cuban waters. However, a condition for this clearance required the participation of two Cuban nationals to be designated by Cuban authorities. This was in line with what was requested for the previous GOMECC cruise. However, in the context of the pandemic, with limited flight options, travel restrictions and vaccination requirements we were not able to find a way to bring the Cuban nationals to the US to join the cruise. We suggested a number of alternatives to the Cuban authorities but did not receive a reply and therefore had to forego the Cuban section of the cruise. This impacted the Cancun and the Florida straits lines, that could only be occupied in Mexican and US waters, respectively.
2. Bahamian clearance. Bahamas set up a new portal to request clearance in 2021 that included fees and potential for fines that prevented us from submitting a request. This impacted the 27N and the Florida Straits lines, that could only be occupied in US waters.
3. Order of stations in the Brownsville line. The ship had to remain by the Brownsville port for 2 days to accommodate consular requirements for the science party and ship's crew exchange. In order to maximize operations, several stations were occupied during the nighttime, out of order.
4. Winch issues: The forward winch had been experiencing issues since the cruise prior to GOMECC-4. Eventually almost 2 full days were spent troubleshooting where the issue may be, switching back and forth between the forward and the aft winch, re-terminating cables, switching sensors, deck boxes, and testing everything until identifying the issue was with the cable itself. Chopping a little over 100m of cable solved the issue. The impact was the loss of several stations in the Galveston line.

2.3. 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*. Funded and non-funded investigators in the project and members of NOAA's Ocean Acidification Program contributed to the successful planning and execution of the cruise. Special thanks go to our Mexican colleagues at CICESE, and UABC (Drs. Herzka, and Hernández-Ayón) who were instrumental in helping us

obtain Mexican clearance. We would not have been able to get appointments at the Mexican consulate in Brownville, let alone in a way that met our covid bubble requirements were it not for the tireless work of Ms. Emy Rodriguez, from NOAA AOML. We are also extremely grateful for Capt. Shoup's work to develop a plan that allowed the science crew to obtain Mexican visas while still maintaining a COVID bubble.

Cruise participants had to isolate for 8 days before the cruise and follow strict covid prevention guidelines. Then crew and scientists were required to wear face masks for more than half the cruise, working in the heat and humidity of the summer in the Gulf of Mexico. Their high degree of professionalism and good nature was greatly appreciated and guaranteed a successful outcome for this cruise.

All officers, deck crew, engineers, and galley staff contributed to the success of this long cruise. Their assistance is gratefully acknowledged.

The GOMECC cruises are sponsored by NOAA's Ocean Acidification Program.

Clearance was requested and granted from the sovereign nation of Mexico (permit number EG0052021) for research conducted in their declared territorial waters. The permission to execute the research effort in their waters was critical for the success of the GOMECC-4 objectives and is greatly appreciated.

3. Description of Measurements from Vertical Profiles

3.1. CTD/Hydrographic measurements

CTD Operations

The basic CTD measurements consisted of pressure, temperature, salinity, dissolved oxygen and optical (for determining the chlorophyll maximum) from CTD profiles (Table 3). A total of 141 CTD/rosette casts were made, usually to within 10 m of the bottom. Several shallow coastal stations were within 5 m of the bottom.

i. CTD Electronics and Water Sampling Package

CTD/rosette casts were performed with a package consisting of a 24-place, 12-liter rosette frame (AOML's pink frame), a 24-place water sampler (SBE32) and 24, 11-liter Bullister-style bottles. This package was deployed on all stations/casts. Underwater electronic components consisted of a Sea-Bird Electronics (SBE) 9 plus CTD with dual pumps and the following sensors: dual temperature (SBE3), dual conductivity (SBE4), dual dissolved oxygen (SBE43), reference temperature (SBE35), a Wet Labs EDO-AFL/FL fluorometer, a Wet Labs CSTAR transmissometer, and a Valeport VA500 altimeter. The other underwater electronic components consisted of two RDI 300 kHz LADCPs.

The CTD's supplied a standard Sea-Bird format data stream at a data rate of 24 frames/second. The SBE9 plus CTD was connected to the SBE32 24-place pylon providing for single-conductor sea cable operation. Power to the SBE 9 plus CTD, SBE32 pylon, auxiliary sensors, and altimeter was provided through the sea cable from the SBE 911plus deck unit in the computer lab. The rosette system was suspended from a UNOLS-standard three-conductor 0.322" electro-mechanical sea cable.

The CTD was mounted vertically attached to the bottom center of the rosette frame. All SBE4 conductivity and SBE3 temperature sensors and their respective pumps were mounted vertically as recommended by SBE outboard of the CTD. Primary temperature, conductivity, and dissolved oxygen were plumbed on one pump circuit and secondary temperature, conductivity, and dissolved oxygen on the other. Pump exhausts were attached to outside corners of the CTD cage and directed downward. The altimeter and fluorometer were mounted on the inside of a one side of the support struts adjacent to the bottom frame ring. The transmissometer was mounted on the inside of the support strut on the other side. The LADCPs were vertically mounted inside the bottle rings with one 300 kHz pointing down, the other 300 kHz transducer pointing up. Both of the *R/V Brown's* CTD winches, forward and aft, were used with the 24-place 12-liter rosette throughout the cruise. There were several issues during the cruise with the forward winch that required switching to the aft winch, while the troubleshooting of the forward winch was done.

The deck watch prepared the rosette typically within a few minutes prior to each cast. All valves, vents, and lanyards were checked for proper orientation. The bottles were cocked and all hardware and connections rechecked. Once on station, the syringes were removed from the CTD sensor intake ports. The CTD was powered-up and the data acquisition system started. The CTD package was put in the water and taken down 10 m for 2-3 minutes to remove any air bubble from the sensor lines and to make sure the sensors were behaving appropriately. After recovery of the CTD package on deck, it was brought into the staging bay for sampling. The bottles and rosette were examined before samples were taken and anything unusual noted on the sample log.

Routine CTD maintenance included soaking the conductivity and DO sensors in a solution of de-ionized water as recommended by Sea-Bird between casts to maintain sensor stability. Rosette maintenance was performed on a regular basis. O-rings were changed as necessary and bottle maintenance was performed each day to ensure proper closure and sealing. Valves were inspected for leaks and repaired or replaced as needed.

Instrument	Stations	S/N	Use	Other
Sea-Bird SBE 32 24-place Carousel Water Sampler	1-7	32-1090		
Sea-Bird SBE 32 24-place Carousel Water Sampler	8-141	32-1087		
Sea-Bird SBE9plus CTD	1-56	957		
Paroscientific Digiquartz Pressure Sensor	1-56	115173		
Sea-Bird SBE9plus CTD	57-141	1335		
Paroscientific Digiquartz Pressure Sensor	57-141	135375		
Sea-Bird SBE3plus Temperature Sensor	1-141	4799	Primary	
Sea-Bird SBE3plus Temperature Sensor	1-141	5171	Secondary	

Sea-Bird SBE33 Reference Temperature Sensor	1-141	97		
Sea-Bird SBE4C Conductivity Sensor	1-141	3860	Primary	
Sea-Bird SBE4C Conductivity Sensor	1-141	3858	Secondary	
Sea-Bird SBE43 Dissolved Oxygen Sensor	1-141	140	Primary	
Sea-Bird SBE43 Dissolved Oxygen Sensor	1-141	2691	Secondary	
Sea-Bird SBE5T Pump	1-51	1027	Primary	
Sea-Bird SBE5T Pump	1-51	7739	Secondary	
Sea-Bird SBE5T Pump	52-141	7889	Primary	
Sea-Bird SBE5T Pump	52-141	1072	Secondary	
Valeport VA500	1-141	48591	range 100	15 scale
Transmissometer CSTAR	1-141	339DR		
WET Labs EDO-AFL/FL Fluorometer	1-141	2125		
RDI LADCP - 300 kHz Workhorse (AOML)	1-141	24616	Upward	
RDI LADCP - 300 kHz Workhorse (AOML)	1-141	1856	Downward	

Table 3: Equipment used during GOMECC-4.

ii. Real-Time CTD Data Acquisition System

The CTD data acquisition system consisted of an SBE-11plus (V2) deck unit and a networked generic PC workstation running Windows located in the computer room. SBE Seasave software version 7.26.7.107 was used for data acquisition and to close bottles on the rosette.

The deck watch prepared the rosette typically after sampling the previous cast. All valves, vents, and lanyards were checked for proper orientation. The bottles were cocked and all hardware and connections rechecked. Fifteen minutes or so prior to station the deck unit was powered on and an on-deck pre-cast pressure was obtained. Once on station, the syringes were removed from the CTD sensor intake ports. Tag lines were necessary for deployments during this cruise and an air tugger was used during recoveries for positioning the CTD on the platform. As soon as it was in the water, the CTD deck unit was powered on and the data acquisition system started. As directed by the deck watch leader, the CTD was taken down to 10 m for 2 minutes to remove any air bubble from the sensor lines and to make sure the sensors were behaving appropriately. The CTD was brought back to just below the surface with the console operator hitting "Mark Scan" before beginning the descent. The profiling rate was no more than 30 m/min to 50 m, 45 m/min to 200 m, and no more than 60 m/min deeper than 200 m. Upon recovery, the CTD deck unit was turned off one on deck. The rosette was brought inside the staging bay for sampling. The bottles and rosette were examined before samples were taken and anything unusual noted on the sample log.

The console watch monitored the progress of the deployment and quality of the CTD data through interactive graphics and operational displays. Additionally, the watch created a sample log for the deployment that would be later used to record the correspondence between rosette bottles and analytical samples taken. The altimeter channel, CTD pressure, wire-out and bathymetric depth were all monitored to determine the distance of the package from the bottom, usually allowing a safe approach to within 10 m.

On the up cast, the winch operator was directed to stop at each bottle trip depth. The CTD console operator waited 30 seconds before tripping a bottle using a ``point and click'' graphical trip button and 8 seconds after to allow the reference temperature sensor to sample. The data acquisition system responded with trip confirmation messages and the corresponding CTD data in a rosette bottle trip window on the display. All tripping attempts were noted on the console log. The console watch then directed the winch operator to raise the package up to the next bottle trip location. After the last bottle was tripped, the console watch directed the deck watch to bring the rosette on deck.

iii. Shipboard CTD Data Processing

Shipboard CTD data processing was performed automatically at the end of each deployment using SEABIRD SBE Data Processing version 7.26.7.114 and AOML Matlab processing software. The raw CTD data and bottle trips acquired by SBE Seasave on the Windows workstation were copied onto the CTD processing laptop, and processed to a 1-dbar series and a 1-second time series. Bottle trip values were extracted and a 1-decibar (dbar) down cast pressure series created. The Sea-Bird Data Processing for primary calibrated data (1 dbar averages) uses the following routines in 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), depth, pressure, t0 ITS-90 C, t1 ITS-90 C, c0 S/m, c1 S/m, salinity (PSU), salinity 2 (PSU), oxygen voltage V, oxygen 2 voltage V, altimeter, oxygen umol/kg, oxygen 2 umol/kg, oxygen ml/l, oxygen 2 ml/l, oxygen dv/dt, oxygen dv/dt 2, potential temperature, potential 2 temperature, sigma-theta, sigma-theta 2, latitude, longitude, and Voltage channel 6 (transmissometer). The scan range offset is 0 seconds and the scan range duration is 5.5 seconds. MARKSCAN was used to determine the number of scans acquired on deck and while priming the system to exclude these scans from processing.
- 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 are automatically advanced by 0.073 seconds and both oxygen are advanced by an additional 1.073 seconds.
- BOTTLESUM - creates a summary of the bottle data. Bottle position, date, and time were output automatically. Pressure, temperature, conductivity, salinity, oxygen voltage and preliminary oxygen values were averaged over a 5.5 second interval.
- WILDEDIT - computes the standard deviation of 300 point bins, and then makes two passes through the data. The first pass flags points that differ from the mean by more than 2

standard deviations. A new standard deviation is computed excluding the flagged points and the second pass marks bad values greater than 20 standard deviations from the mean. For this data set, data were kept within a distance of 100 of the mean (i.e., all data).

- 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. The value used for the thermal anomaly amplitude (alpha) was 0.03°C. The value used for the thermal anomaly time constant (1/beta) was 7.0°C.
- LOOPEDIT - removes scans associated with pressure slowdowns and reversals. If the CTD velocity is less than 0.25 m/s 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 salinities.
- 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 the computed oxygen variable.
- TRANS - converts the binary data file into ASCII format.
- SPLIT - separates the cast into upcast and downcast values.

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, a program computes values of density locally referenced between every 1 dbar of pressure to compute N^2 and linearly interpolates temperature, conductivity, and oxygen voltage over those records where N^2 is less than or equal to -1×10^{-5} per s^2. These data were retained but flagged as questionable in the final WOCE formatted files.

Final calibrations are applied to delooped data files. ITS-90 temperature, salinity, and oxygen are computed, and WOCE quality flags are created.

CTD data were examined at the completion of each deployment for clean corrected sensor response and any calibration shifts. As bottle salinity and oxygen results became available, they were used to refine shipboard conductivity and oxygen sensor calibrations.

A total of 141 casts were processed.

iv. CTD Calibration Procedures

Laboratory calibrations of the CTD pressure, temperature, and conductivity sensors were all performed at SBE. Secondary temperature, conductivity and dissolved oxygen (T2, C2 and DO2) sensors served as calibration checks for the reported primary sensors. In-situ salinity and

dissolved O_2 check samples collected during each cast were used to calibrate the conductivity and dissolved O_2 sensors. A reference temperature sensor is used to calibrate the temperature sensor. Sensor used during the cruise are listed in Table 3. Both sets of sensors behaved well compared to each other and when compared to the bottle and reference temperature data. After calibrating the primary and secondary sensors, the primary sensors were chosen for final calibrations.

v. CTD Pressure

Pressure sensor calibration coefficients derived from the pre-cruise calibrations were applied to raw pressure data during each cast. Residual pressure offsets between the first and last near surface pressures and before and after on deck pressures were examined to check for calibration shifts (see Table 4 and Figure 2).

Pressure sensor s/n 0957 was used for stations 1-56 with an initial pressure offset in the configuration file of -3.01953 dbar. On deck pressure before and after the cast were stable at 1.3957 +/- 0.07 dbar and 1.3605 +/- 0.09 dbar, respectively. Near surface pressure values at the start and end of the cast were stable at 4.1064 +/- 0.8 dbar and 4.1435 +/- 0.7 dbar, respectively. During final processing a pressure offset of -1.3378 was applied to the configuration file for a total pressure offset of -4.3573 dbar.

Pressure sensor s/n 1335 was used for stations 57-141 with an initial pressure offset in the configuration file of 0.0 dbar. On deck pressure before and after the cast were stable at 0.42 +/- 0.22 dbar and 0.34 +/- 0.20 dbar, respectively. Near surface pressure values at the start and end of the cast were stable at 3.3285 +/- 1.0 dbar and 3.376 +/- 0.6 dbar, respectively. During final processing a pressure offset of -0.3811 was applied to the configuration file for a total pressure offset of -0.3811 dbar.

sta#	cast	mark scan	start pr	end pr	start sfc btl prs	end sfc btl prs
1	1	30330	1.4642	1.428	6.7371	7.61
2	1	30203	1.337	1.36	4.5382	4.945
3	1	12025	1.26	1.32	7.5573	4.141
4	1	21455	1.37	1.34	6.5263	3.58
5	1	13170	1.3837	1.36	4.7391	4.269
6	1	12499	1.3461	1.41	5.3052	5.057
7	1	10356	1.35	1.37	4.4218	4.498
8	1	15390	1.4582	1.41	5.2499	5.173
9	1	15505	1.4	1.16	4.249	3.887
10	1	14194	1.3914	1.1682	4.2105	5.538
11	1	11230	1.4	1.14	4.658	4.771
12	2	9991	1.16	1.13	4.4891	4.517
13	1	15241	1.3228	1.3542	4.1318	3.897
14	1	10149	1.4031	1.3859	4.0617	4.055
15	1	15574	1.3662	1.3905	3.5953	4.215
16	1	11458	1.3	1.4	4.2833	4.223
17	1	6836	1.4	1.38	3.7127	3.72
18	1	22059	1.362	1.4237	3.6355	3.68

19	1	8570	1.4168	1.39	3.7137	4.23
20	1	7969	1.45	1.3751	3.4997	3.64
21	1	10128	1.3286	1.361	3.5071	3.393
22	1	19829	1.3527	1.3683	4.0915	4.174
23	1	20861	1.3743	1.4023	3.6632	4.283
24	1	9061	1.446	1.38	4.3964	4.556
25	1	8612	1.4	1.4	3.5254	3.81
26	1	11031	1.5	1.38	3.7666	4.146
27	1	7729	1.5	1.4	4.0371	3.903
28	1	8734	1.3637	1.1377	4.4213	3.346
29	1	17025	1.3869	1.13	3.6657	3.348
30	1	9091	1.5	1.2	4.1282	3.403
31	1	14994	1.3873	1.3689	5.1263	4.62
32	1	8305	1.4113	1.3473	4.1213	3.977
33	1	13185	1.3388	1.343	4.4652	4.423
34	1	15358	1.4	1.3	4.2657	3.959
35	1	13548	1.5	1.2	3.819	3.816
36	1	14775	1.3352	1.2073	3.9946	3.925
37	1	22350	1.4	1.2	4.5636	4.37
38	1	18111	1.5	1.3	4.1279	4.575
39	1	6868	1.5144	1.3499	4.5794	4.503
40	1	14793	1.3585	1.277	3.8295	3.951
41	1	23115	1.355	1.3734	3.2311	4.149
42	1	5441	1.5	1.2	4.1876	3.954
43	1	17838	1.5	1.3931	3.9807	4.208
44	1	7441	1.4	1.3	3.7849	4.135
45	1	18005	1.3	1.3	3.4853	3.893
46	1	8401	1.4	1.4	3.8101	3.706
47	1	6773	1.5	1.4	3.3931	4.14
48	1	21454	1.3486	1.3481	3.621	3.678
49	1	5841	1.5	1.4	3.4792	4.149
50	1	5265	1.52	1.45	3.7664	3.596
51	1	9486	1.422	1.4322	4.0382	3.873
52	5	13201	1.5	1.4	4.0647	4.686
53	1	12492	1.3	1.3	4.0383	4.662
54	1	13448	1.3	1.3	5.0543	4.662
55	1	9445	1.3235	1.3448	4.3441	3.842
56	2	12457	1.3605	1.3955	4.1387	4.448
57	1	12283	0.5	0.3	5.1369	4.393
58	1	11951	0.5	0.5	3.7636	4.873
59	1	6673	0.62	0.55	9.8588	3.527
60	1					
61	1	9223	0.3584	0.3854	3.1443	3.175
62	1	14144	0.48	0.46	3.9824	3.574
63	1	7532	0.56	0.58	3.5133	3.674
63	2	19557	0.5542	0.5527	2.6271	3.31
64	1	12280	0.42	0.41	3.4451	3.487

65	1	6942	0.5	0.46	3.0088	3.585
66	1	24072	0.5345	0.5448	3.715	3.451
67	1	19622	0.4453	0.46	3.3986	2.704
68	1	7479	0.6	0.46	2.6066	2.87
69	1	5686	0.64	0.4	2.6987	3.235
70	1	13730	0.5	0.211	3.4052	3.113
71	1	17690	0.556	0.214	3.254	3.022
72	1	13181	0.48	0.27	2.9567	2.683
73	1	13419	0.4	0.27	2.5419	2.819
74	1	16547	0.4654	0.1628	3.5732	3.154
75	1	15179	0.511	0.4607	3.9955	3.903
76	1	14282	0.6244	0.4358	4.753	4.338
77	1	18087	0.4559	0.4578	3.9113	4.036
78	1	11160	0.5035	0.4772	4.8011	4.329
79	1	5207	0.62	0.57	3.6376	3.66
80	1	7465	0.5	0.38	3.5056	3.282
81	1	15144	0.3735	0.2448	3.4526	3.858
82	1	15246	0.4952	0.1583	4.2213	4.208
83	1	6881	0.51	0.23	2.9404	2.315
84	1	11521	0.4416	0.2711	4.1292	3.796
85	1	15822	0.4965	0.3426	4.062	3.922
86	1	13056	0.52	0.4	2.9438	3.853
87	1	10639	0.51	0.44	3.4789	3.268
88	1	19464	0.4082	0.4473	3.7736	4.286
89	1	28729	0.5163	0.4303	3.7278	4.093
90	1	10664	0.3638	0.2041	5.6275	3.615
91	1	20077	0.3945	0.446	3.5137	4.062
92	1	9913	0.51	0.38	2.5295	2.801
93	1	11205	0.48	0.37	2.7404	2.955
94	1	19036	0.3809	0.3789	3.8511	3.517
95	1	10413	0.3482	0.3814	2.9253	2.757
96	1	7290	0.52	0.33	2.6965	2.733
97	1	8713	0.51	0.42	2.7123	3.163
98	2	17675	0.4497	0.1398	4.1627	3.73
99	1	10044	0.5	0.36	2.8421	3.542
100	1	8156	0.39	0.31	2.5378	2.691
101	1	10169	0.5077	0.4719	3.7707	3.677
102	1	16424	0.4223	0.4278	3.8298	3.821
103	1	21771	0.4134	0.4054	4.8889	3.637
104	1	7389	0.53	0.21	2.9663	2.589
105	1	4385	0.52	0.1336	2.5794	3.793
106	1	11786	0.3854	0.1371	4.7392	3.6
107	1	9613	0.0023	0.3333	2.7861	3.297
108	2	44884	0.3613	0.3422	3.7222	3.319
109	1	12712	0.3533	0.373	2.5256	3.134
110	1	5063	0.54	0.37	3.3622	3.394
111	1	7008	0.52	0.42	2.6363	3.331

112	1	9605	0.33	0.43	2.4881	3.196
113	1	5486	0.5	0.44	2.5985	2.792
114	1	12457	0.39	0.45	2.7319	2.845
115	1	22395	0.4154	0.397	3.4403	3.122
116	1	19430	0.3596	0.4263	3.6481	3.513
117	1	10675	0.4067	-0.0156	4.8799	3.076
118	1	17230	-0.0487	-0.1638	2.7034	2.338
119	1	4289	0.1	0.25	2.1175	2.872
120	1	7536	-0.16	0.23	2.1085	2.57
121	1	22165	0.0472	-0.0357	2.8422	2.742
122	1	18574	0.0286	-0.0915	2.5399	2.913
123	1	15074	-0.086	-0.1082	2.564	2.986
124	1	15475	0.018	0.07	3.2482	3.505
125	1	6078	0.11	0.08	3.675	3.274
126	1	16625	0.04	0.05	3.2307	3.552
127	1	13905	0.0729	-0.0365	2.9793	3.376
128	1	9352	0.08	-0.08	2.6152	2.967
129	1	11833	0.05	0.01	3.3285	3.239
130	1	6295	-0.05	-0.12	3.1938	3.71
131	1	15295	-0.0385	-0.0235	3.0827	3.806
132	1	12336	-0.0159	-0.0185	3.7216	3.396
133	1	10438	-0.0292	-0.0316	3.7014	3.664
134	1	16408	-0.0529	-0.0214	3.6037	3.319
135	1	12624	-0.0245	-0.0107	3.1762	3.492
136	1	14698	-0.0573	0.02	2.9144	2.98
137	1	5520	0.09	-0.04	3.2893	3.106
138	1	11272	0.15	-0.03	2.7282	3.4
139	1	6987	0.14	-0.001	2.3984	3.205
140	1	8724	0.007	0.42	4.0606	5.572
141	1	11389	0.64	0.26054	4.0717	4.099

Table 4: Near surface pressure values and scan number used to remove surface soak and on-deck values. The highlighted line marks when SBE9plus pressure sensor, s/n 0957, was replaced with s/n 1335.

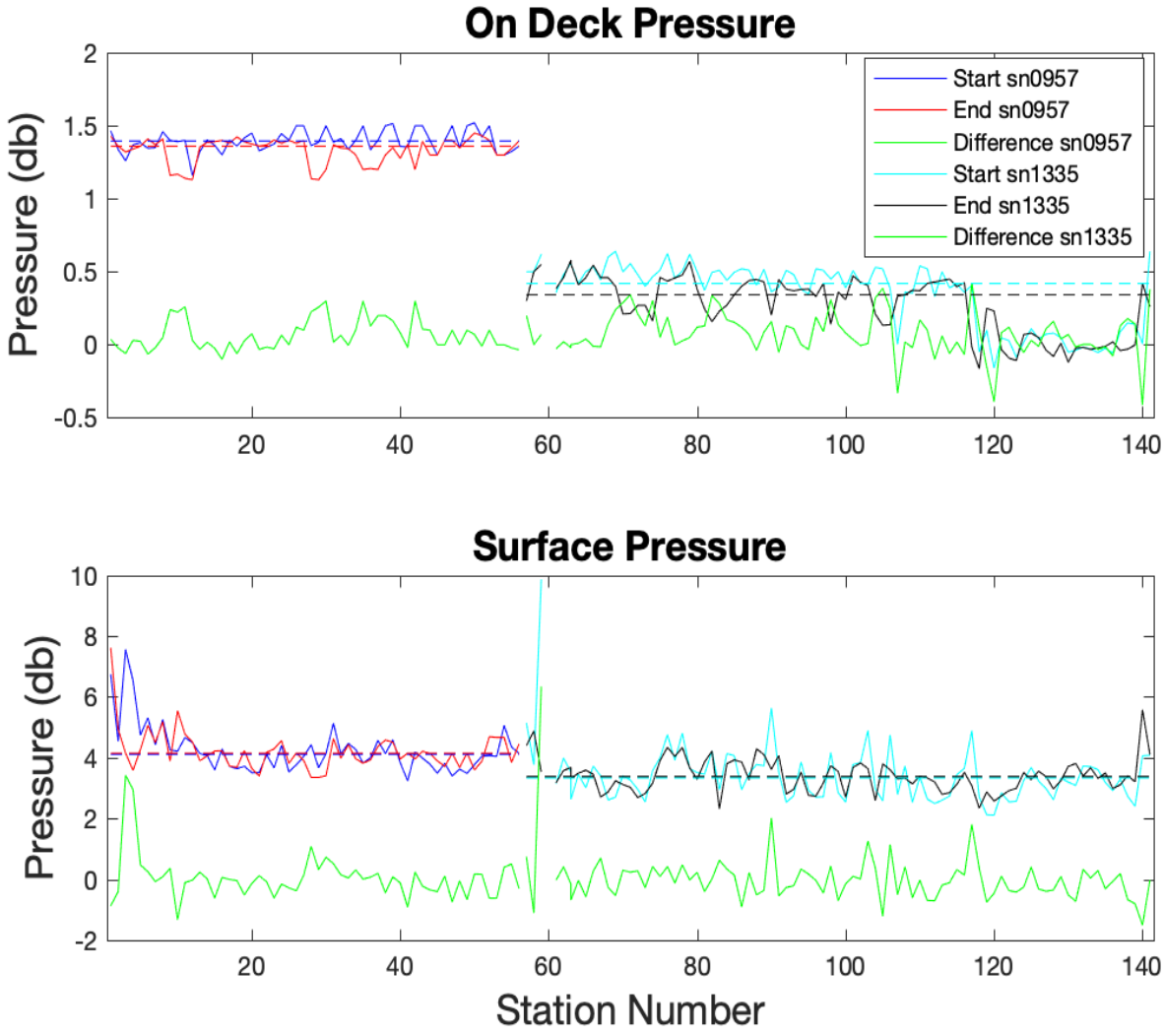

Figure 2: On deck and surface pressures before and after the CTD cast. Top panel are the pressures (s/n 0957, s/n 1335) measured on deck before the cast (blue,cyan), at the end of the upcast (red,black) and the difference (green). Bottom panel are the near sea surface pressure values measured at the start of the downcast (blue,cyan), at the end of the upcast (red,black) and the difference (green).

vi. CTD Temperature

Temperature sensor calibration coefficients derived from the pre-cruise calibrations were applied to raw primary and secondary temperature data during each cast. Calibration accuracy was examined by comparing T1-T2 over a range of station numbers and pressures (bottle trip locations) for each cast. For the entire cruise, only one set of temperature sensors was used (Table 3). These comparisons are summarized in Figure 3, which shows a median temperature difference between the two sensors of -0.001 ºC and a standard deviation of 0.02 ºC (-0.0009 ºC and a standard deviation of 0.0007 ºC below 1000m).

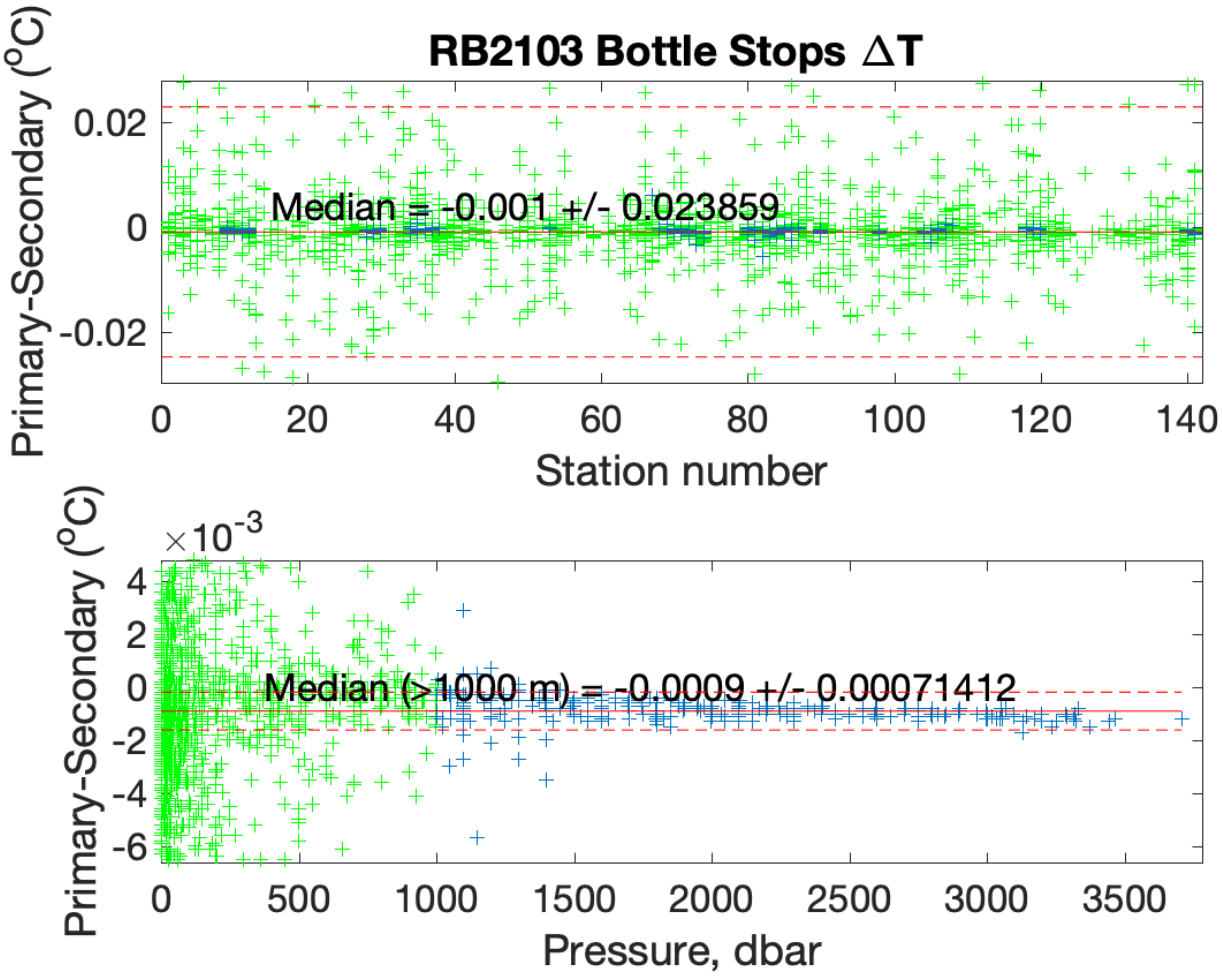

Figure 3: Uncalibrated temperature sensor differences between primary and secondary temperature sensors.

A SBE 35RT reference temperature was used during the cruise as a check to monitor the behavior of the primary and secondary temperature sensors. This allows for corrections to be made if there is any significant pressure dependence or offset seen in the sensors throughout the cruise. Both temperature sensors behaved well when compared to the reference temperature. The primary temperature sensor was chosen for final calibrations. The primary median difference is -7.2 x 10-5 ºC +/- 0.0017 ºC and -0.0003 ºC +/- 0.0018 ºC below 1000 m (Figure 4). After calibration the

primary median difference is -1.3 x 10-5 ºC +/- 0.0016 ºC and 1.4 x 10-5 ºC +/- 0.0002 ºC below 1000 m (**Error! Reference source not found.**).

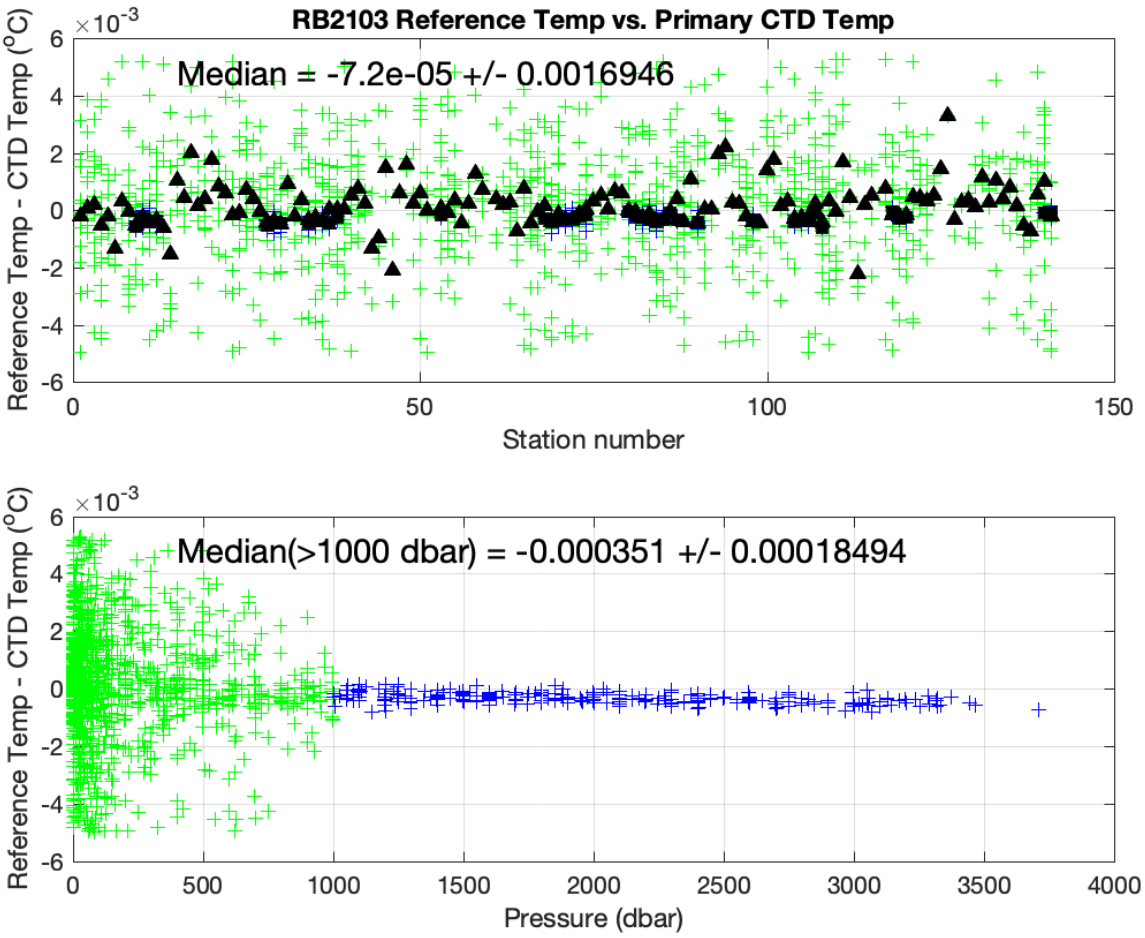

Figure 4: Uncalibrated temperature sensor differences between primary temperature sensor and reference temperature.

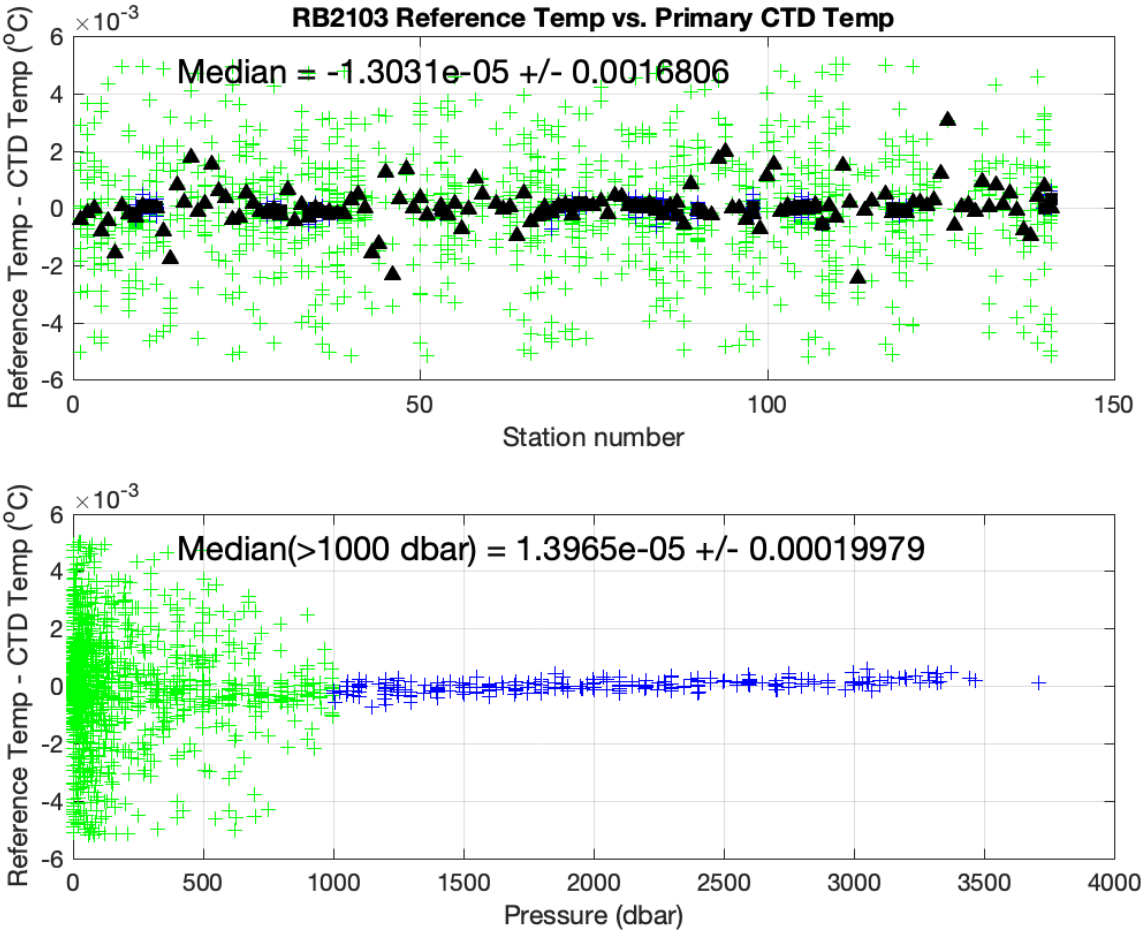

Figure 5: Calibrated temperature sensor differences between primary temperature sensor and reference temperature.

vii. CTD Conductivity

Conductivity sensor calibration coefficients derived from the pre-cruise calibrations were applied to raw primary and secondary conductivities. Comparisons between the primary and secondary sensors and between each of the sensors to check sample conductivities (conductivity calculated from bottle salinities) were used to derive conductivity corrections. Uncorrected C1-C2 are shown in Figure 6 to help identify sensor drift. For the entire cruise, only one set of conductivity sensors was used (Table 3), both tracked each other extremely well. The two sensors show a median difference of -0.0007 mS/cm and a standard deviation of 0.03 mS/cm (0.0004 mS/cm and a standard deviation of 0.0005 mS/cm below 1000m). The primary conductivity sensor was chosen for final calibrations. The primary median difference is -0.003 mS/cm +/- 0.008 mS/cm and -0.001 mS/cm +/- 0.002 mS/cm below 1000 m (Figure 7). After calibration the primary median difference is -0.0001 mS/cm +/- 0.008 mS/cm and -0.0003 mS/cm +/- 0.002 mS/cm below 1000 m (Figure 8).

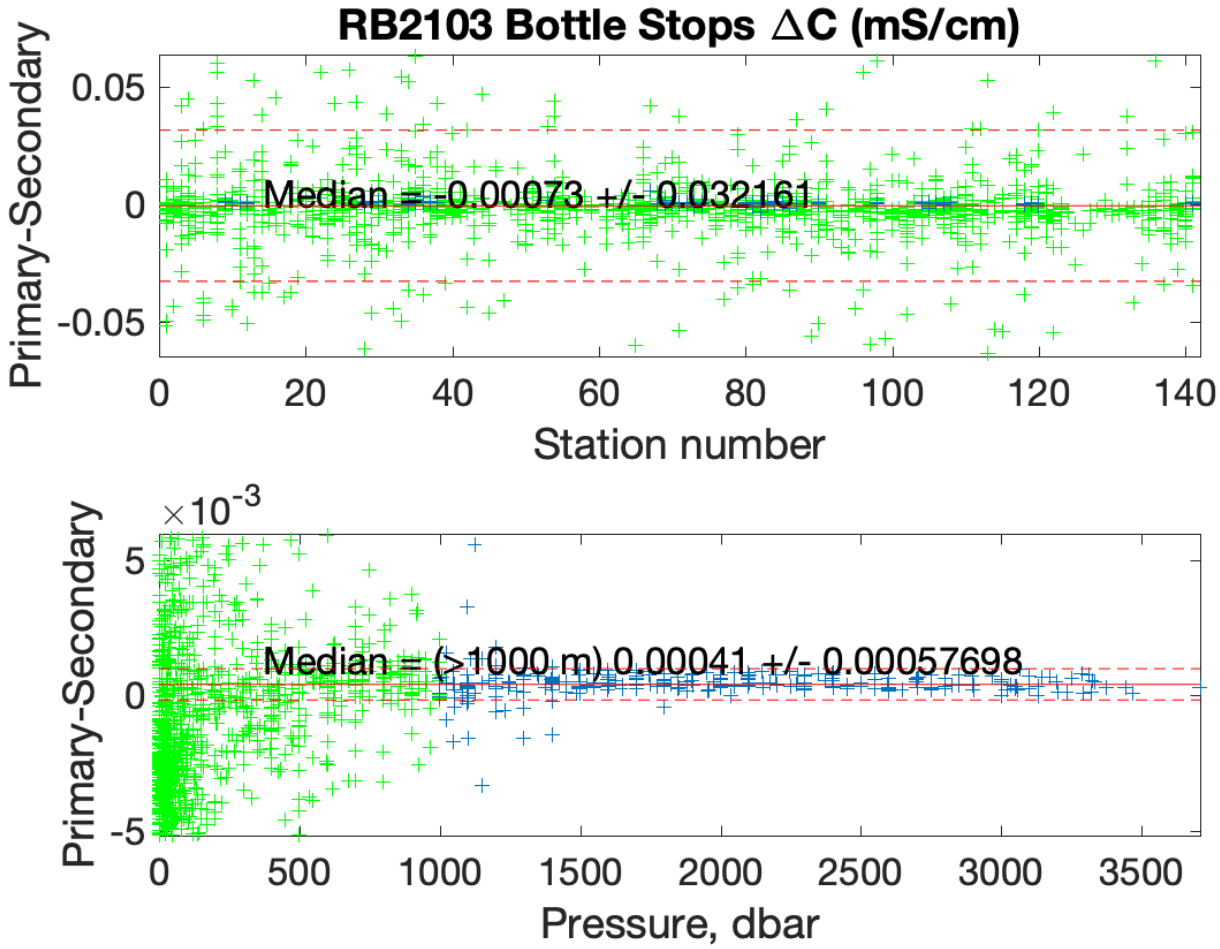

Figure 6: Uncalibrated conductivity differences between primary and secondary conductivity (salinity) sensors.

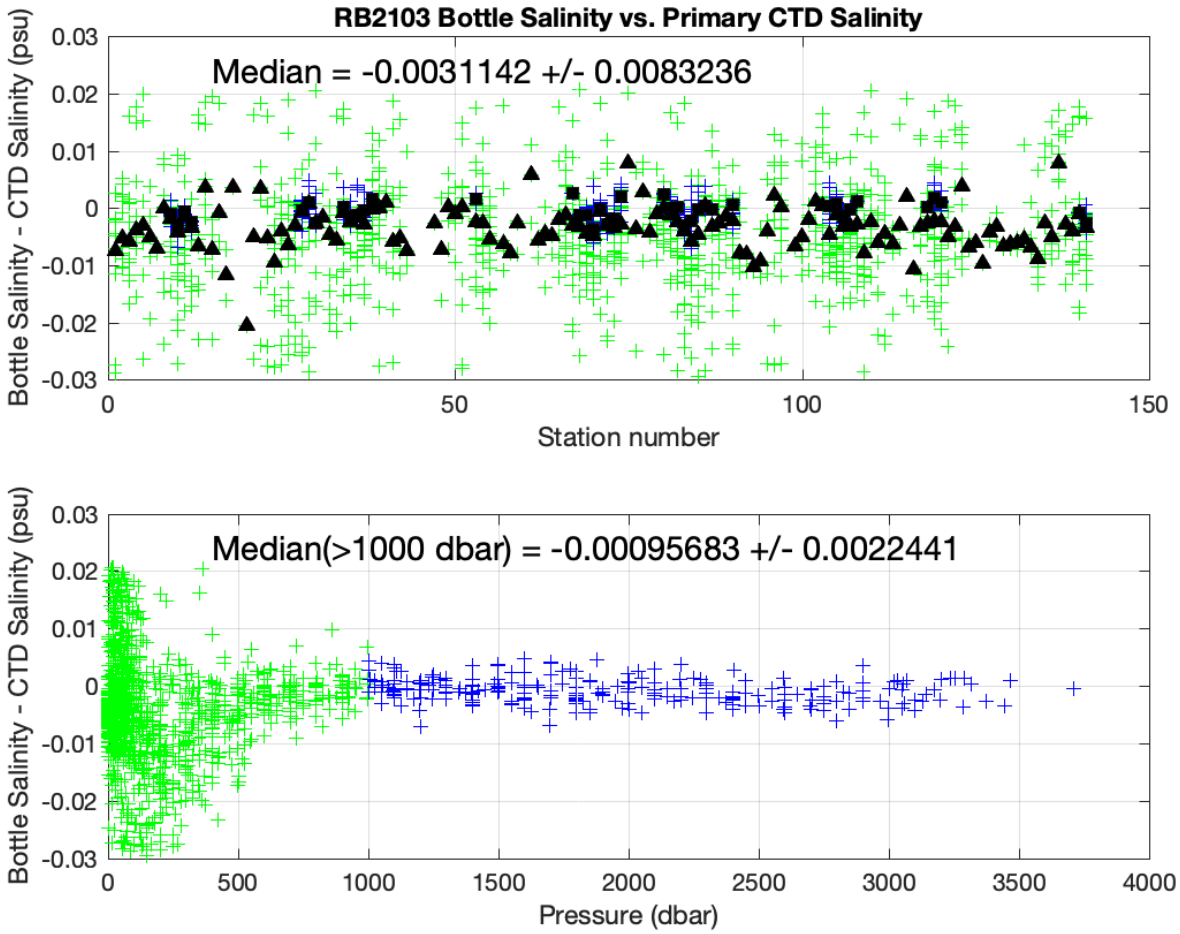

Figure 7:Uncalibrated conductivity differences between primary conductivity (salinity) sensor and bottle salinity.

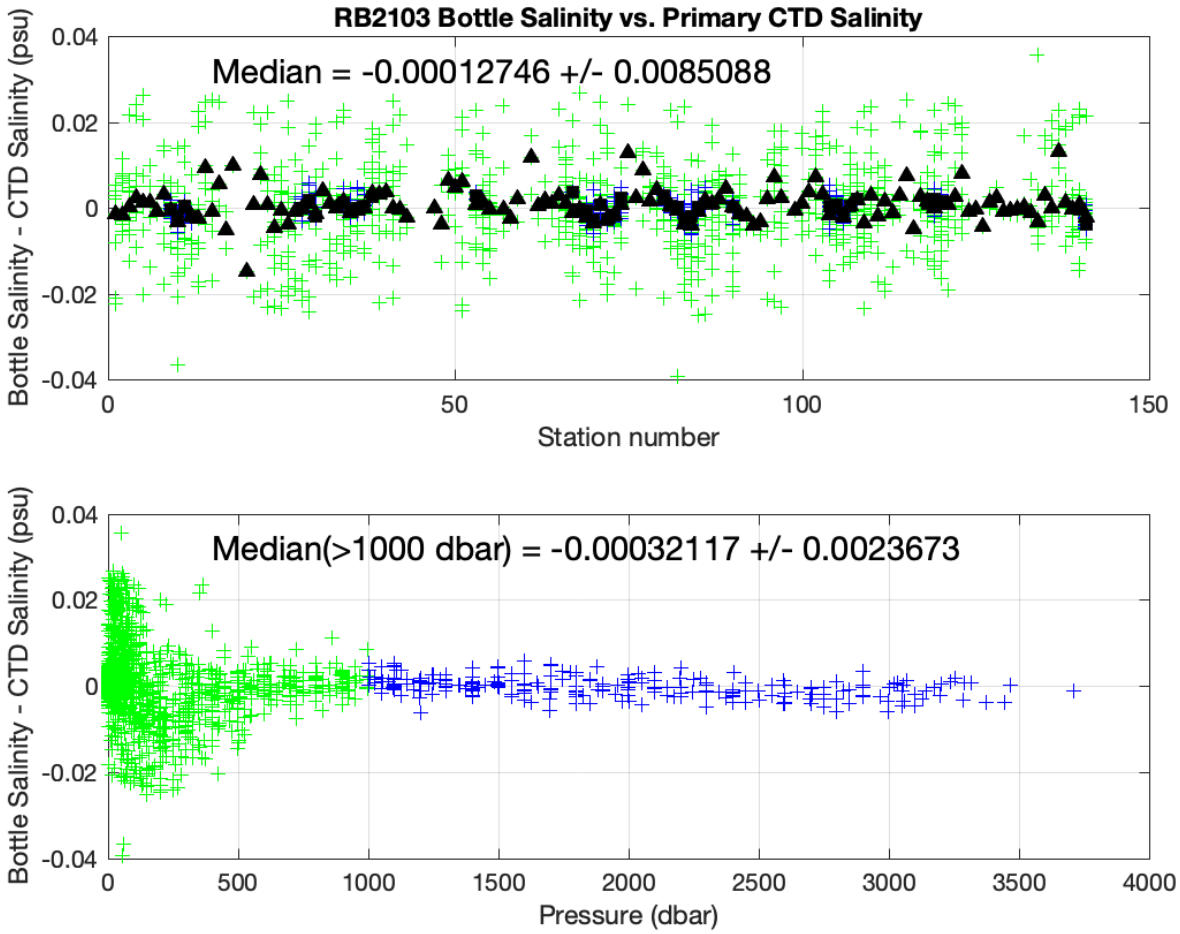

Figure 8:Calibrated conductivity differences between primary conductivity (salinity) sensor and bottle salinity.

viii. CTD Dissolved Oxygen

Two SBE43 dissolved O_2 (DO) sensors were used on this leg (Table 3). Both sensors tracked each other well (Figure 9). The sensors show a median difference of -0.51 µmol/kg and a standard deviation of 1.04 µmol/kg below (-0.81 µmol/kg and a standard deviation of 0.67 µmol/kg below 1000m). The primary oxygen sensor was chosen for final calibrations. The primary median difference with bottle oxygen is 5.09 µmol/kg +/- 2.79 µmol/kg and 9.08 µmol/kg +/- 3.30 µmol/kg below 1000 m (Figure 10). After calibration the primary median difference with bottle oxygen is 0.001 µmol/kg +/- 1.26 µmol/kg and -0.006 µmol/kg +/- 1.03 µmol/kg below 1000 m (Figure 11).

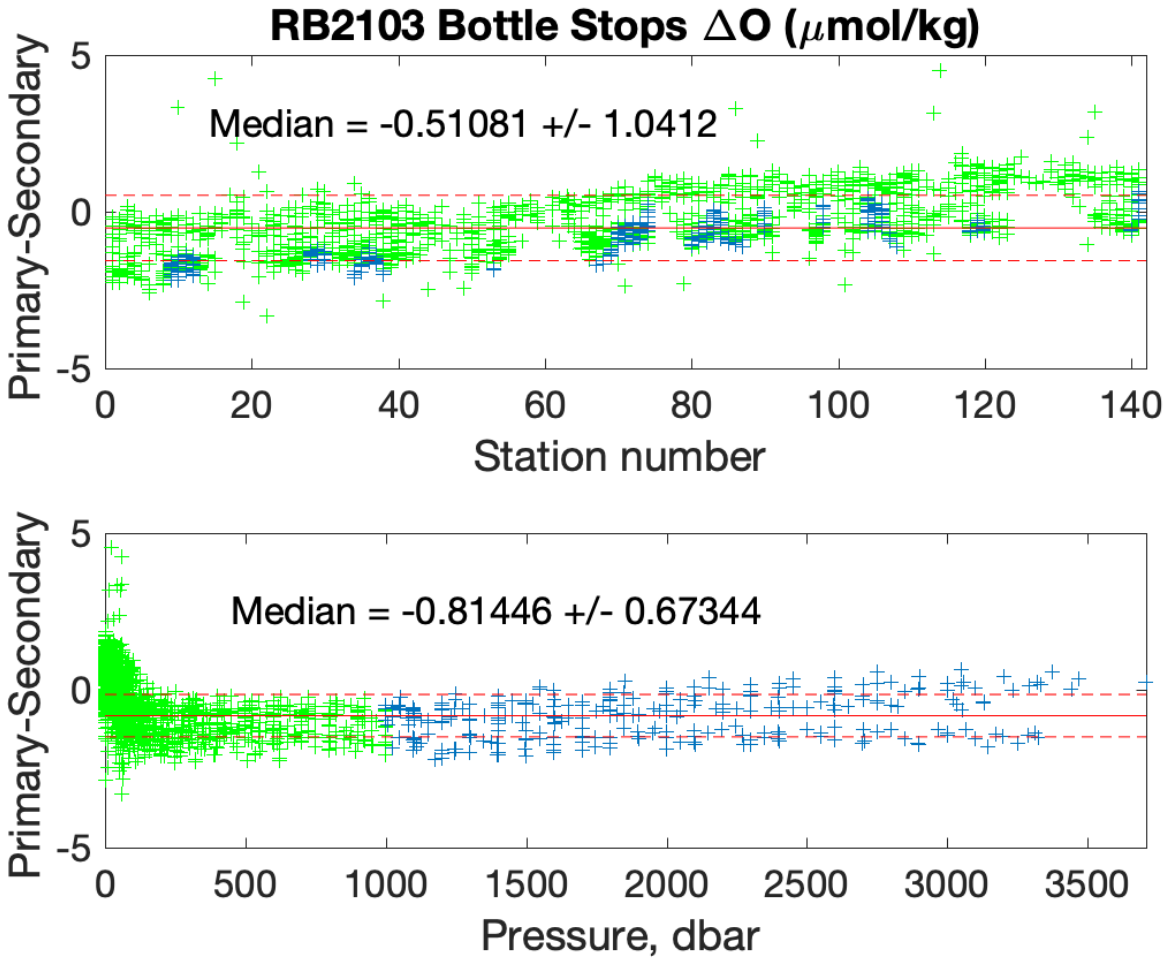

Figure 9:Uncalibrated oxygen differences between primary and secondary oxygen sensors.

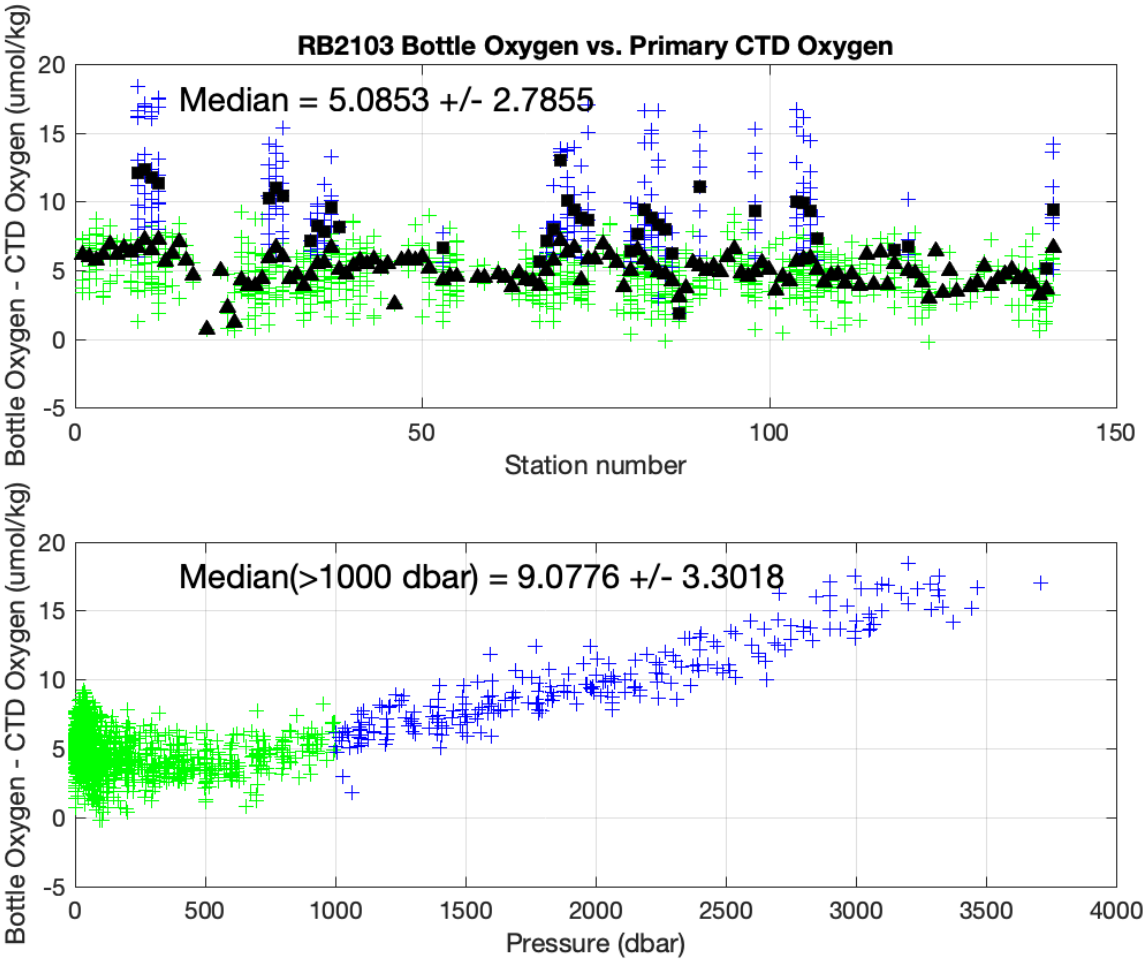

Figure 10: Uncalibrated oxygen differences between primary oxygen sensor and bottle oxygen.

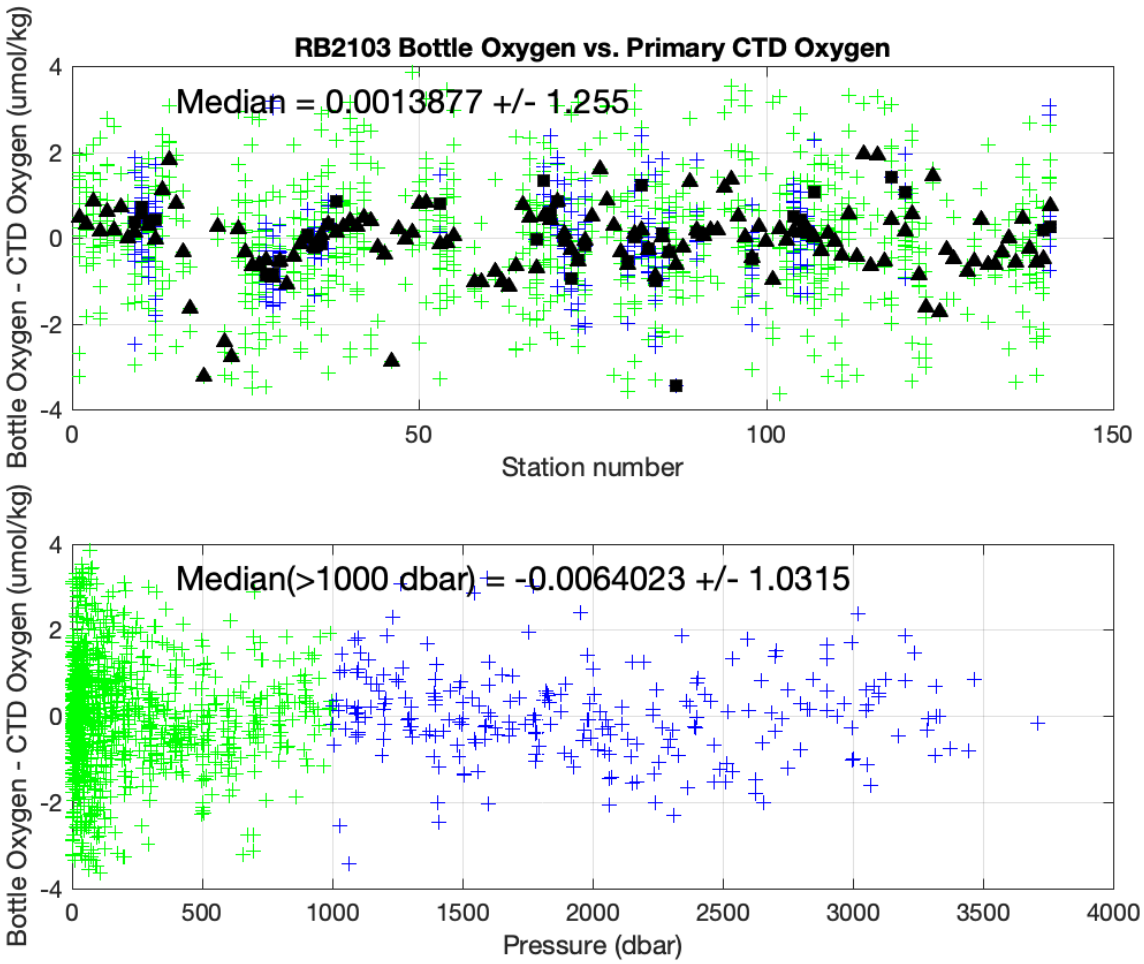

Figure 11: Calibrated oxygen differences between primary oxygen sensor and bottle oxygen.

ix. Preliminary CTD Data Processing

The final calibrated CTD data files were used to produce the section plots that follow. Primary calibrated sensor data as well as bottle distributions of the water samples taken for the main CTD lines are shown in Figures Figure 12 to Figure 59.

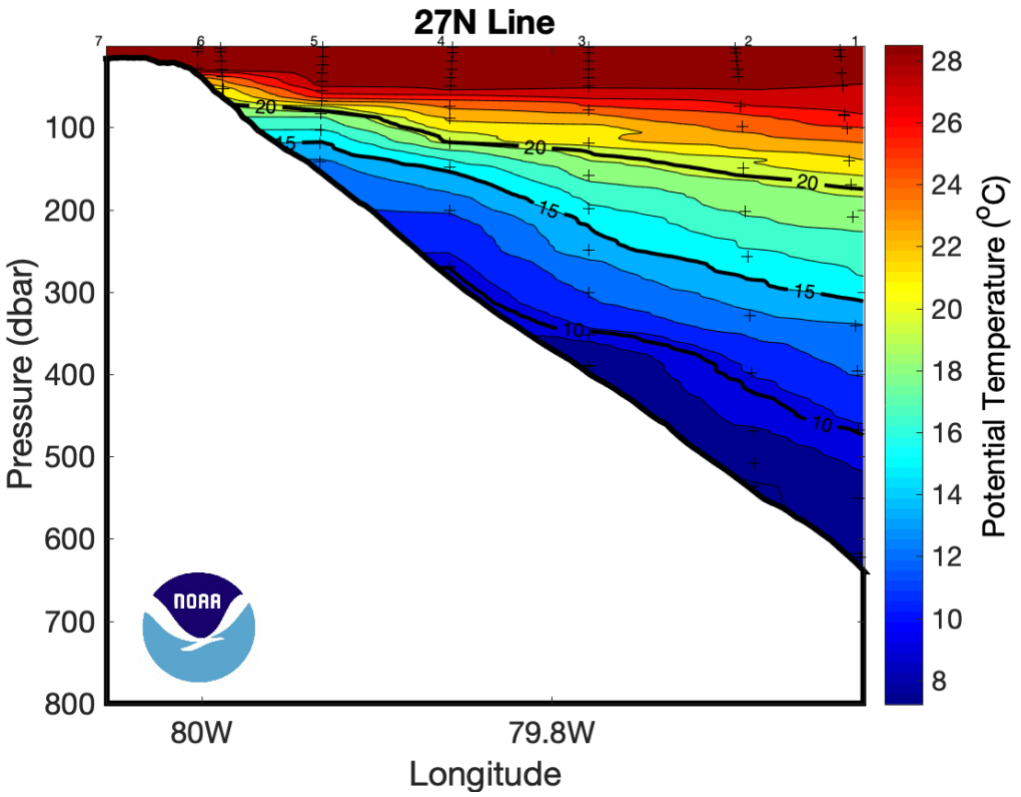

Figure 12: Potential temperature (ºC) for the 27N section. The black crosses represent the bottle trip depths.

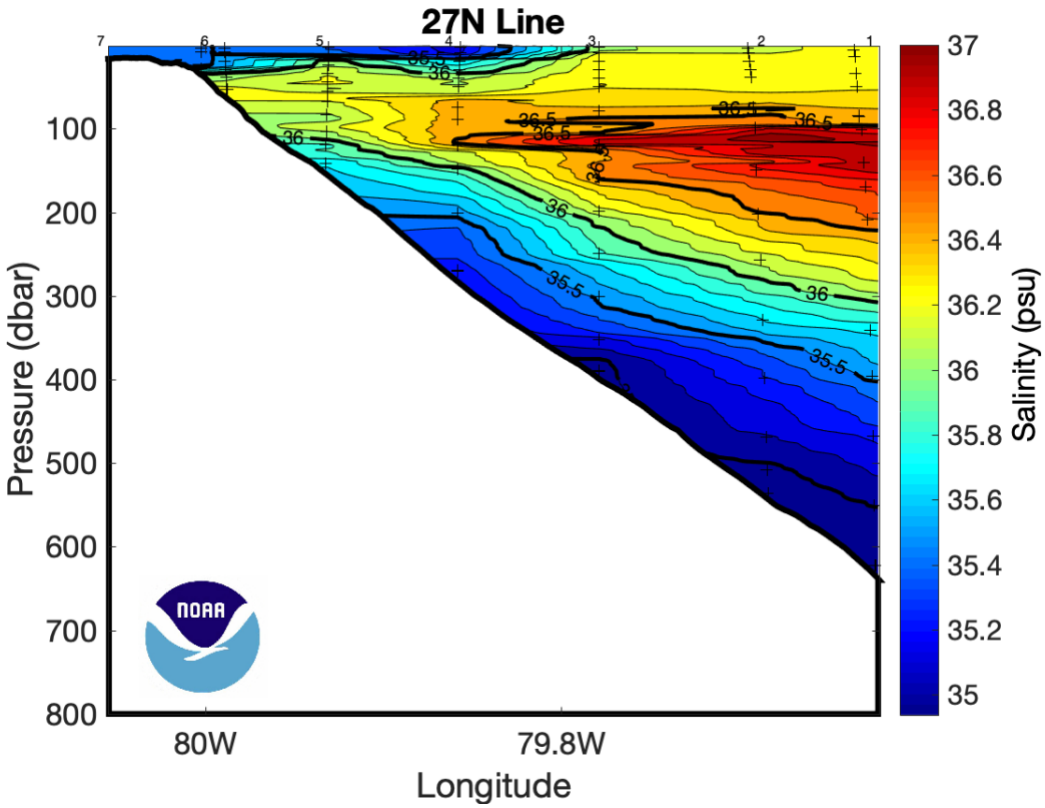

Figure 13: Salinity (PSS 78) for the 27N section. The black crosses represent the bottle trip depths.

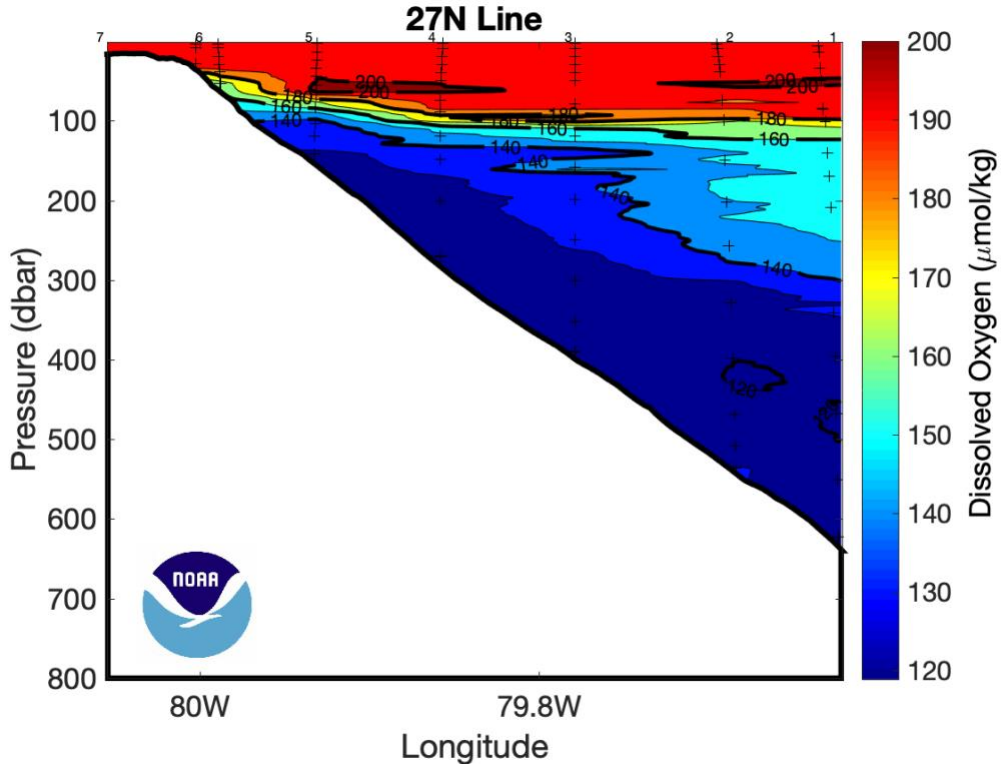

Figure 14: Dissolved oxygen (μmol/kg) for the 27N section. The black crosses represent the bottle trip depths.

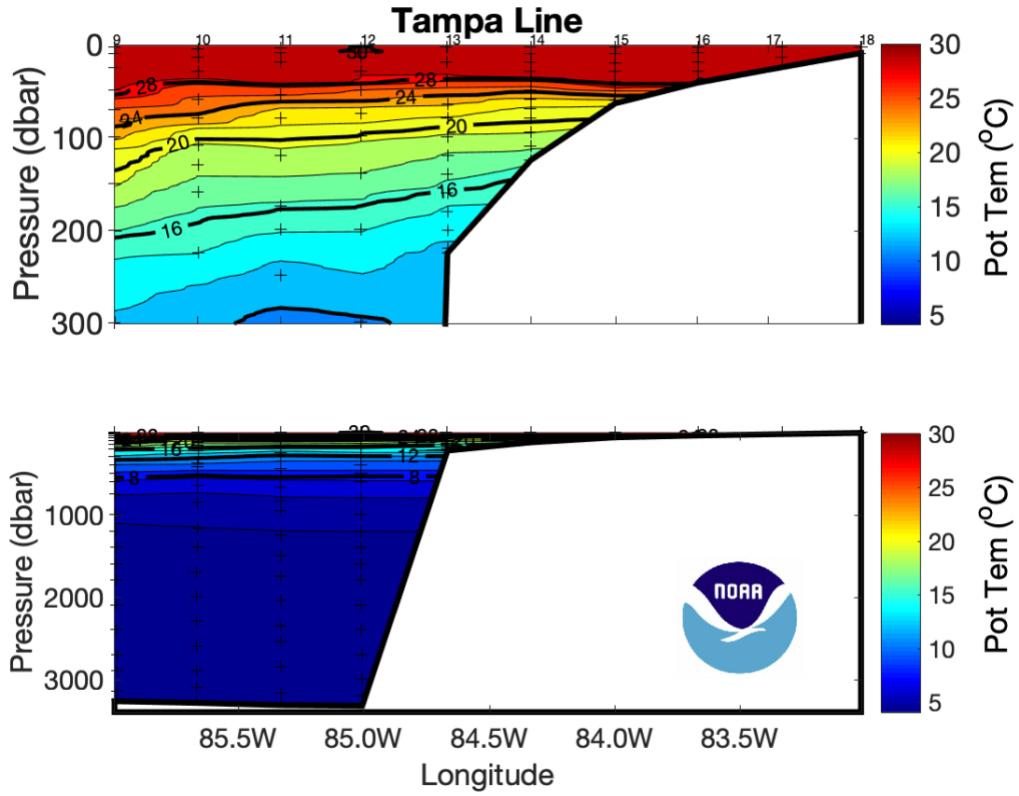

Figure 15: Potential temperature (ºC) for the Tampa section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

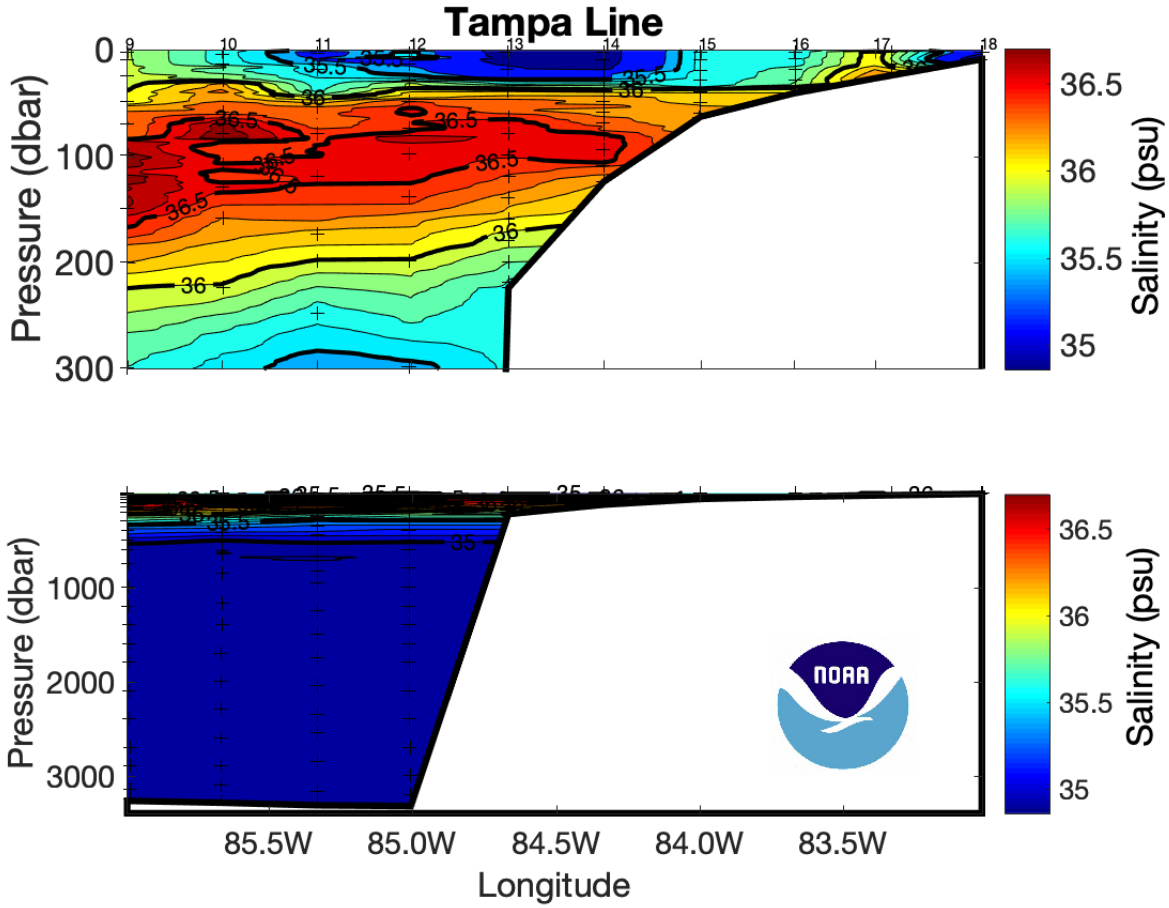

Figure 16: Salinity (PSS 78) for the Tampa section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

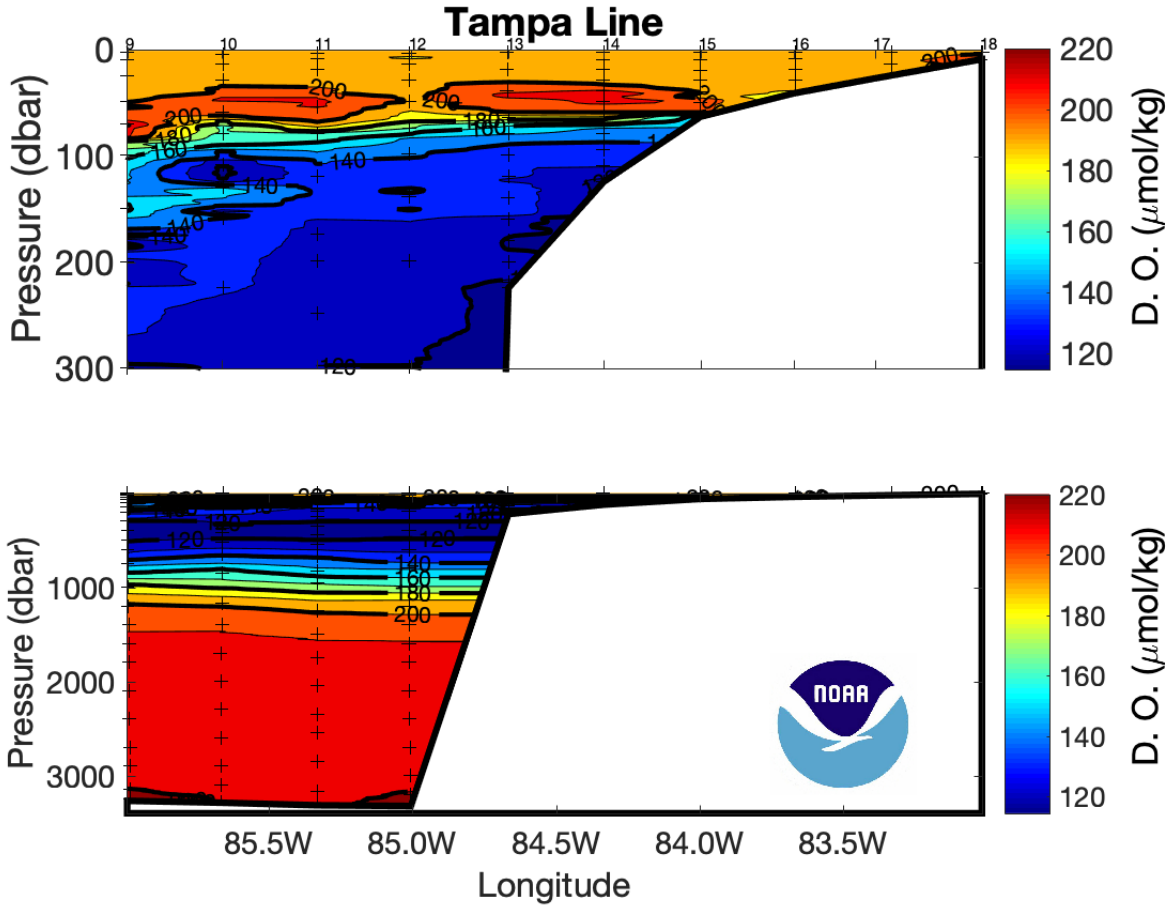

Figure 17: Dissolved oxygen (μmol/kg) for the Tampa section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

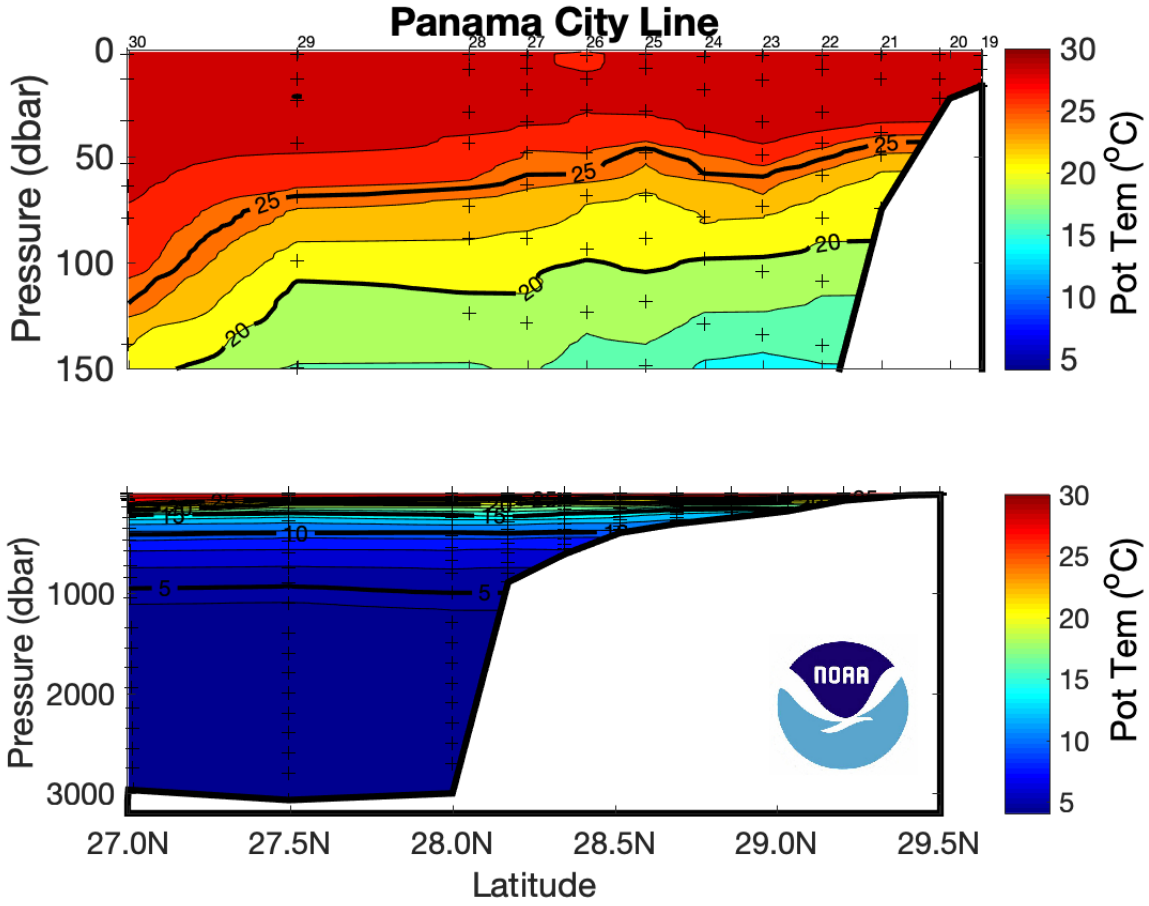

Figure 18: Potential temperature (ºC) for the Panama City section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

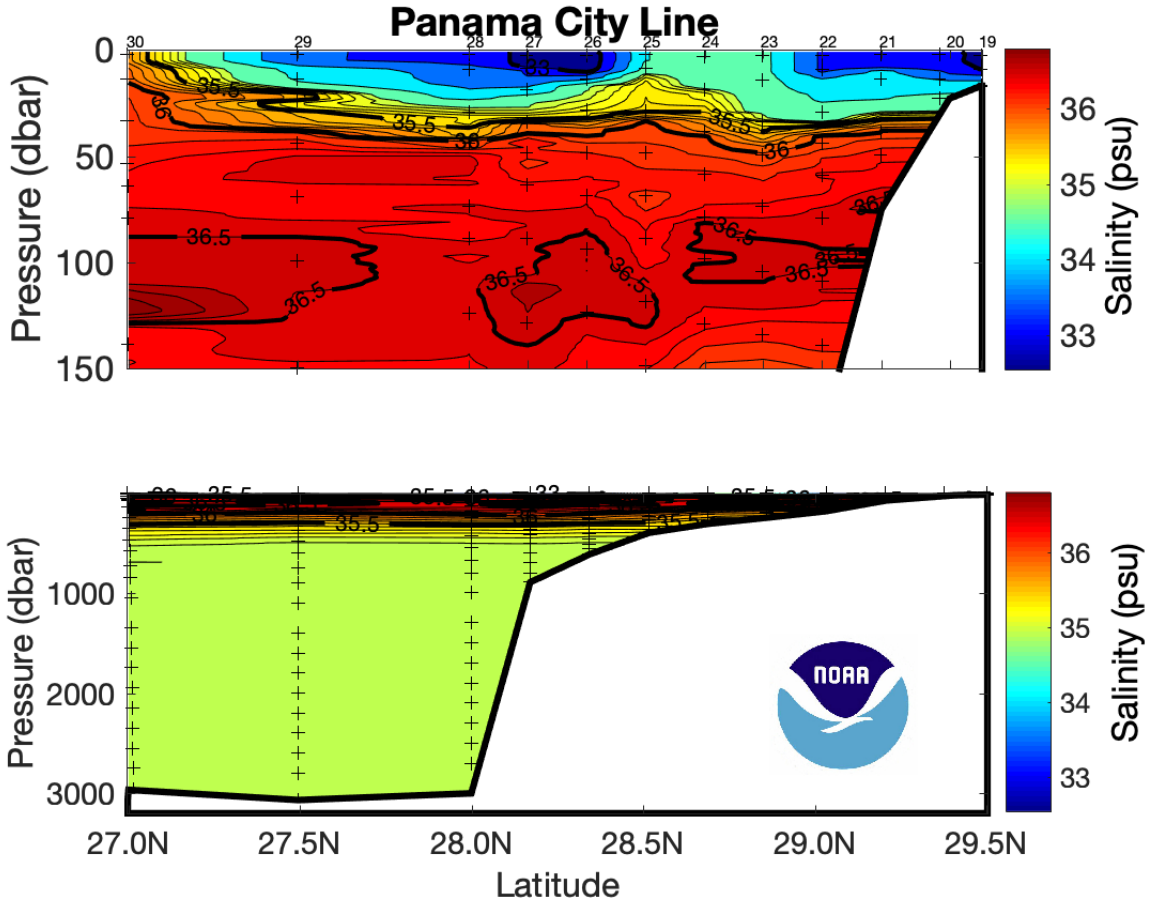

Figure 19: Salinity (PSS 78) for the Panama City section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

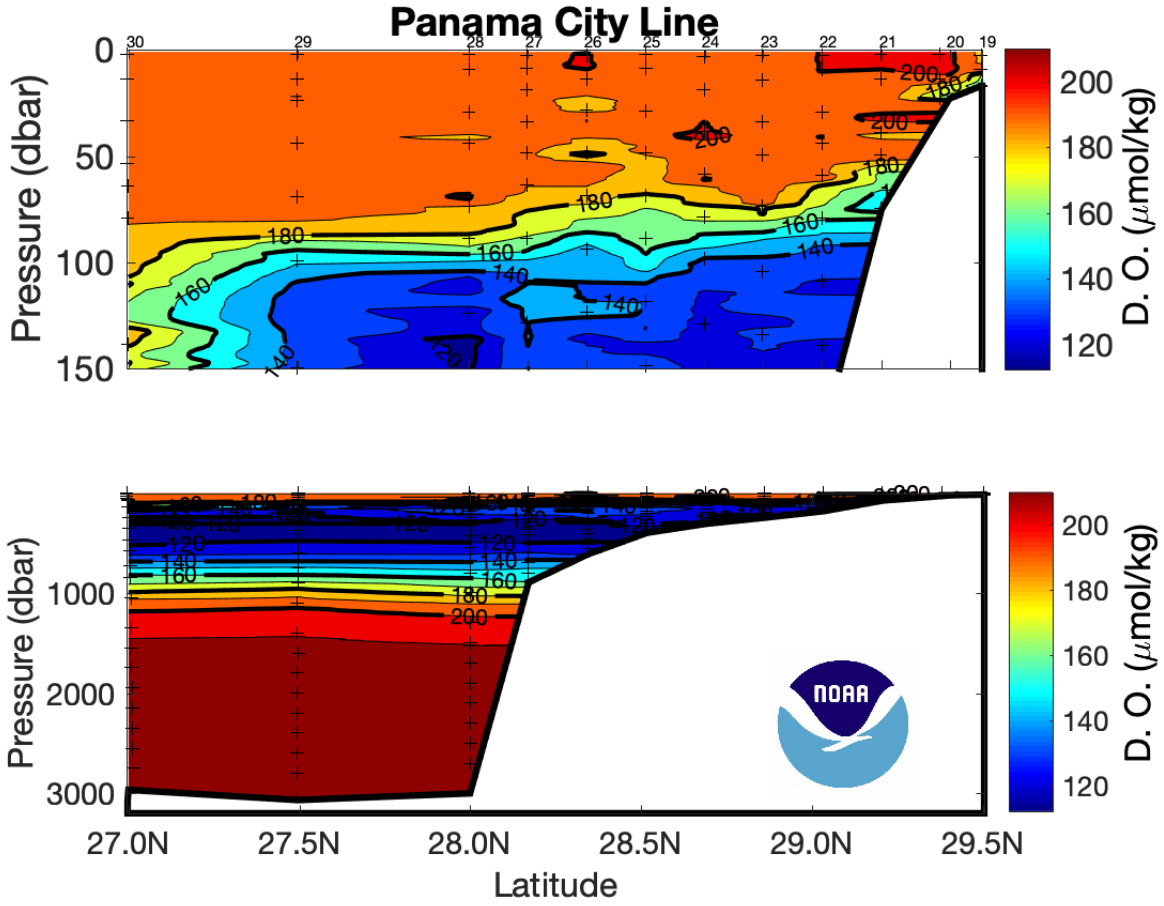

Figure 20: Dissolved oxygen (μmol/kg) for the Panama City section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

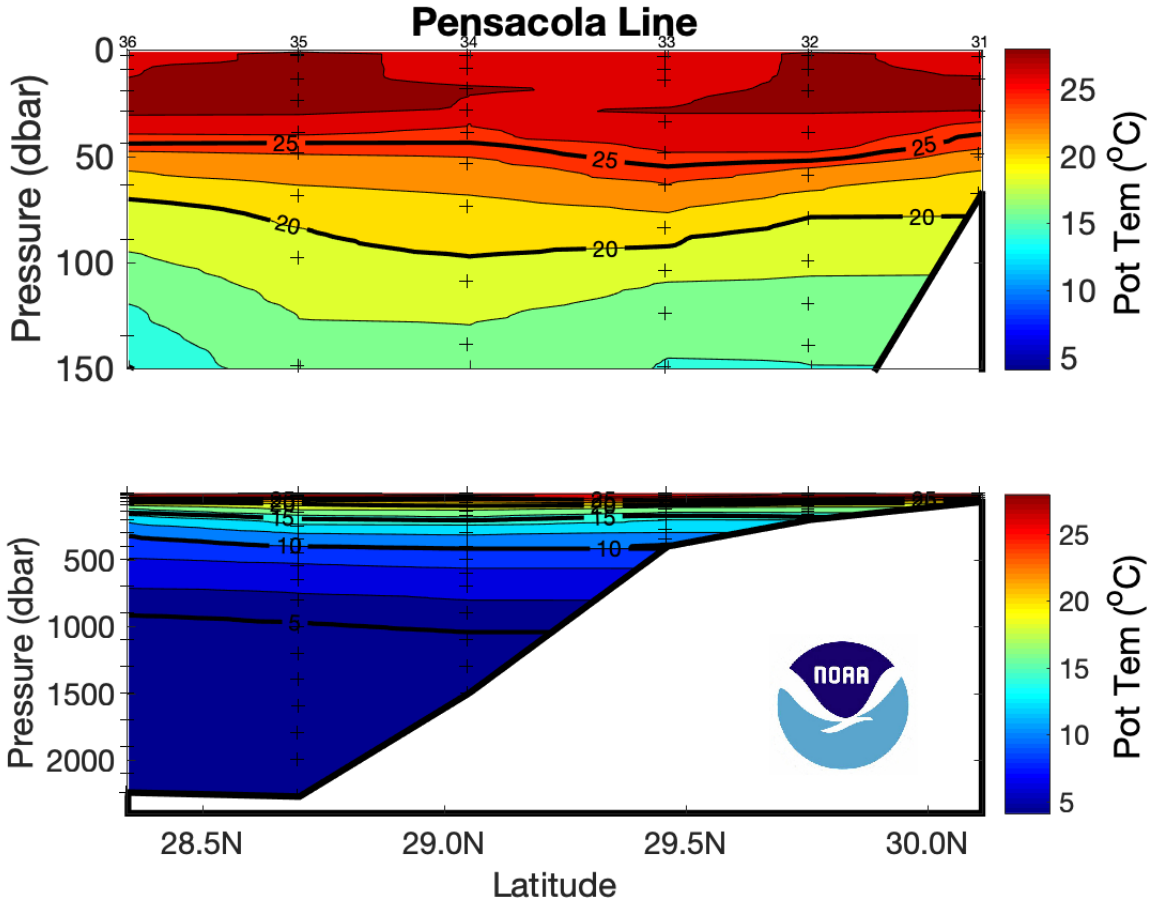

Figure 21: Potential temperature (ºC) for the Pensacola section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

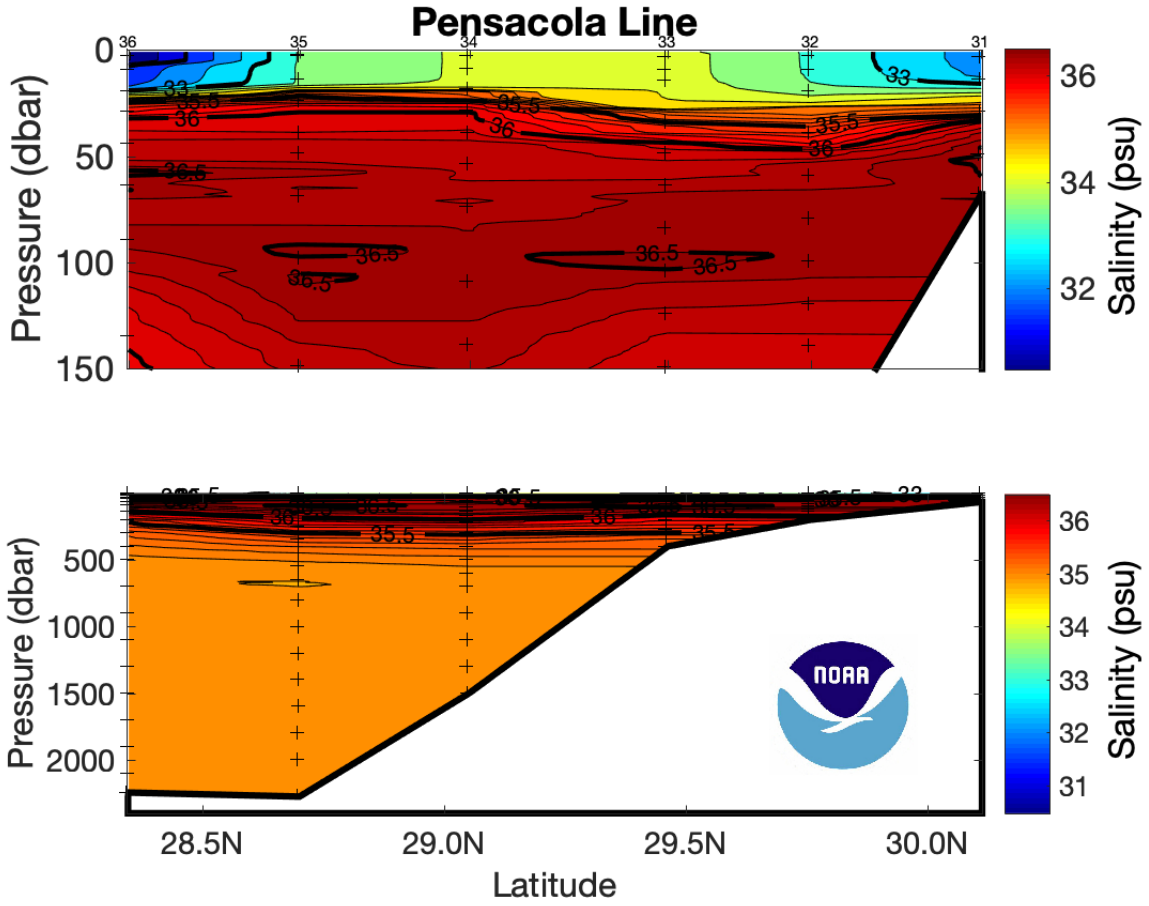

Figure 22: Salinity (PSS 78) for the Pensacola section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

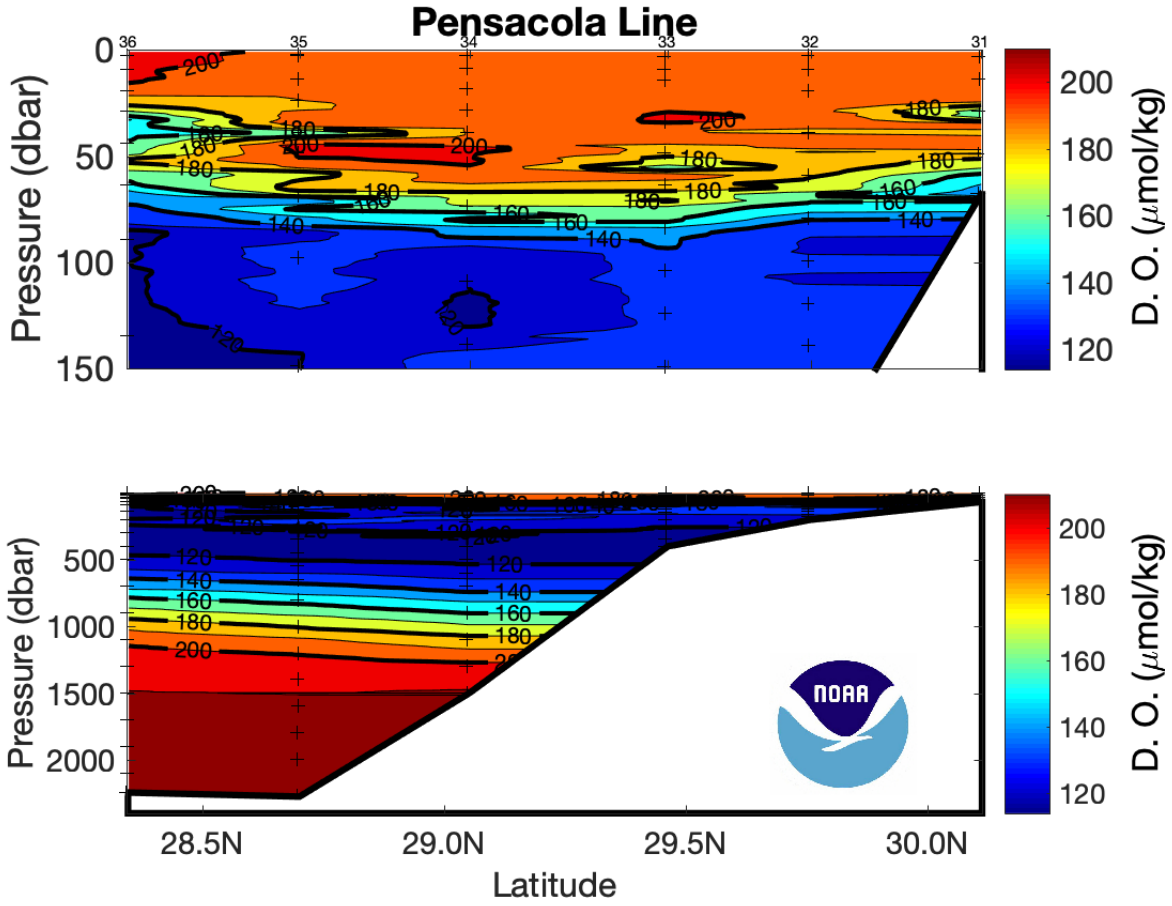

Figure 23: Dissolved oxygen (μmol/kg) for the Pensacola section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

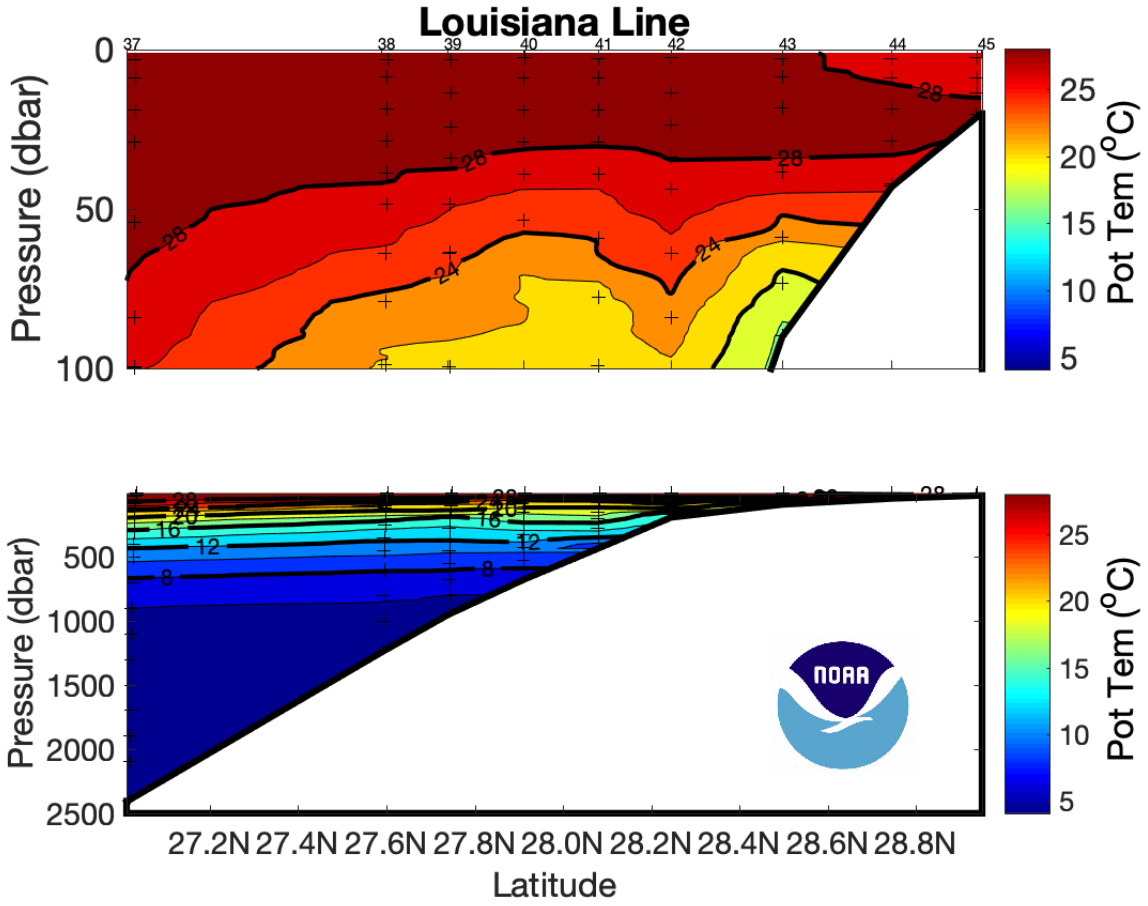

Figure 24: Potential temperature (ºC) for the Louisiana section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

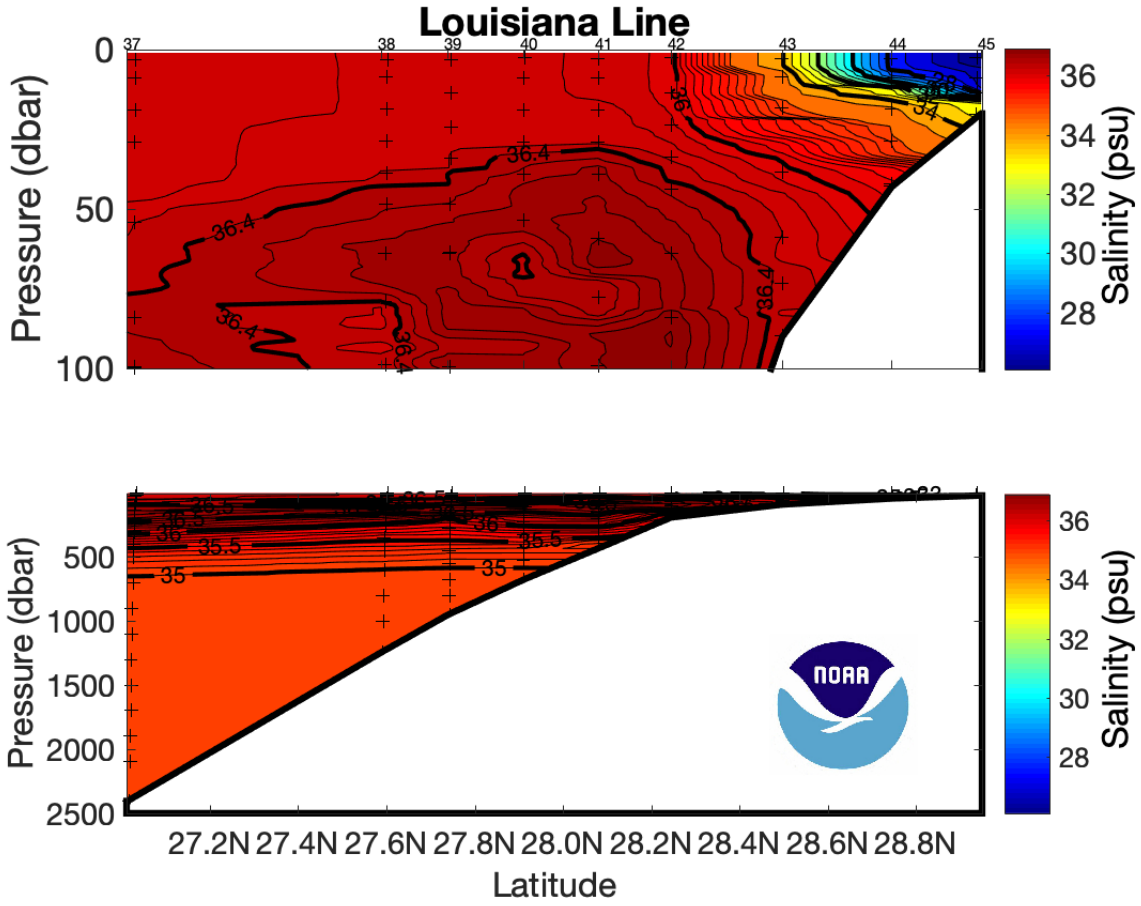

Figure 25: Salinity (PSS 78) for the Louisiana section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

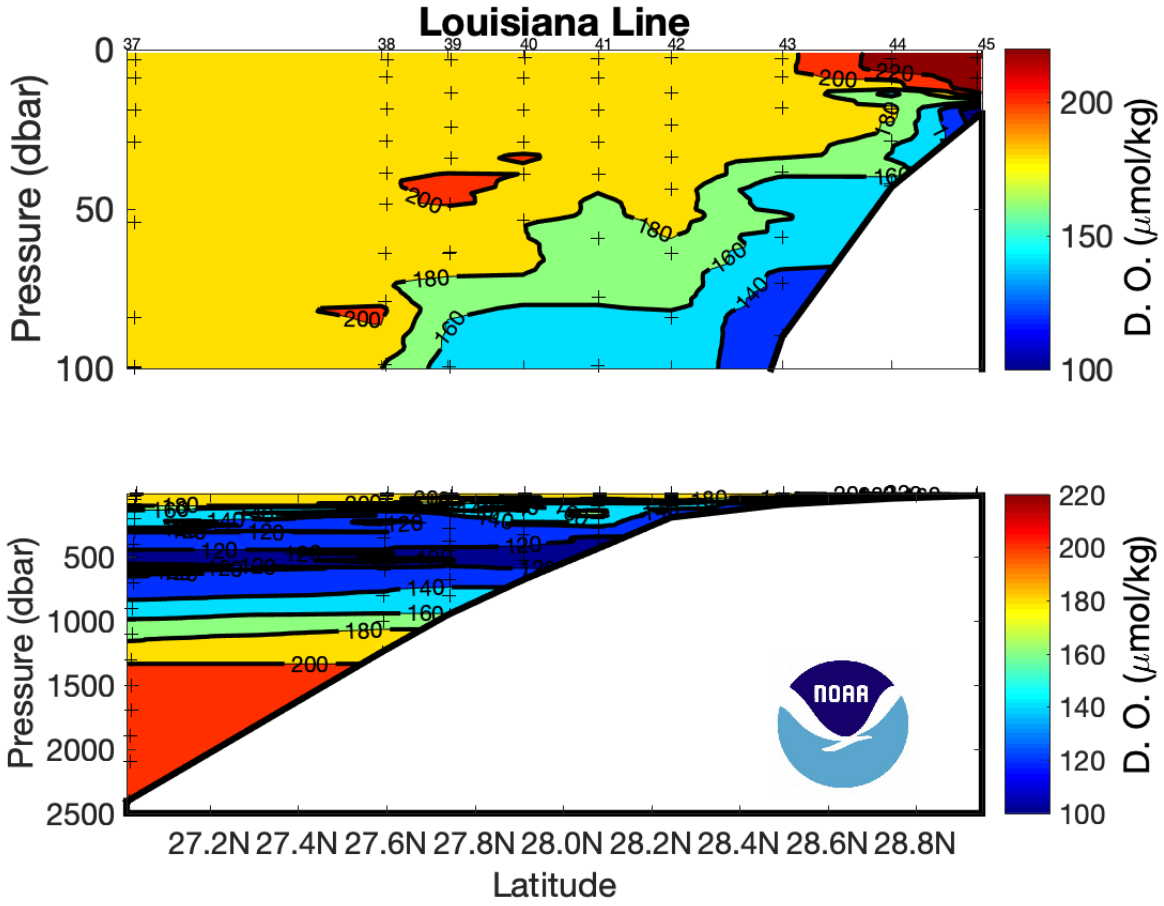

Figure 26: Dissolved oxygen (μmol/kg) for the Louisiana section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

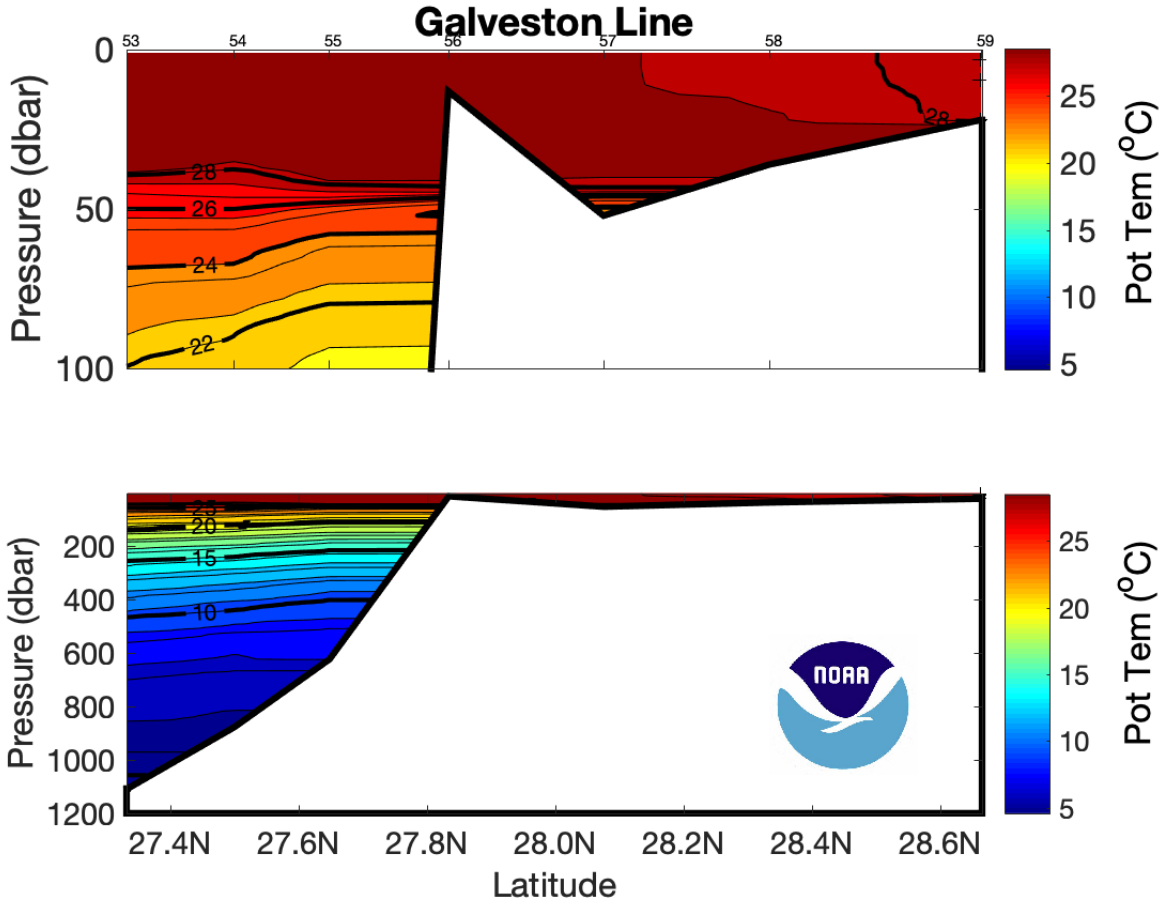

Figure 27: Potential temperature (ºC) for the Galveston section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

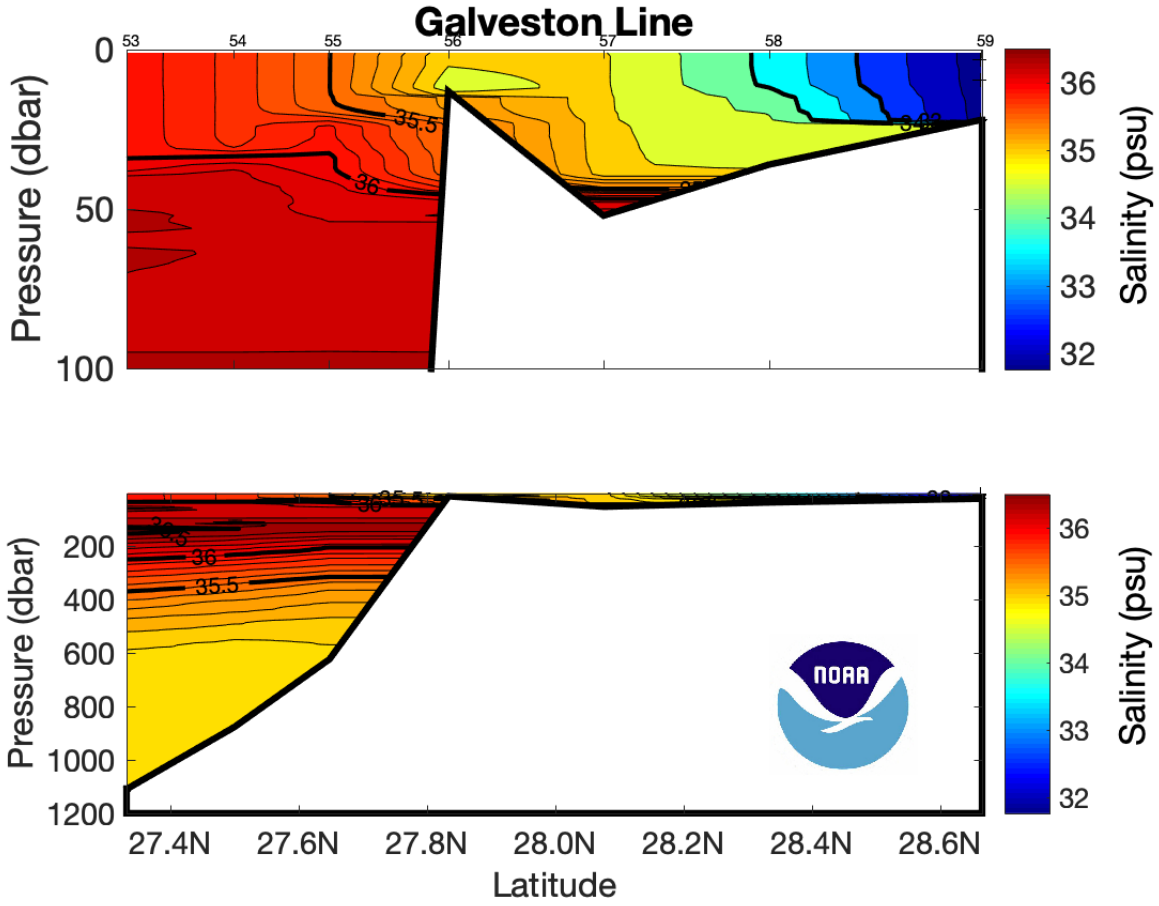

Figure 28: Salinity (PSS 78) for the Galveston section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

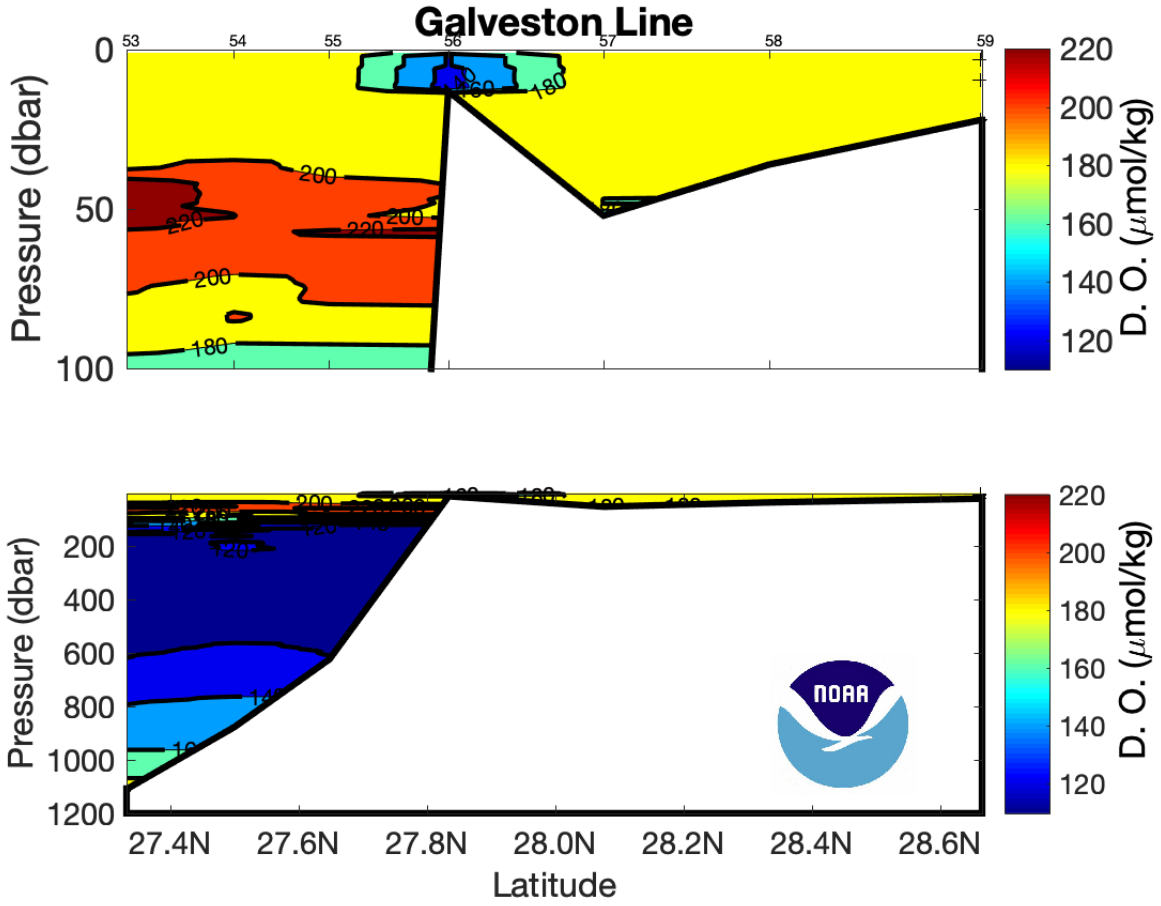

Figure 29: Dissolved oxygen (µmol/kg) for the Galveston section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

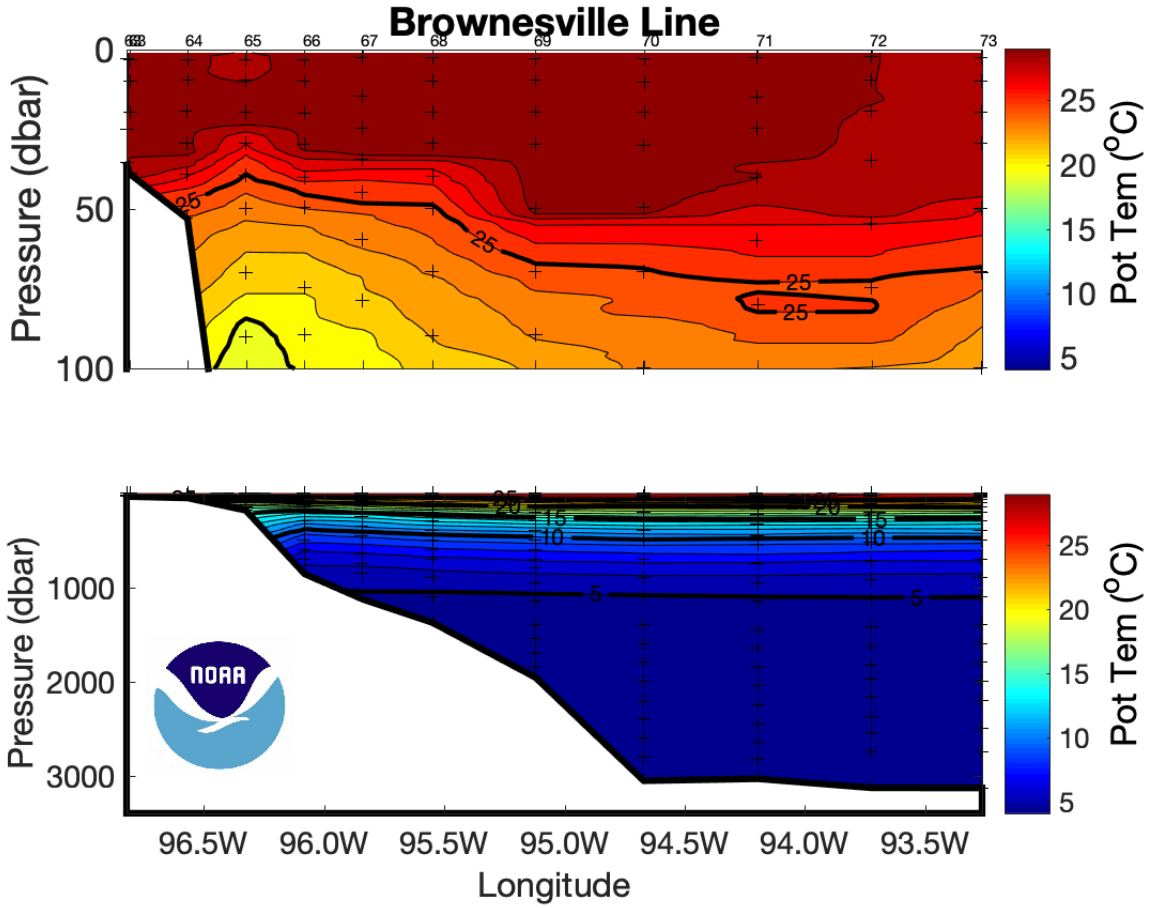

Figure 30: Potential temperature (ºC) for the Brownesville section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

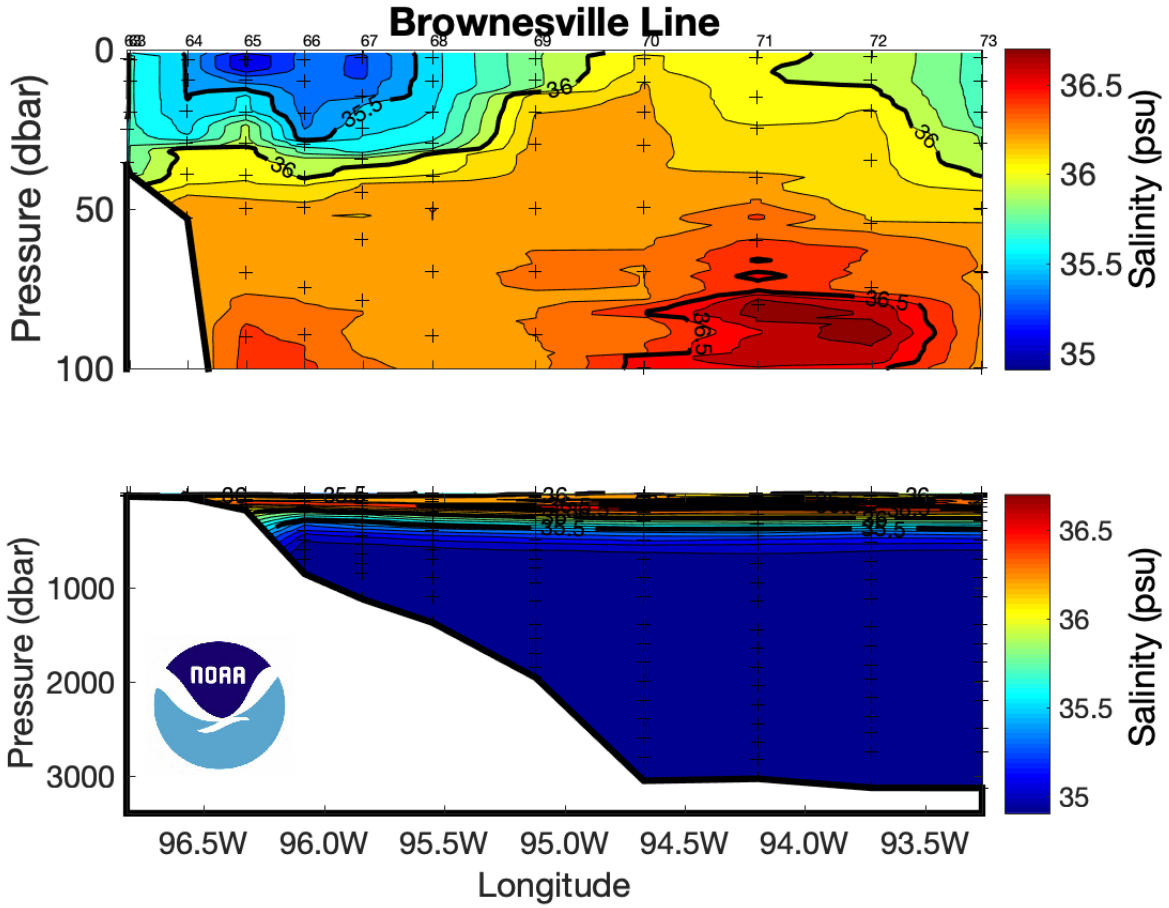

Figure 31: Salinity (PSS 78) for the Brownesville section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

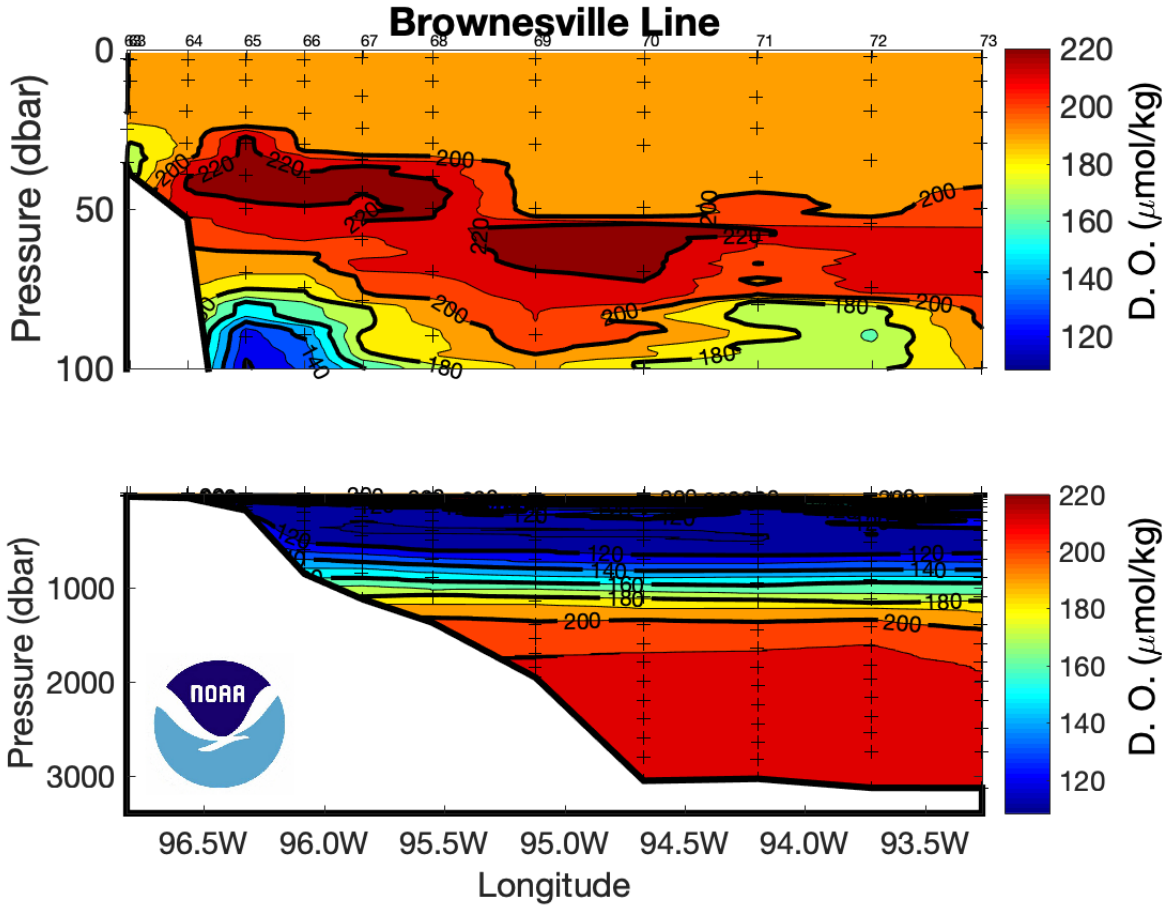

Figure 32: Dissolved oxygen (μmol/kg) for the Brownesville section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

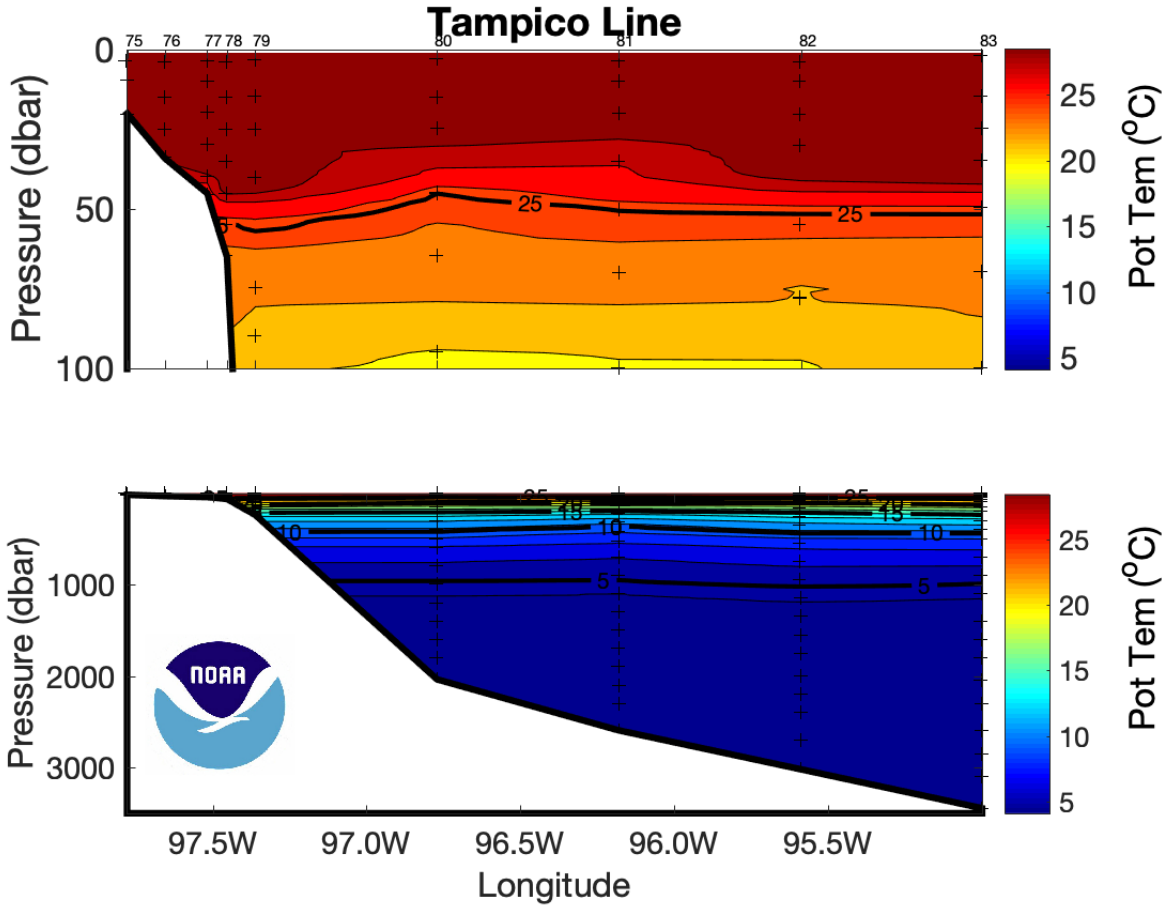

Figure 33: Potential temperature (ºC) for the Tampico section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

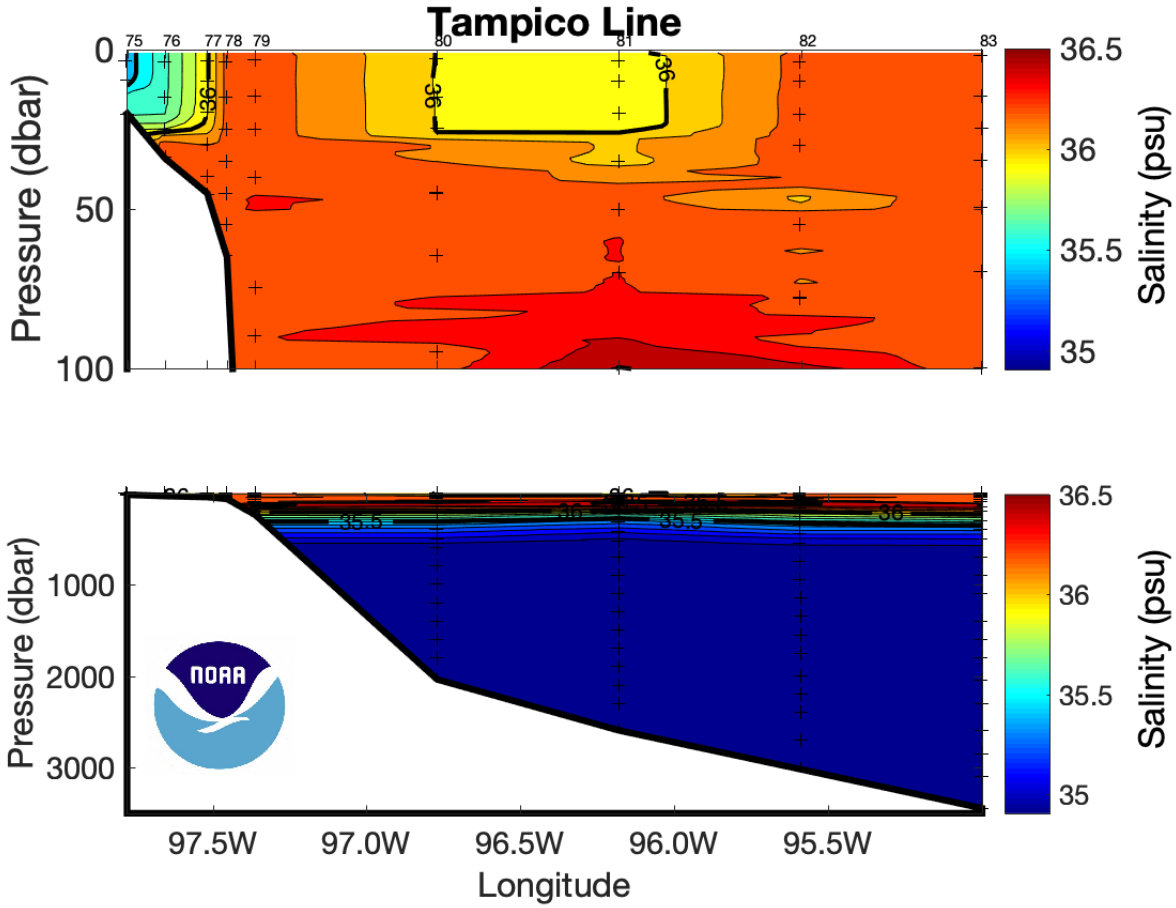

Figure 34: Salinity (PSS 78) for the Tampico section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

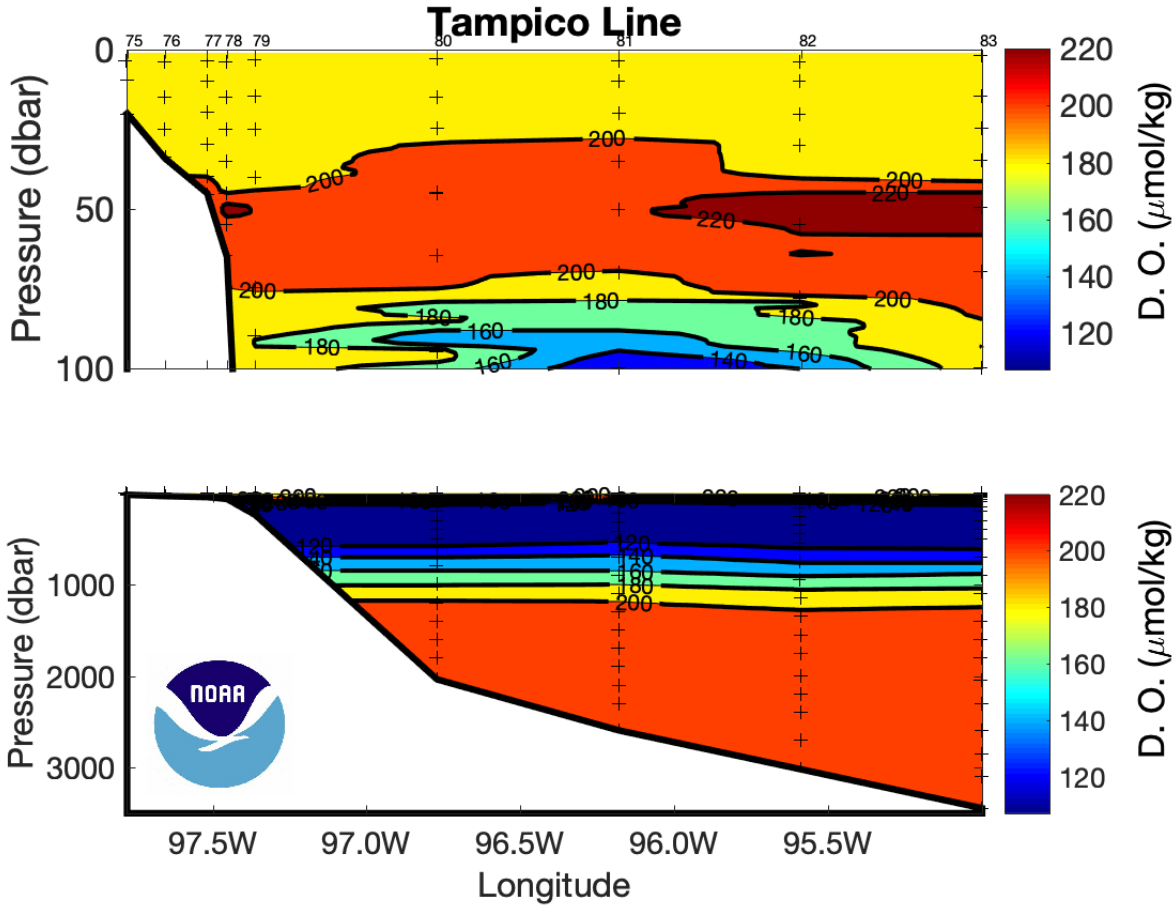

Figure 35: Dissolved oxygen (μmol/kg) for the Tampico section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

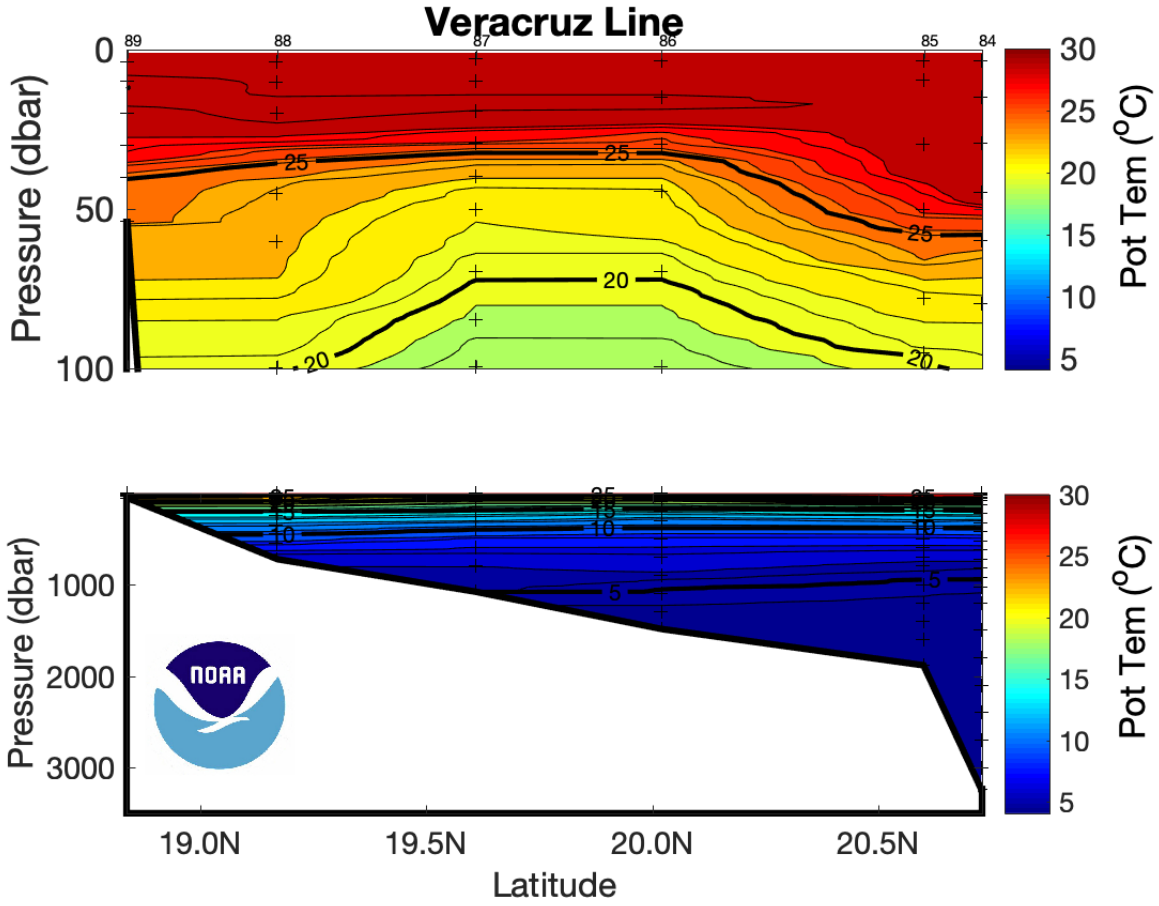

Figure 36: Potential temperature (ºC) for the Veracruz section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

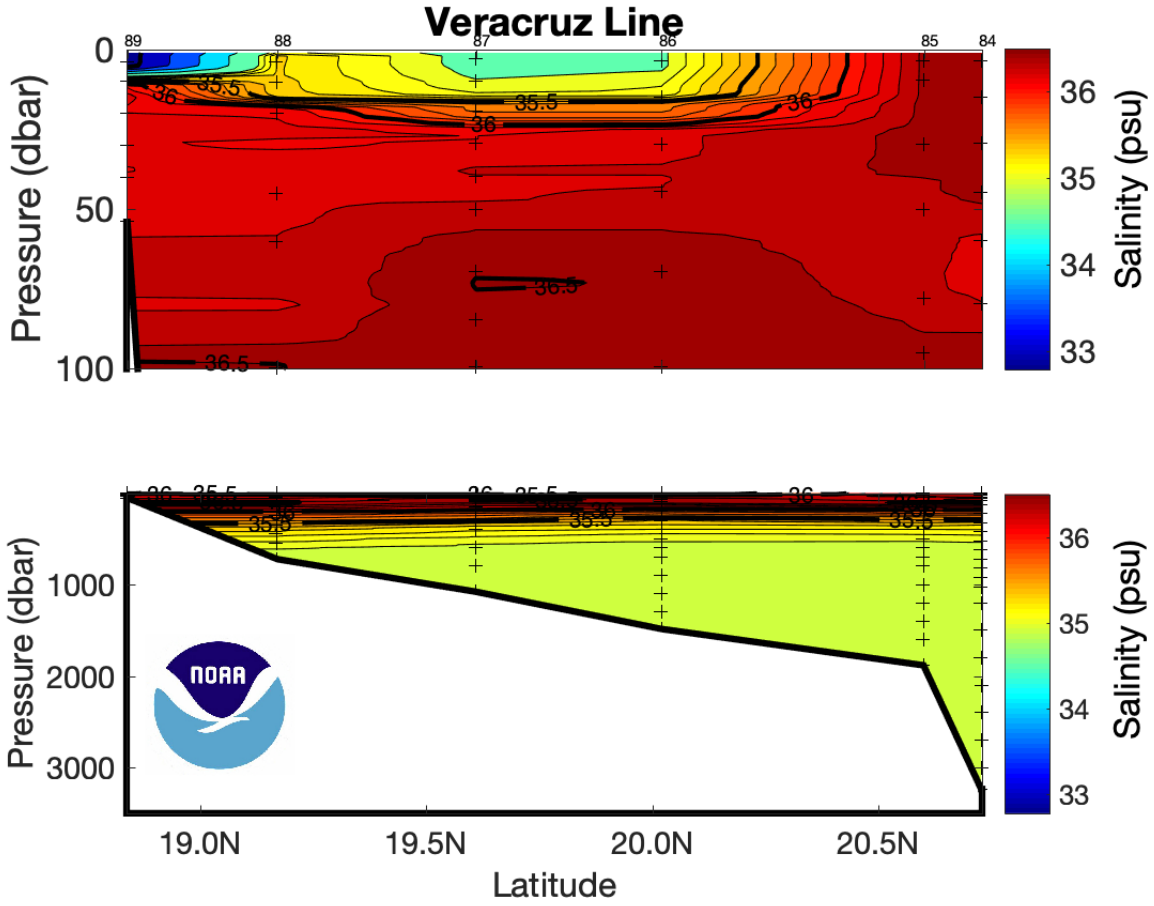

Figure 37: Salinity (PSS 78) for the Veracruz section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

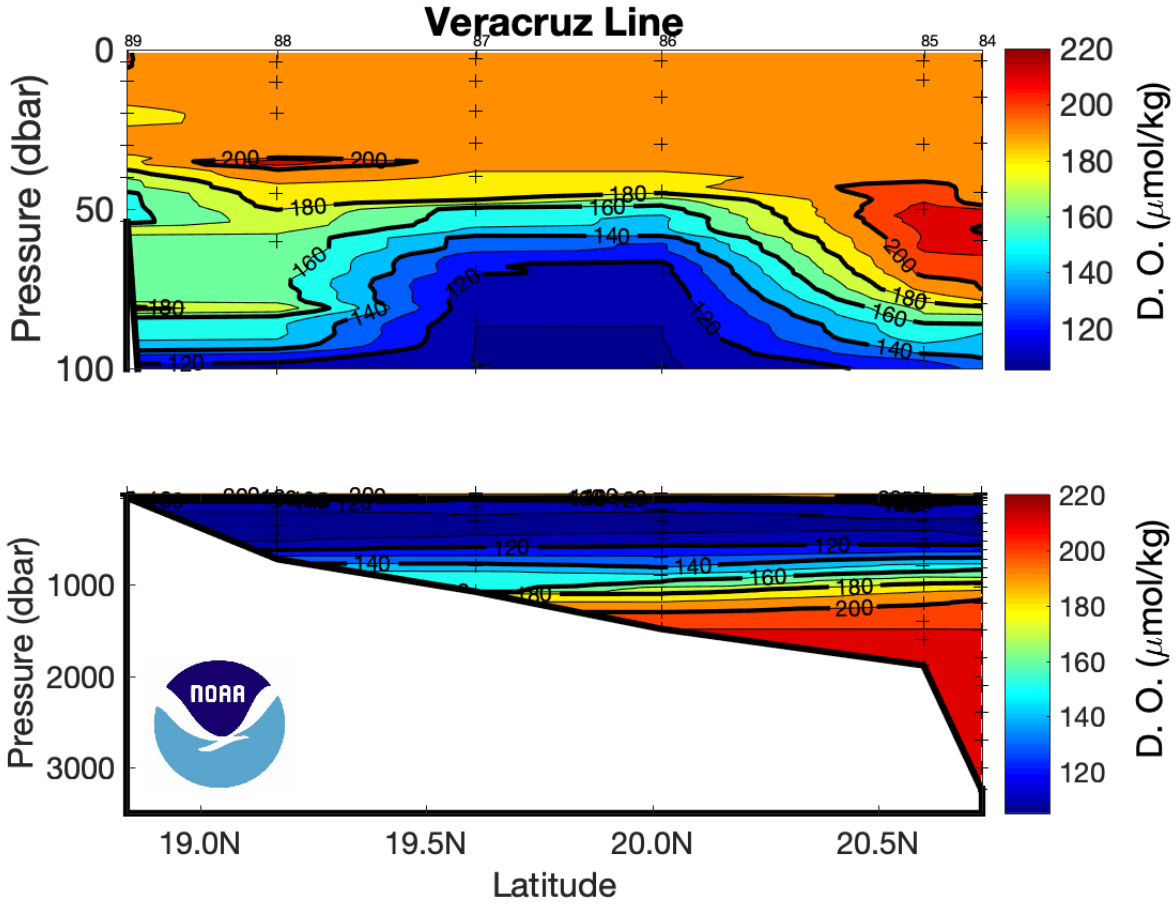

Figure 38: Dissolved oxygen (µmol/kg) for the Veracruz section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

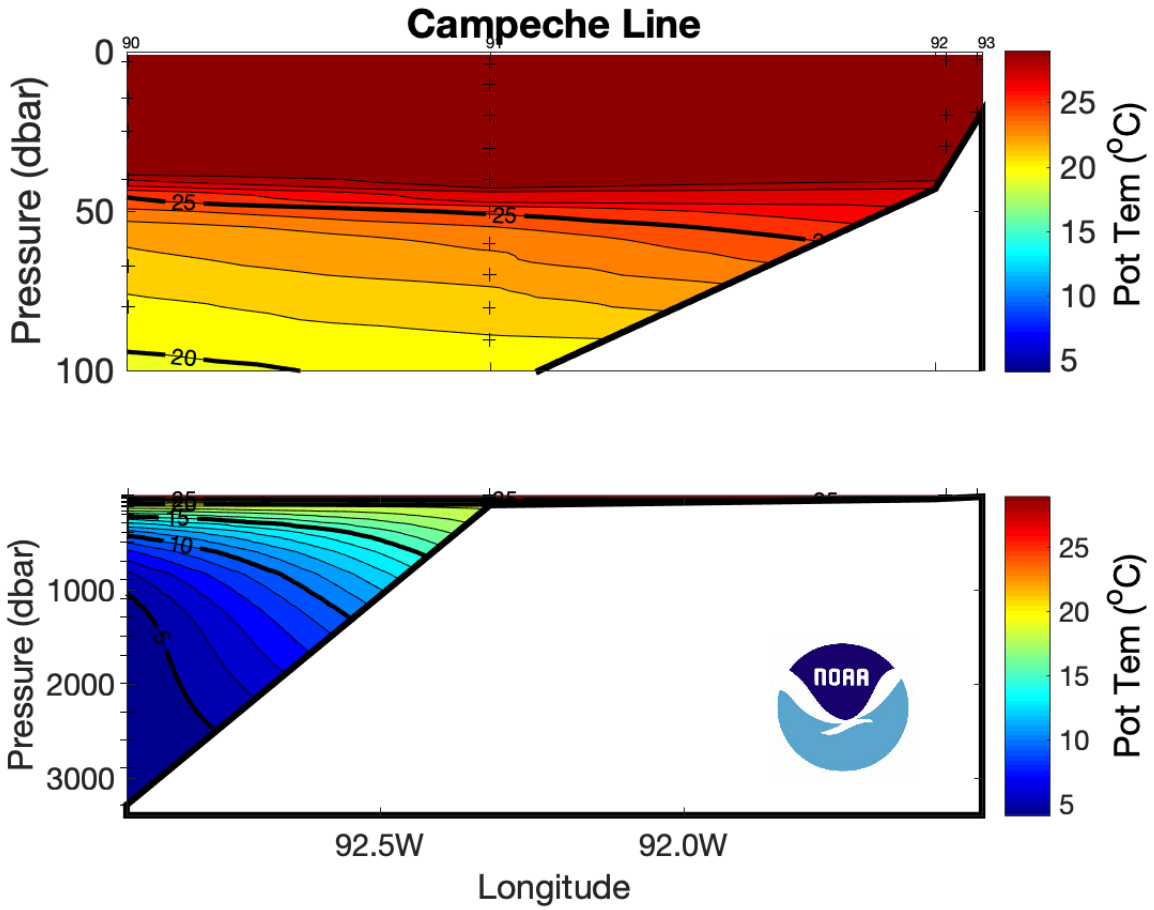

Figure 39: Potential temperature (ºC) for the Campeche section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

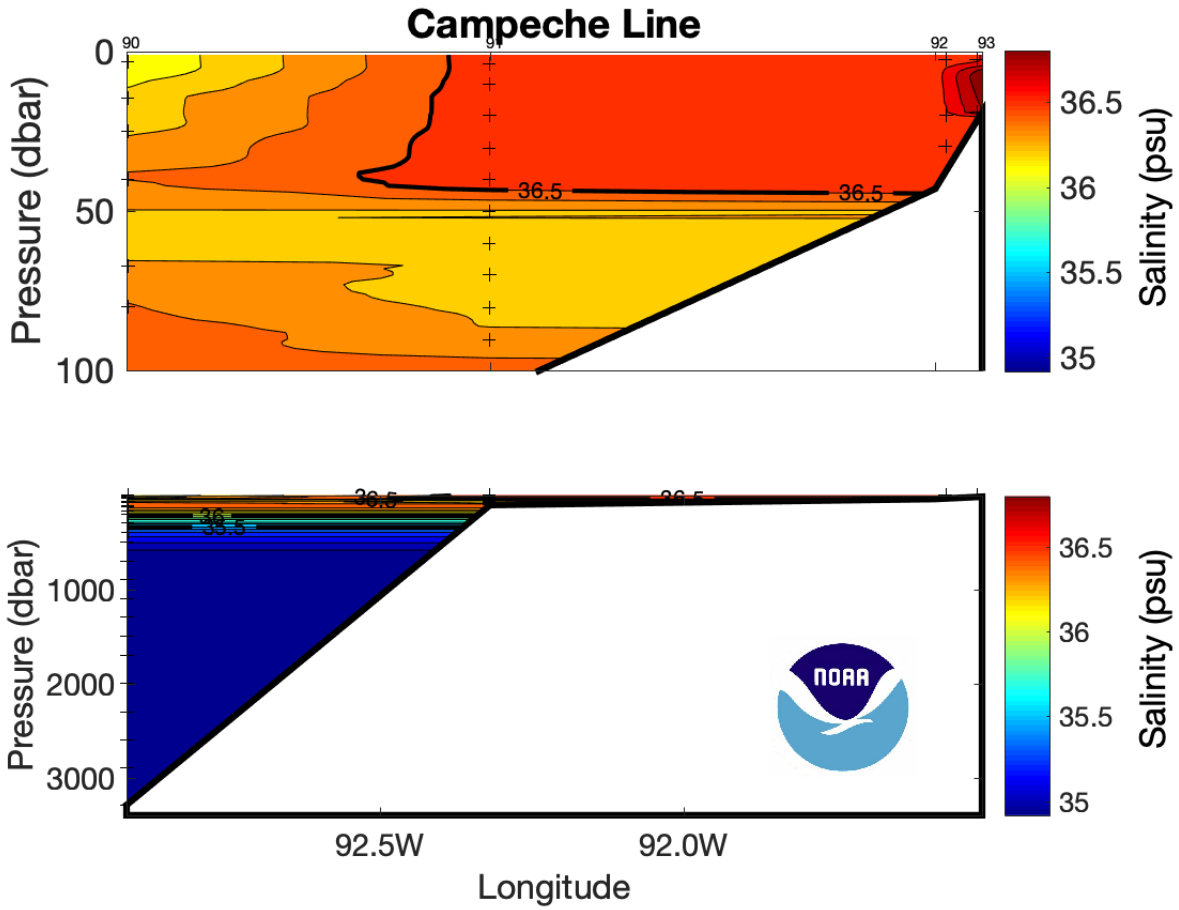

Figure 40: Salinity (PSS 78) for the Campeche section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

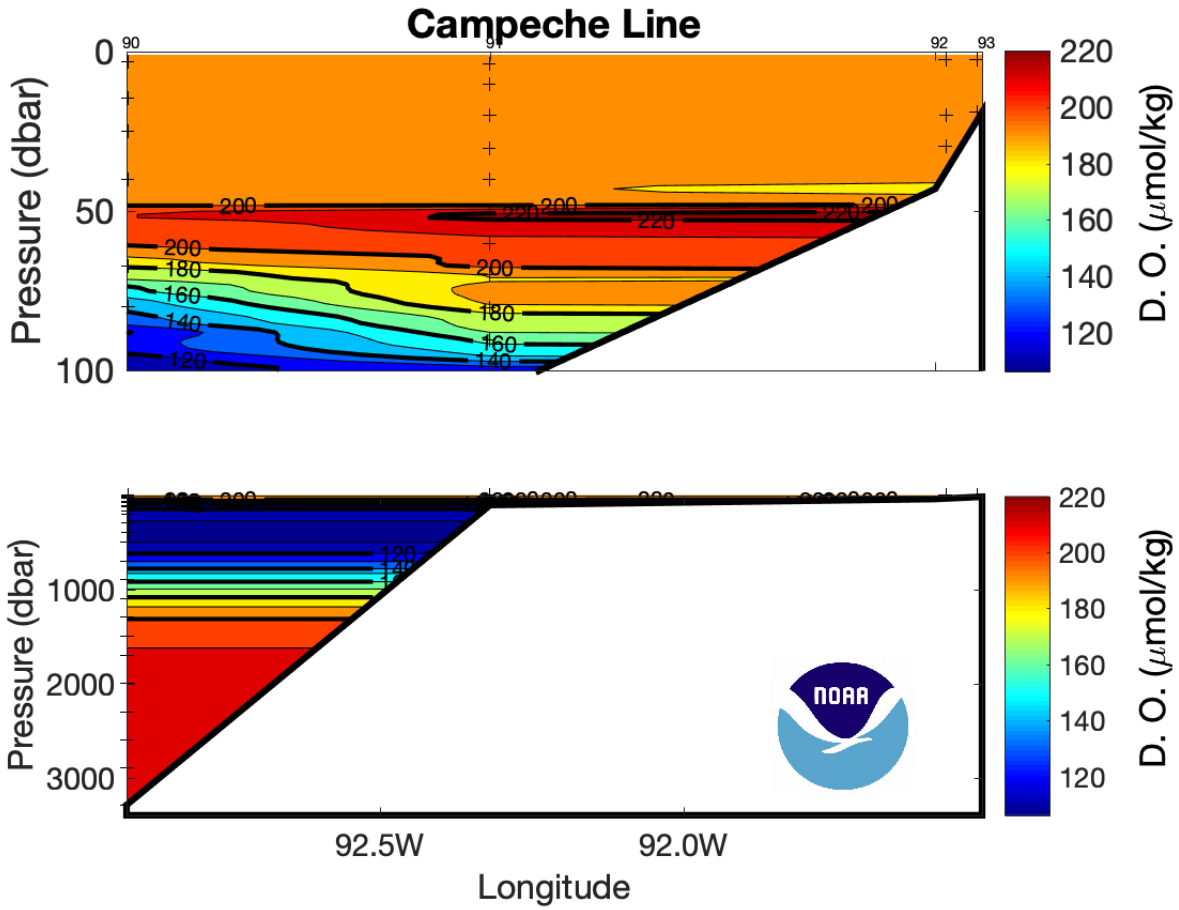

Figure 41: Dissolved oxygen (µmol/kg) for the Campeche section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

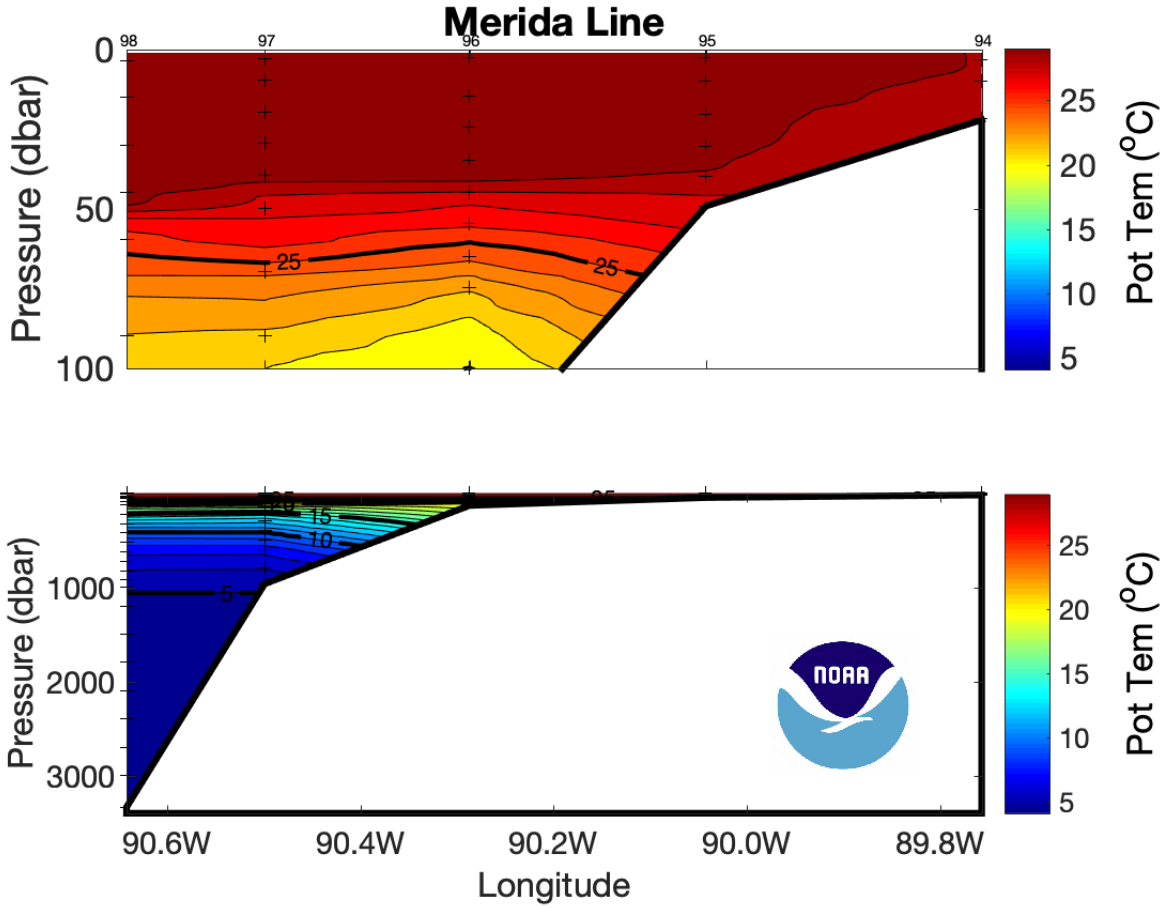

Figure 42: Potential temperature (ºC) for the Merida section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

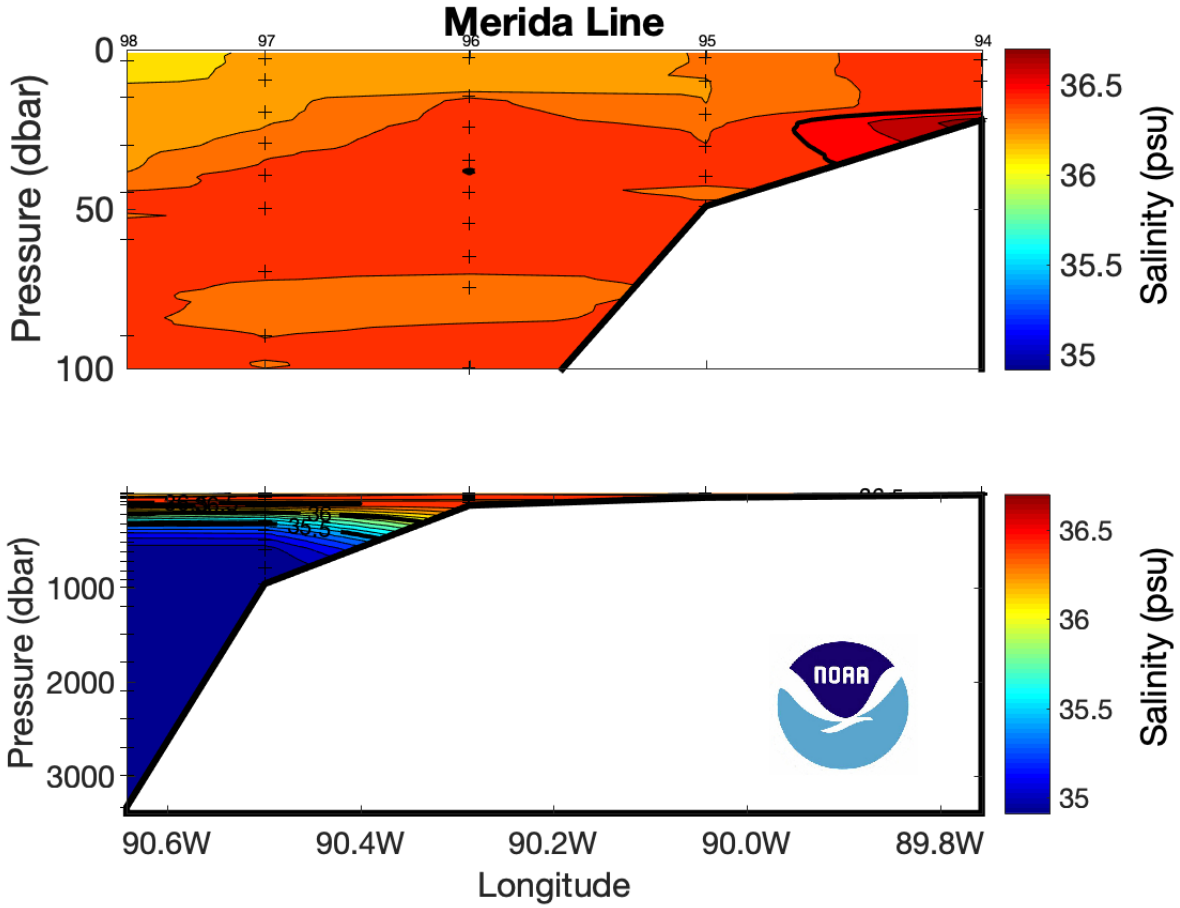

Figure 43: Salinity (PSS 78) for the Merida section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

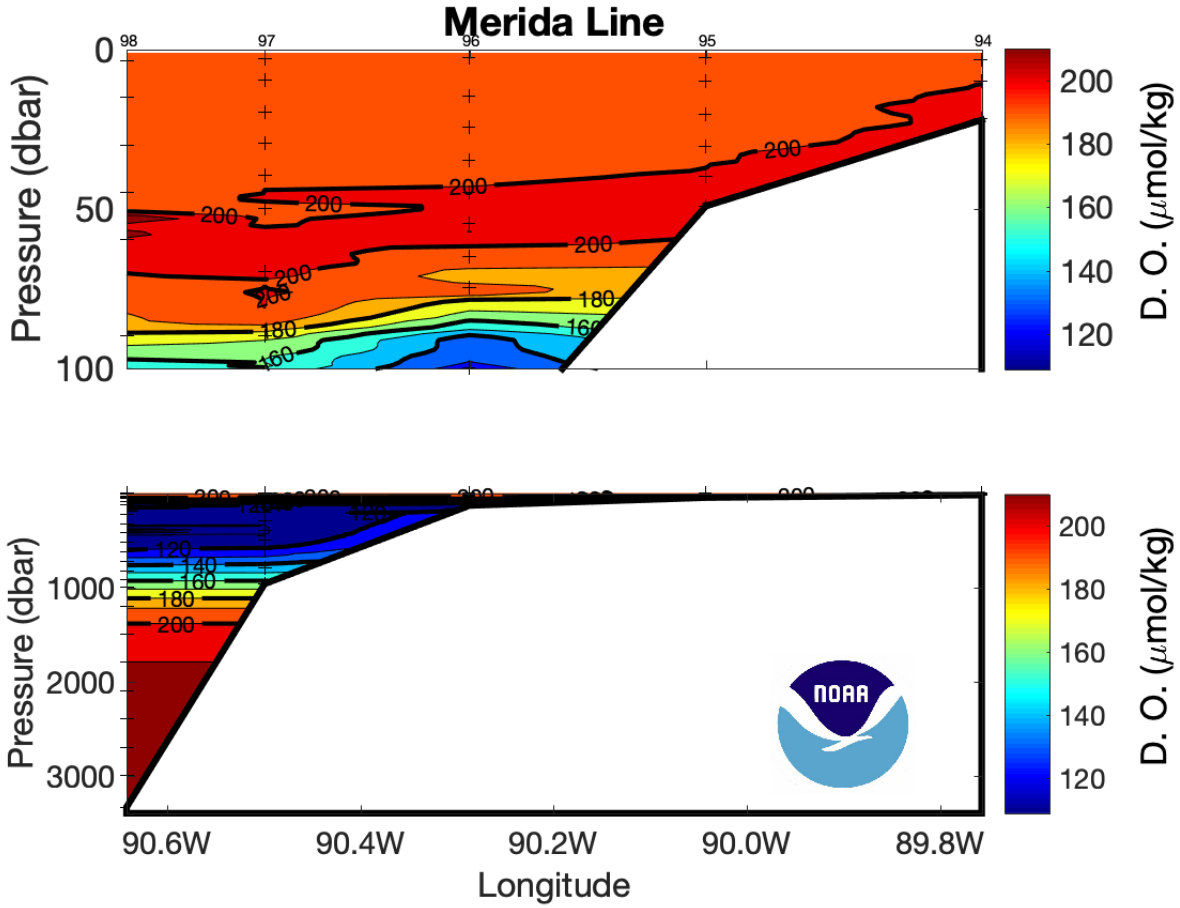

Figure 44: Dissolved oxygen (µmol/kg) for the Merida section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

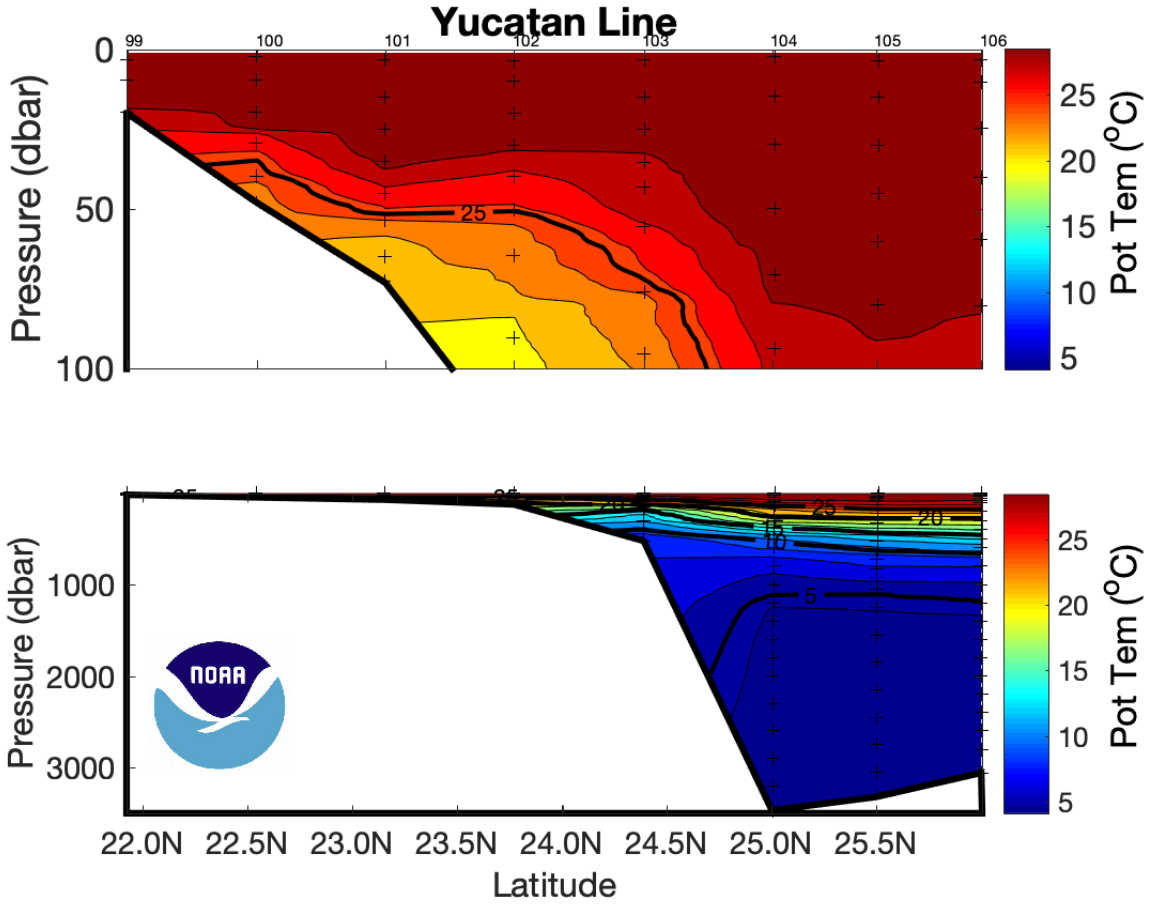

Figure 45: Potential temperature (ºC) for the Yucatan section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

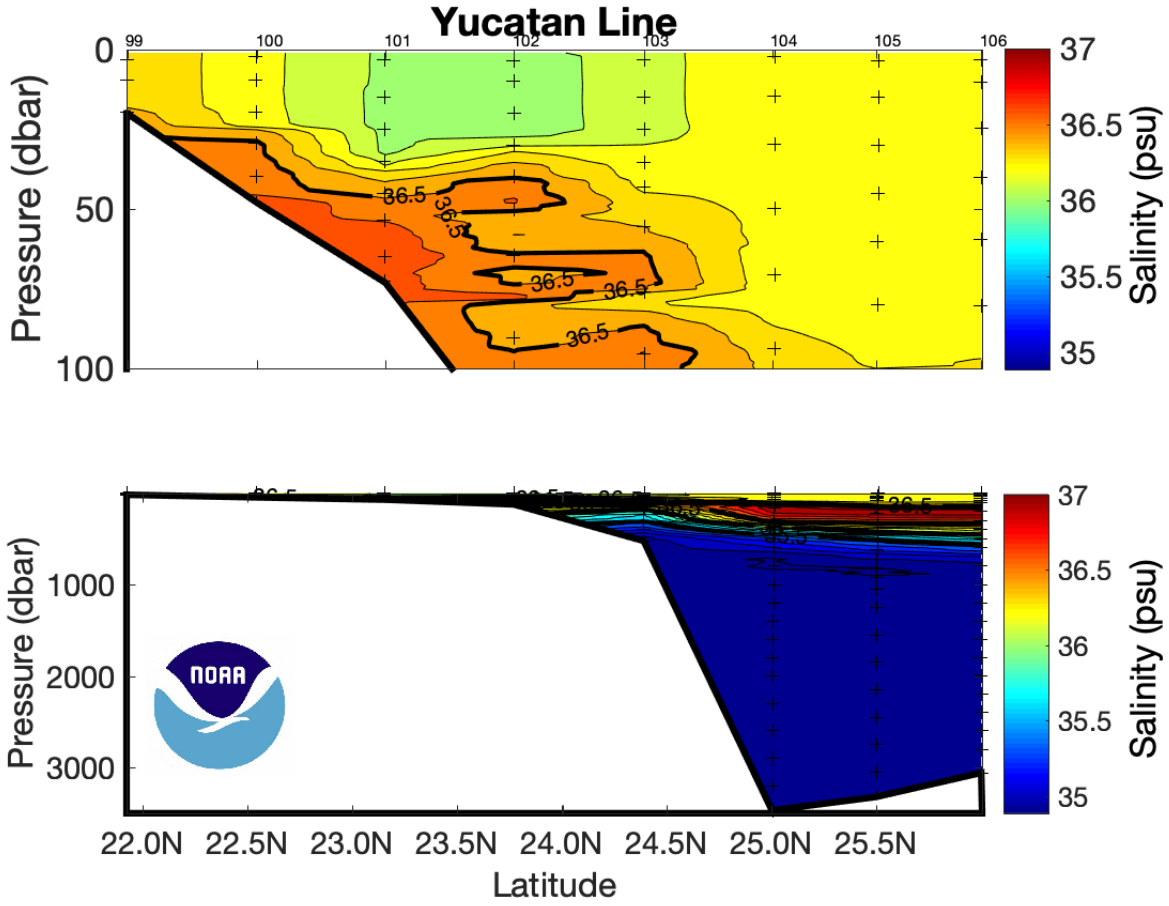

Figure 46: Salinity (PSS 78) for the Yucatan section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

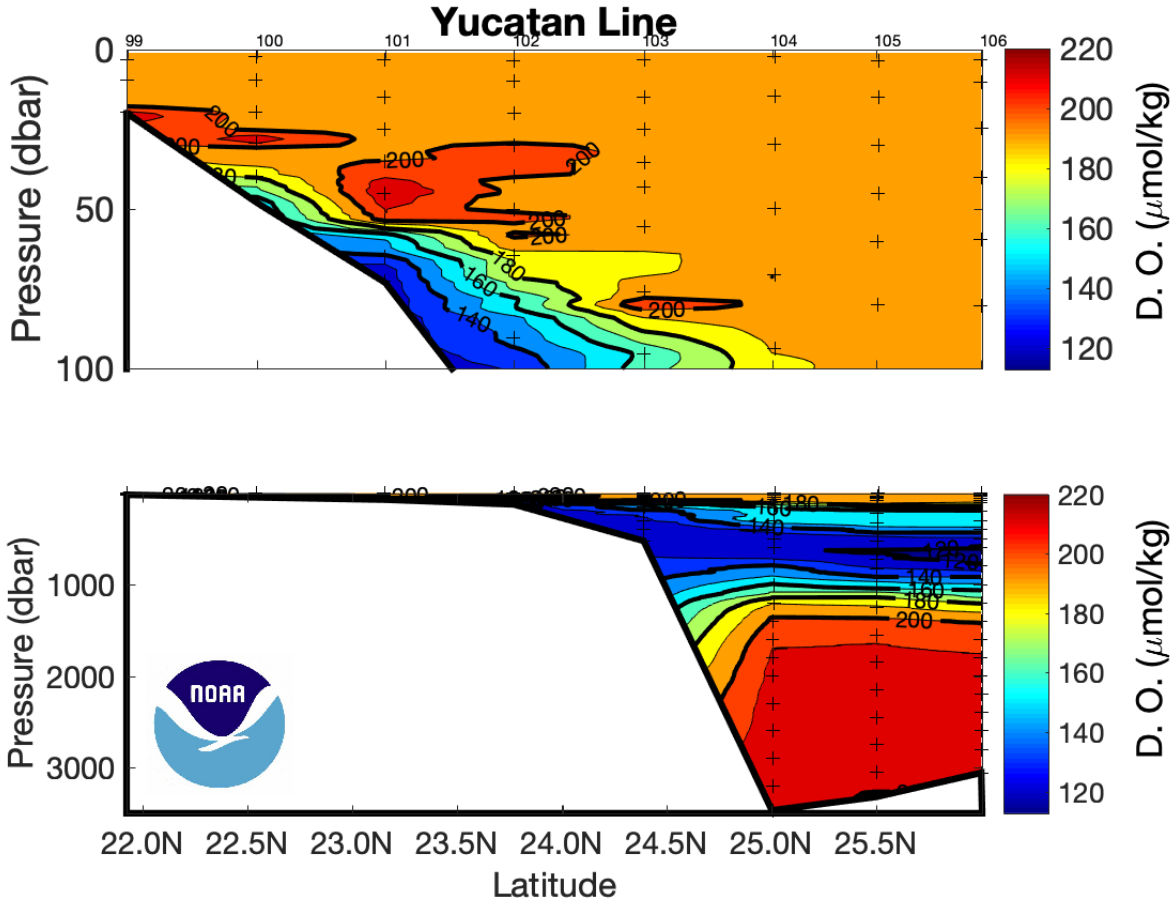

Figure 47: Dissolved oxygen (μmol/kg) for the Yucatan section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

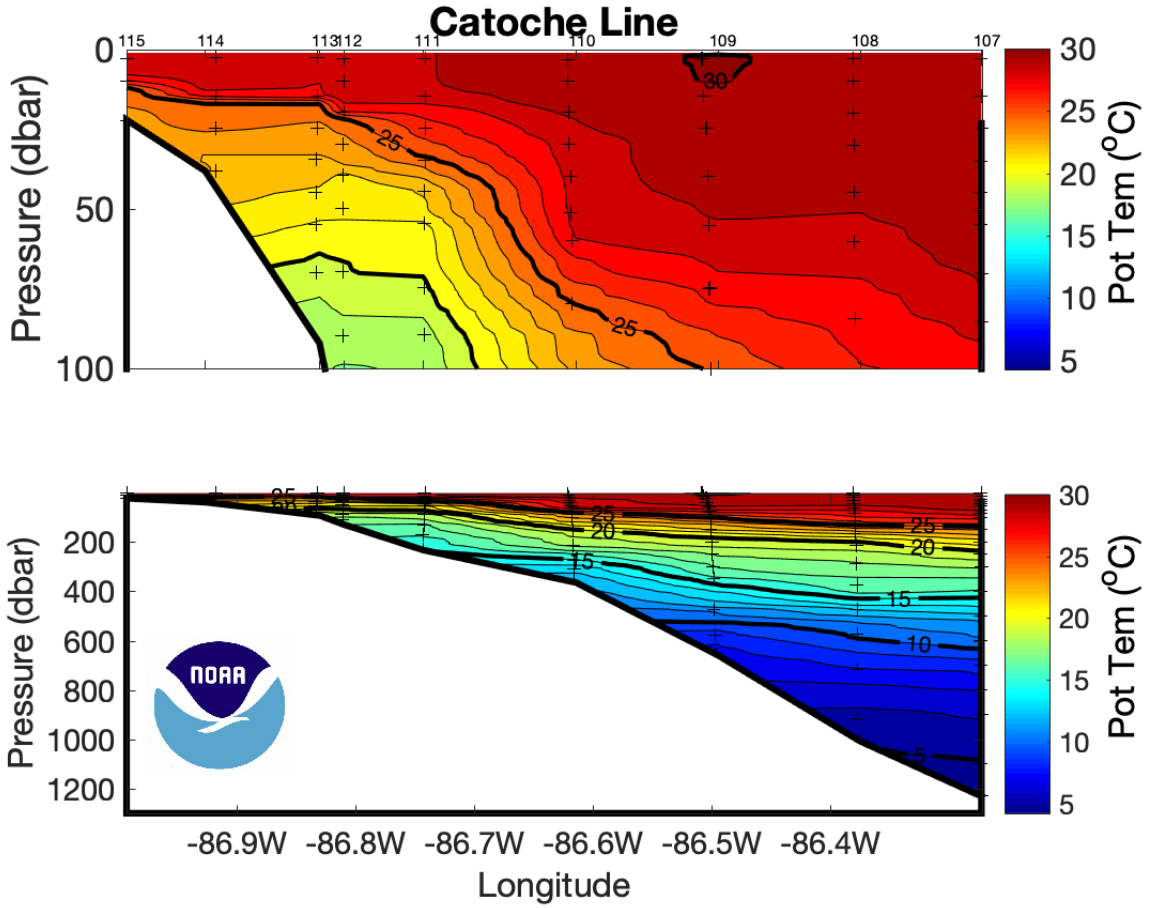

Figure 48: Potential temperature (ºC) for the Catoche section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

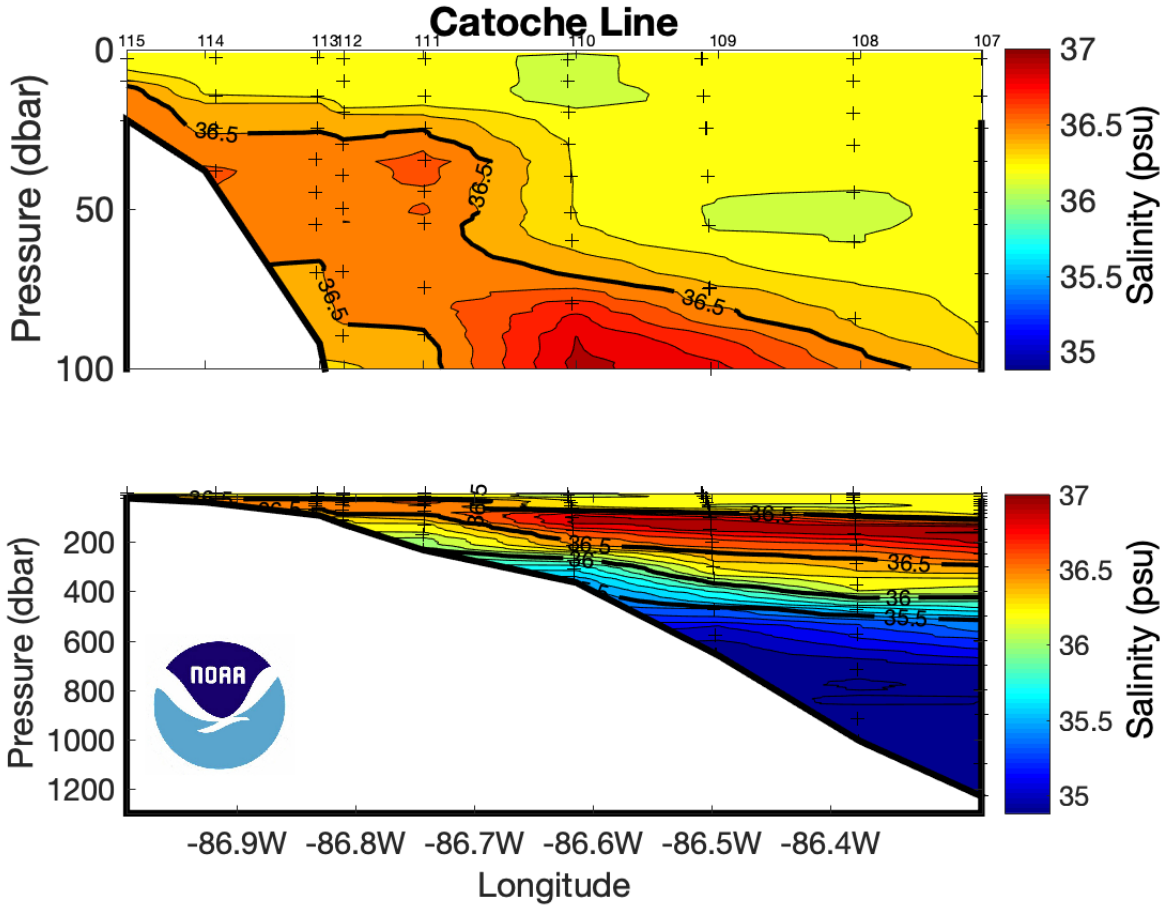

Figure 49: Salinity (PSS 78) for the Catoche section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

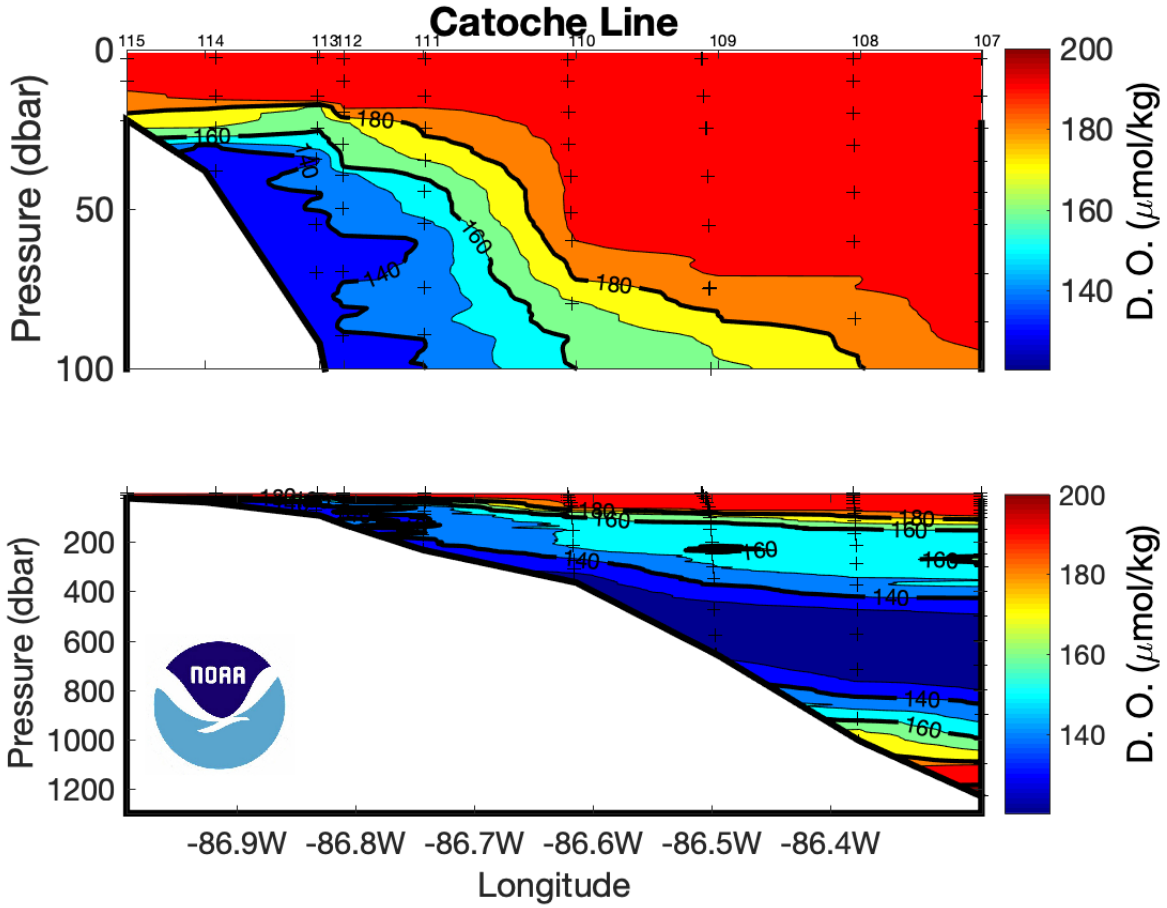

Figure 50: Dissolved oxygen (µmol/kg) for the Catoche section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

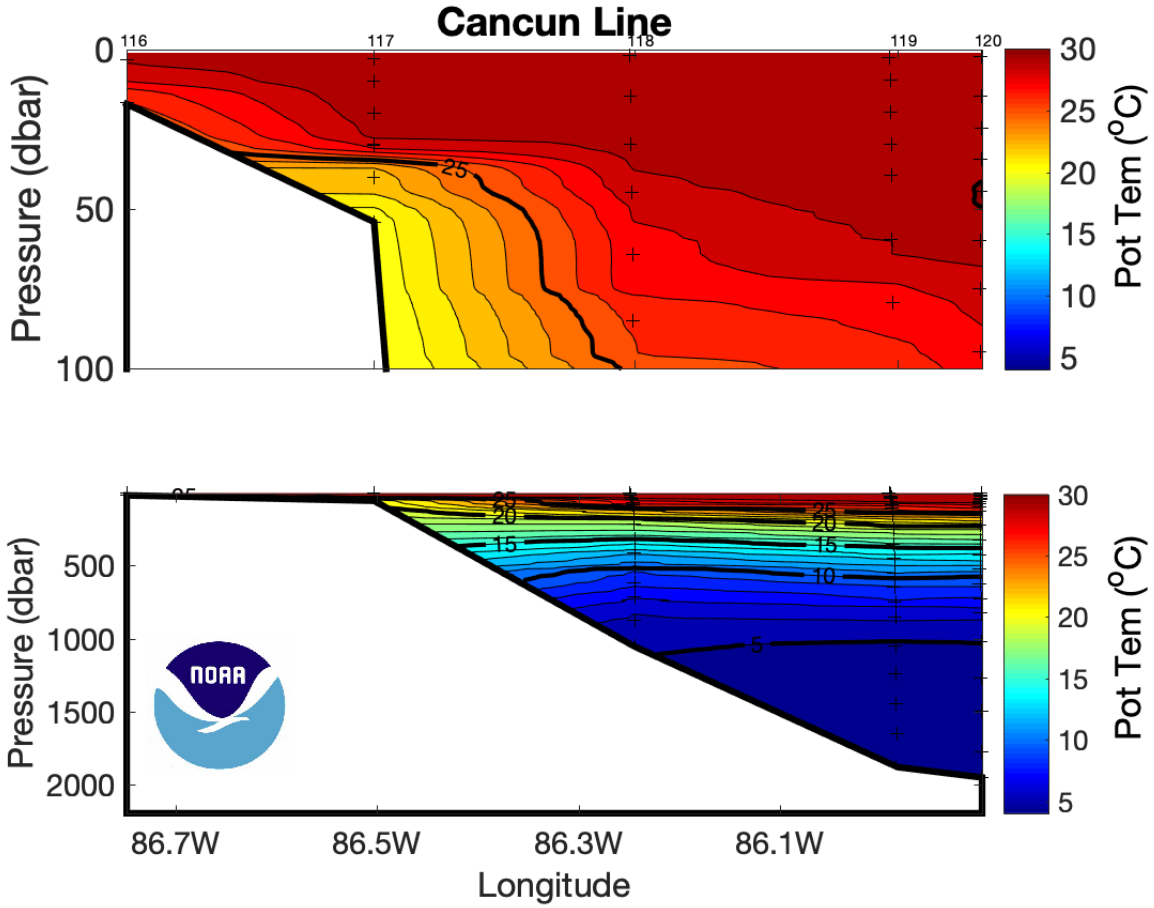

Figure 51: Potential temperature (ºC) for the Cancun section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

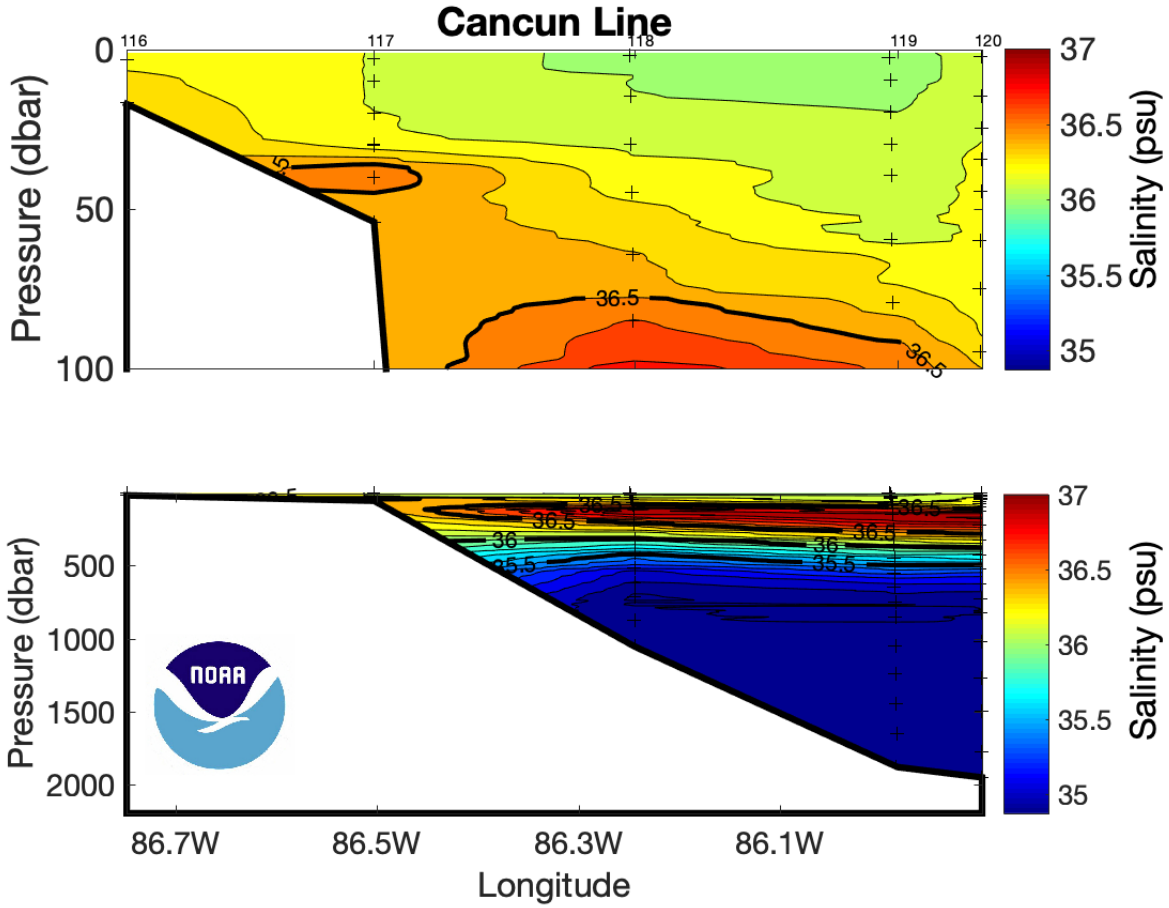

Figure 52: Salinity (PSS 78) for the Cancun section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

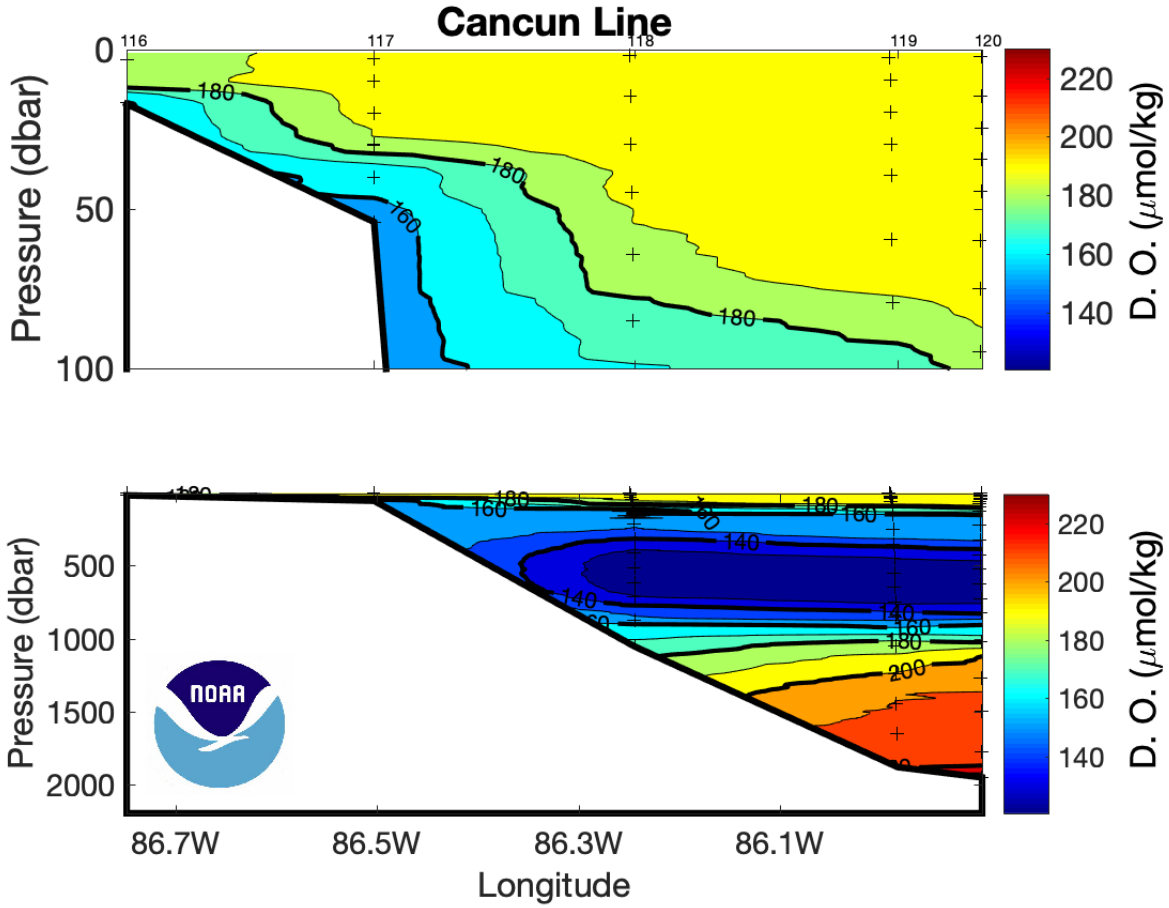

Figure 53: Dissolved oxygen (μmol/kg) for the Cancun section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

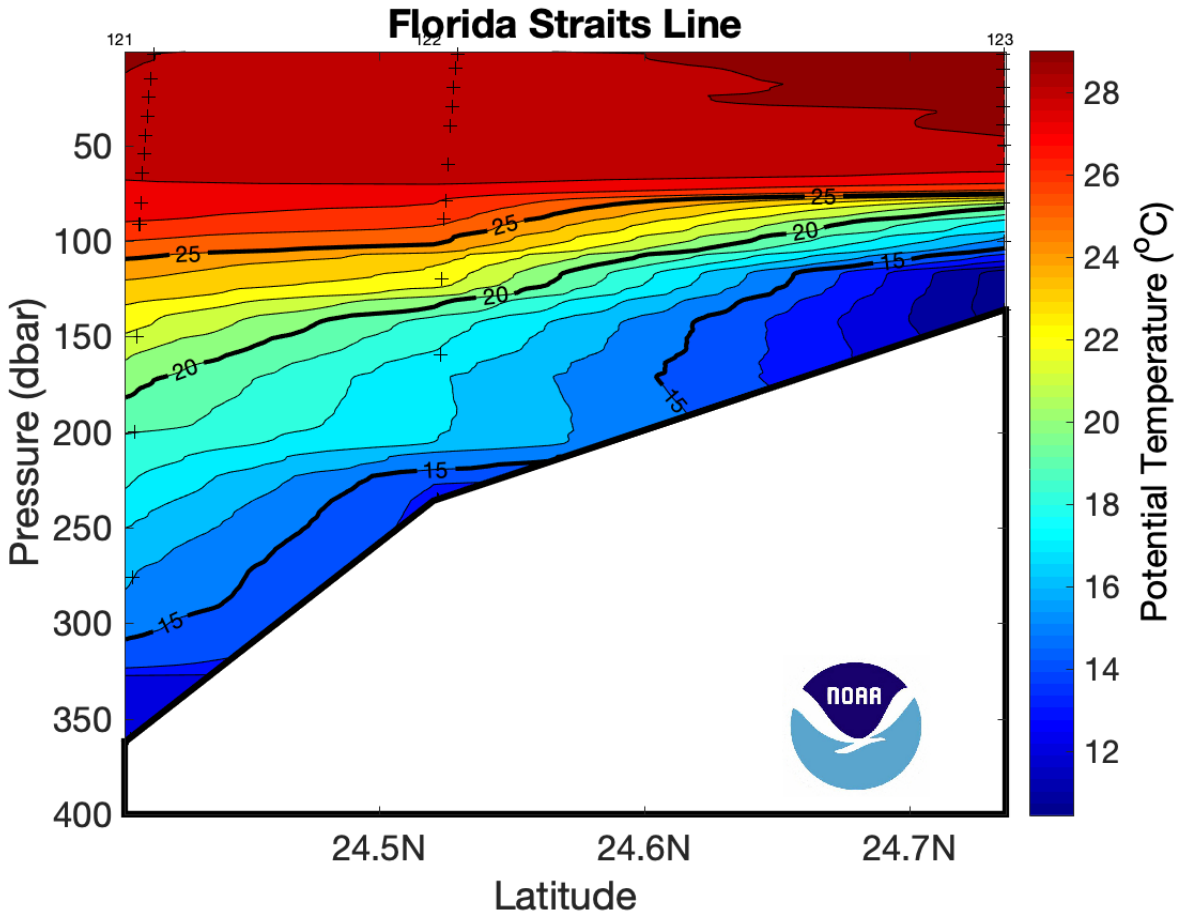

Figure 54: Potential temperature (ºC) for the Florida Straits section. The black crosses represent the bottle trip depths.

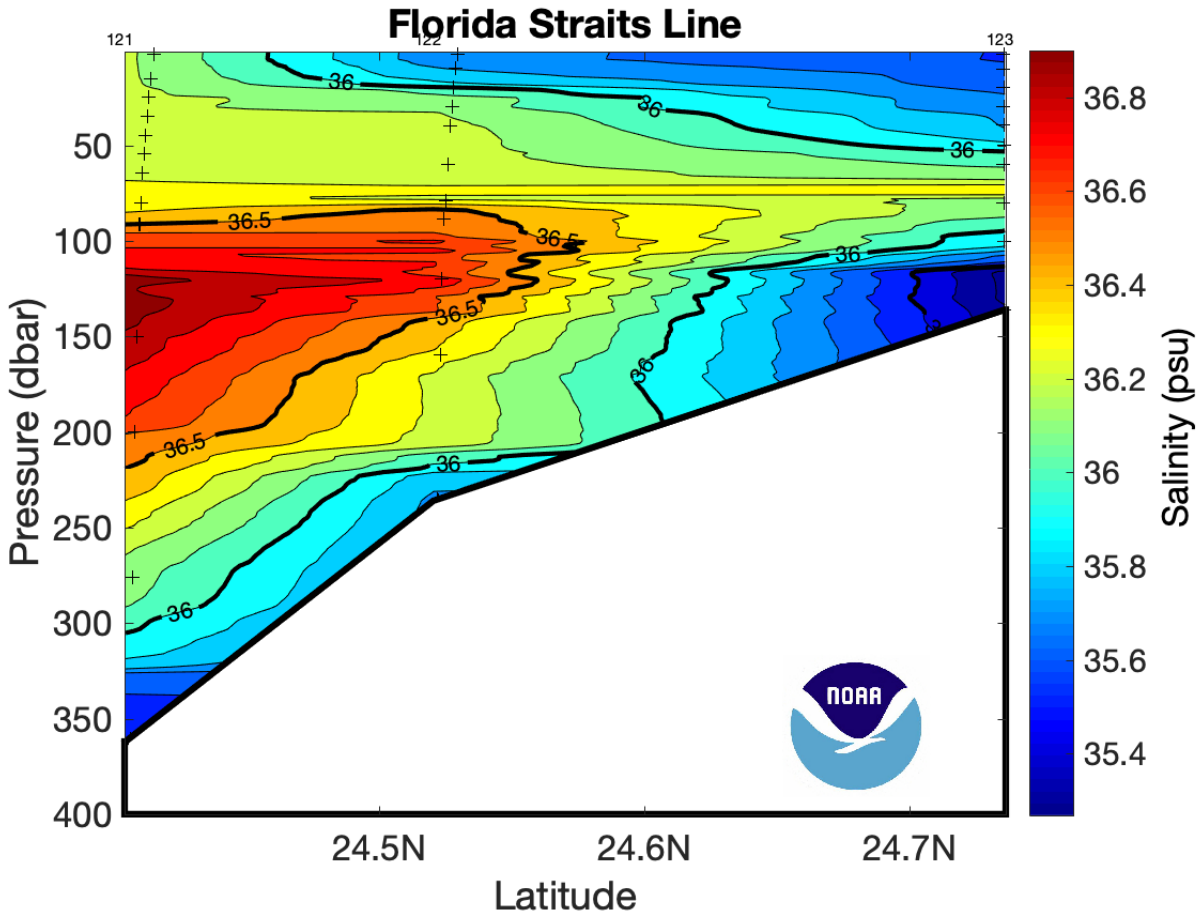

Figure 55: Salinity (PSS 78) for the Florida Straits section. The black crosses represent the bottle trip depths.

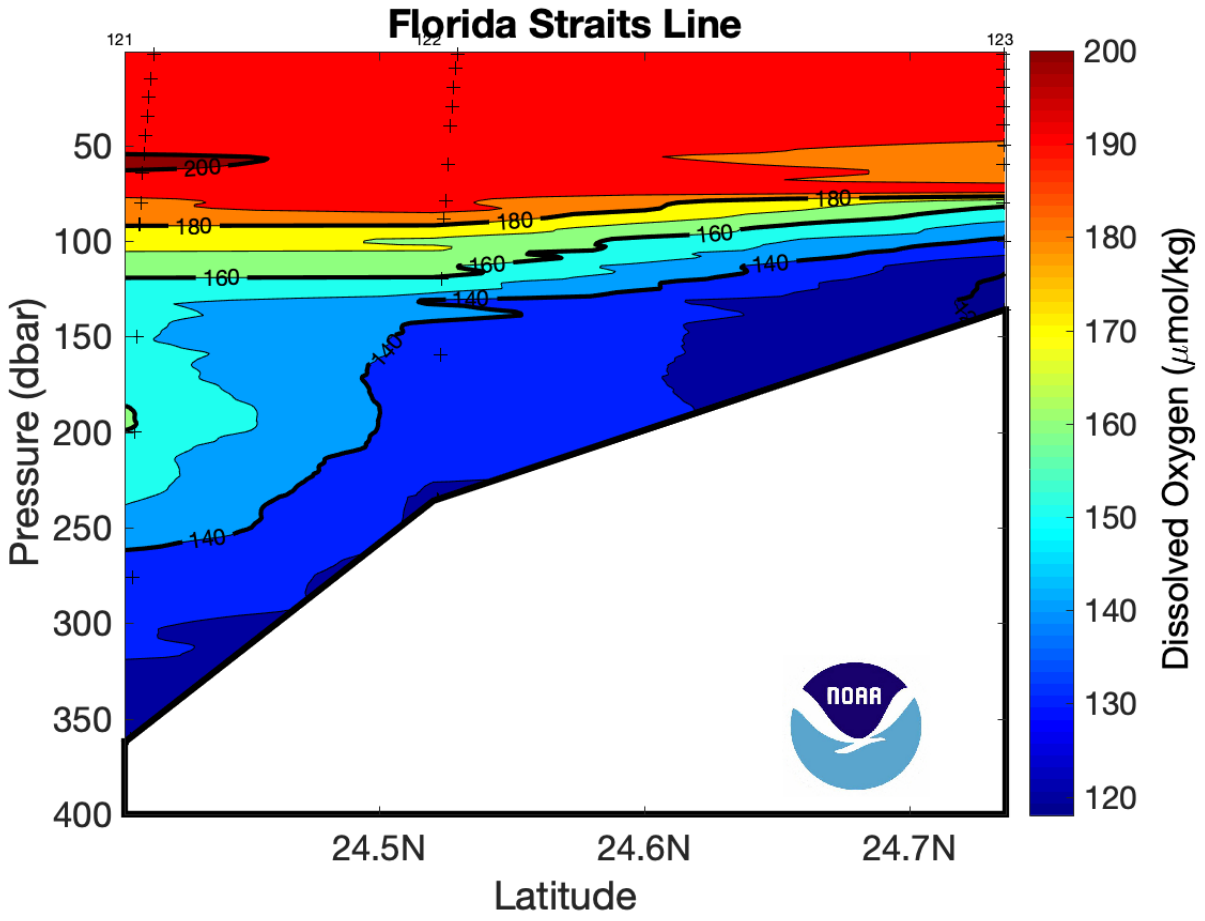

Figure 56: Dissolved oxygen (μmol/kg) for the Florida Straits section. The black crosses represent the bottle trip depths.

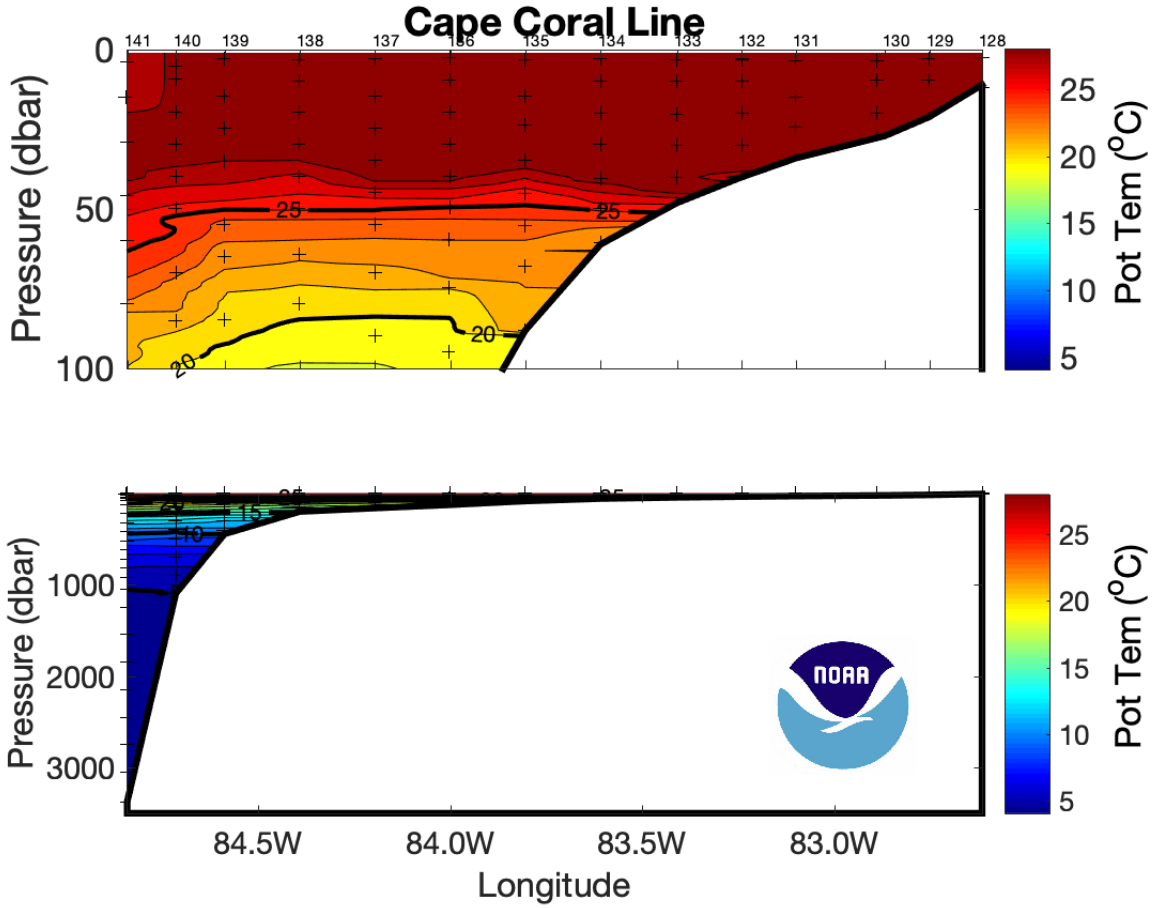

Figure 57: Potential temperature (ºC) for the Cape Coral section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

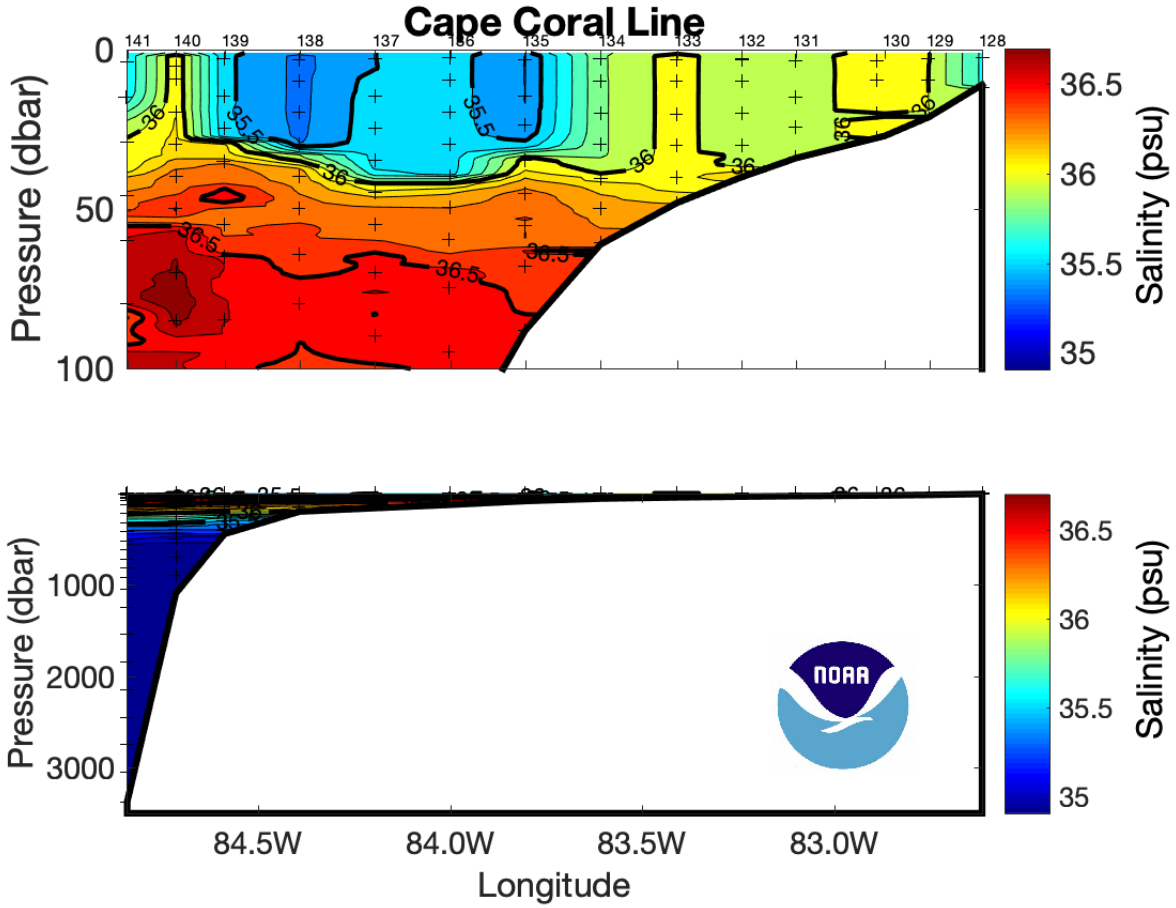

Figure 58: Salinity (PSS 78) for the Cape Coral section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

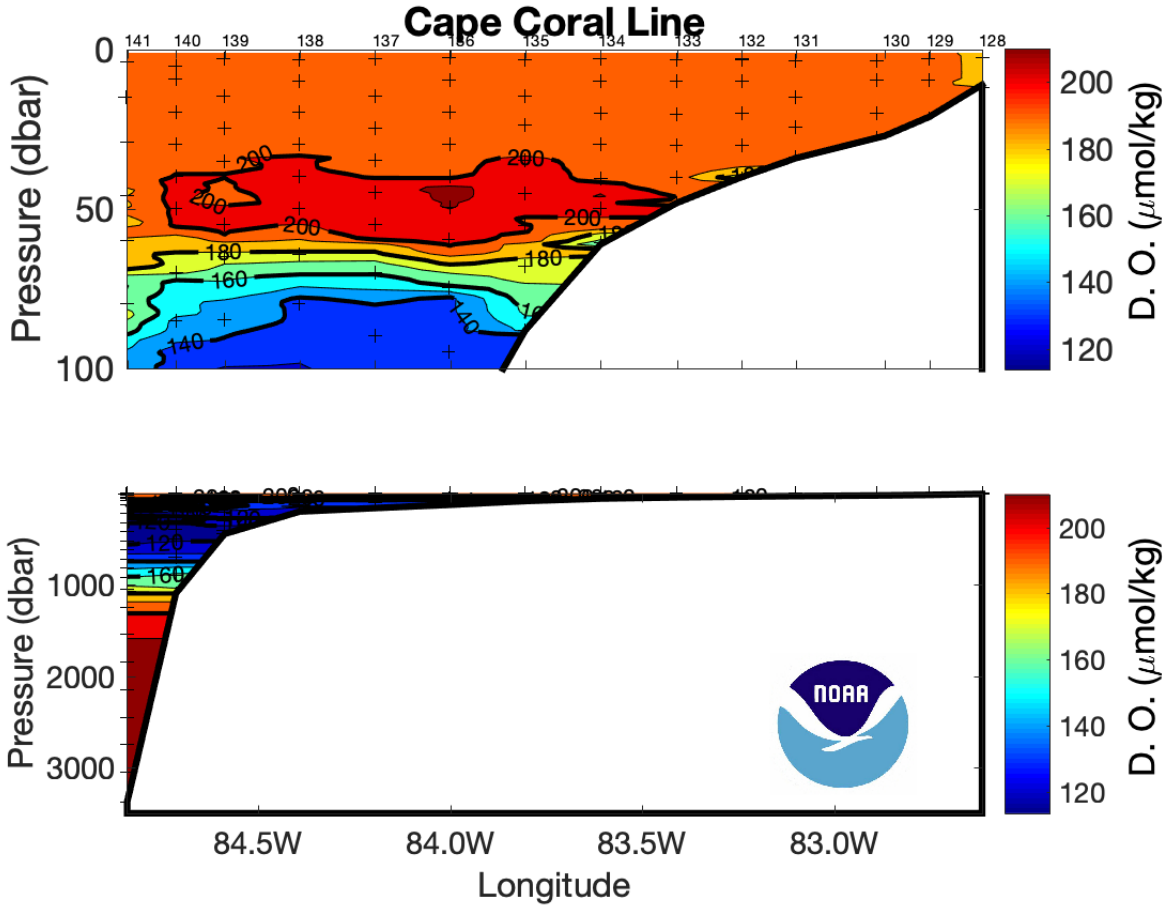

Figure 59: Dissolved oxygen (μmol/kg) for the Cape Coral section. The top figure is the upper 150 dbar of the full depth plot below. The black crosses represent the bottle trip depths.

3.2. Acoustic Doppler Current Profiler Activities

3.2.1. Shipboard Acoustic Doppler Current Profiler (SADCP)

During the GOMECC-4 survey, the NOAA Ship *Ronald H. Brown* was equipped with a hull-mounted (or shipboard) Teledyne RD-Instruments (TRDI) 75 kHz Ocean Surveyor (OS75) acoustic Doppler current profiler (SADCP). The OS75 SADCP provided reliable coverage of upper-ocean current velocities to a depth of approximately 750 m during the cruise. In addition to a primary heading source provided by the ship's gyrocompass, the SADCP was also equipped with a secondary heading input from an Applanix POS MV directional GPS (which also provided position information to the instrument). The addition of a secondary GPS-based heading device like the POS MV allowed for improved heading accuracy when the ship was accelerating (which can impart Shuler Oscillations in the output from a traditional mechanical gyrocompass), as well as when trying to account for long-period drift over the course of an entire cruise.

SADCP data were collected using the University of Hawaii's UHDAS software package (UHDAS: University of Hawaii Data Acquisition System). The software's configuration allowed

for collection of alternating narrowband (greater depth range) and broadband (greater resolution) data. Broadband data were collected with a 4-m bin length, while narrowband data were collected with a 16-m bin length. Broadband data coverage typically extended into the water column to a depth range of 270-400 m (depending on the speed of the vessel), while narrowband data were typically collected to a depth of ~750 m (previously mentioned). The nature of the SADCP installation: including the hull depth of the transducer, the blanking distance required by the instrument (8 meters), and the bin length, results in a data gap at the surface. In some cases, this gap can be greater than the water depth in nearshore survey work, resulting in a loss of data. By collecting broadband data in addition to the narrowband data, scientists were able to collect more usable SADCP data on the shallower legs of the survey than would otherwise have been possible collecting narrowband data alone.

Following the cruise, the GOMECC-4 SADCP data set will be post-processed using the University of Hawaii's CODAS software package (CODAS: Common Ocean Data Access System). CODAS is the industry standard for producing the highest quality SADCP data set possible.

3.2.2 Lowered Acoustic Doppler Current Profiler (LADCP)

Dual, upward-facing and downward-facing, TRDI 300 kHz Workhorse (WH300) acoustic Doppler current profilers (ADCPs) were incorporated into the CTD package used during the GOMECC-4 survey. These *lowered* ADCPs (LADCP) were battery-powered and logged velocity data internally during each CTD cast. Following each cast, data were recovered from the instruments manually using a direct cable connection to an LADCP processing computer onboard the ship.

Data collected from the LADCPs were processed using v10.20 of the *Visbeck* MATLAB routines originally developed by Martin Visbeck while at Lamont-Doherty Earth Observatory (LDEO) and now maintained by Gerd Krahmann at the Helmholtz Center for Ocean Research in Kiel, Germany (part of IMF-GEOMAR). The Visbeck software suite incorporates concurrent GPS position data, as well as supplementary pressure, temperature, and salinity data from the CTD, and SADCP velocity data for the upper ocean, into the LADCP processing to produce a final LADCP ocean velocity profile for the entire depth of the cast. This method for processing LADCP data is considered the best technique for producing the most accurate LADCP velocity profiles possible. Final ocean velocity profiles were generated, at a resolution of 10 m, for each of the CTD casts conducted during the GOMECC-4 research cruise.

3.3. Discrete Salinity Sampling

A single Guildline Autosal, model 8400B (s/n 60555), located in salinity analysis room, was used for all salinity measurements. The salinometer readings were logged on a computer using Ocean Scientific International's logging hardware and software. The Autosal's water bath temperature was set to 24°C, which the Autosal is designed to automatically maintain. The laboratory's temperature is typically set and maintained to just below 24°C, to help further stabilize reading values and improve accuracy. The room temperature was monitored by a digital thermometer. The temperature was used to gauge when the Autosal room temperature was acceptable to run salts. Salinity analyses were performed after samples had equilibrated to

laboratory temperature, usually at least 12 hours after collection. The salinometer was standardized for each group of samples analyzed (usually 2 casts and up to 52 samples) using two bottles of standard seawater: one at the beginning and end of each set of measurements. The salinometer output was logged to a computer file. The software prompted the analyst to flush the instrument's cell and change samples when appropriate. Prior to each run a sub-standard flush, approximately 200 ml, of the conductivity cell was conducted to flush out the DI water used in between runs. For each calibration standard, the salinometer cell was initially flushed 6 times before a set of conductivity ratio reading was taken. For each sample, the salinometer cell was initially flushed at least 3 times before a set of conductivity ratio readings were taken.

IAPSO Standard Seawater Batch P-163, Exp. April 10, 2022, K15=0.99985, salinity: 34.994 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. Laboratory temperature was also monitored electronically throughout the cruise. 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. During GOMECC-4, 1773 salinity measurements were taken, including 88 duplicates, and approximately 70 vials of standard seawater (SSW) were used. Up to two duplicate samples were drawn, primarily for the deep casts (>1000 m), to determine total analytical precision.

The running standard calibration values are shown in Figure 60. Throughout the course of the cruise, the autosal standards had a range of approximately 0.005 in conductivity ratio (about 0.01 in salinity). The duplicates for the bottle salinity had a median of 0.0003 psu +/- 0.006 psu and can be seen in Figure 61.

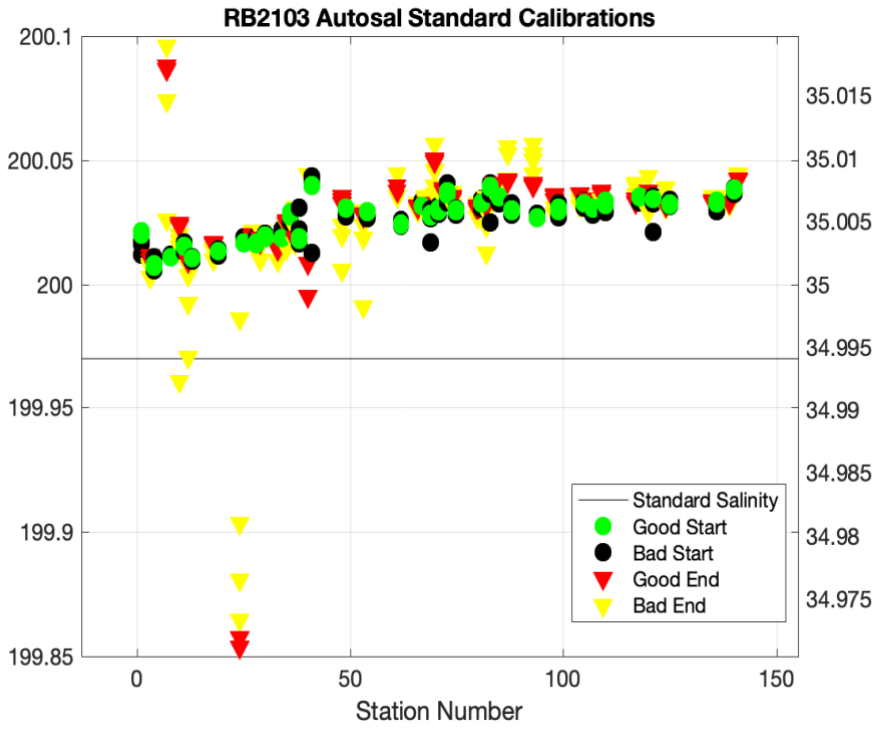

Figure 60: Standard vial calibrations throughout the cruise.

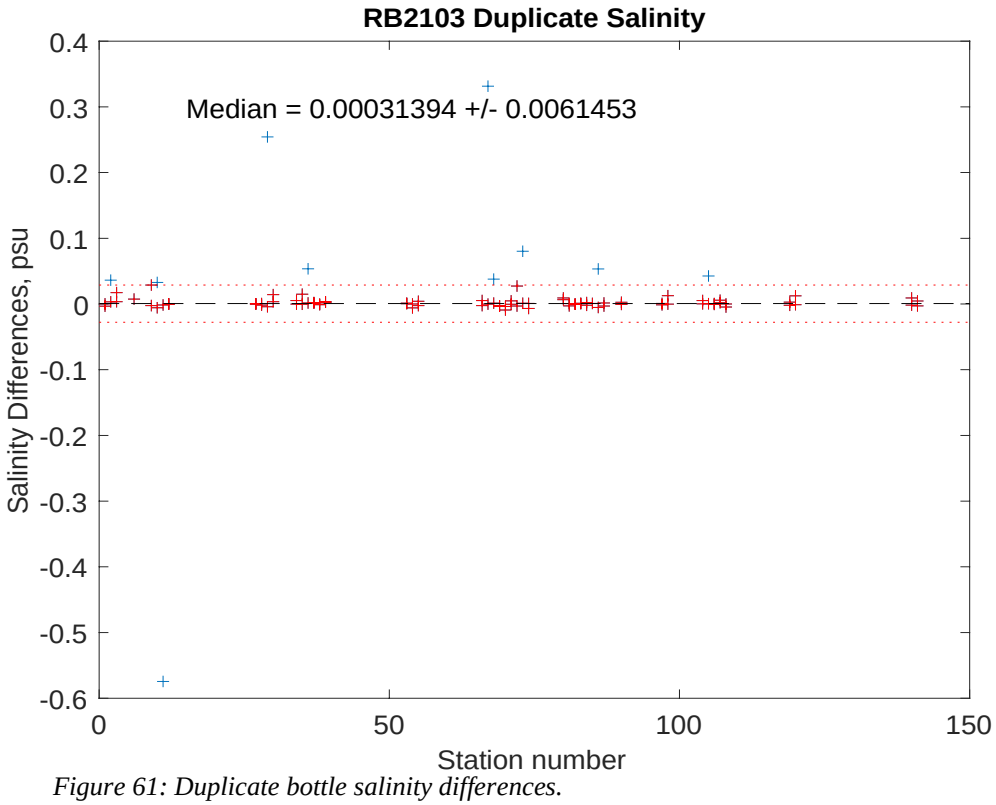

Figure 61: Duplicate bottle salinity differences.

3.4. Dissolved Oxygen Measurements

3.4.1. Equipment and Techniques

Analysts on board:: Mia Andrew-Nandllal, Willem Weinberger
Science lead: Chris Langdon

A total of 141 stations were occupied and 1880 discrete oxygen analyses were performed. One set of duplicates were done at each station.

A total of 1445 analyses were assigned a QF of 2 and 181 analyses were assigned a QF of 3.

A regression of Winkler O_2 against CTD O_2 (Figure 62) yielded an intercept of 0.08 and a slope of 0.9997 and a RMSE of 1.7 umol/kg indicating good agreement between the CTD O_2 sensor and the discrete Winkler analyses .

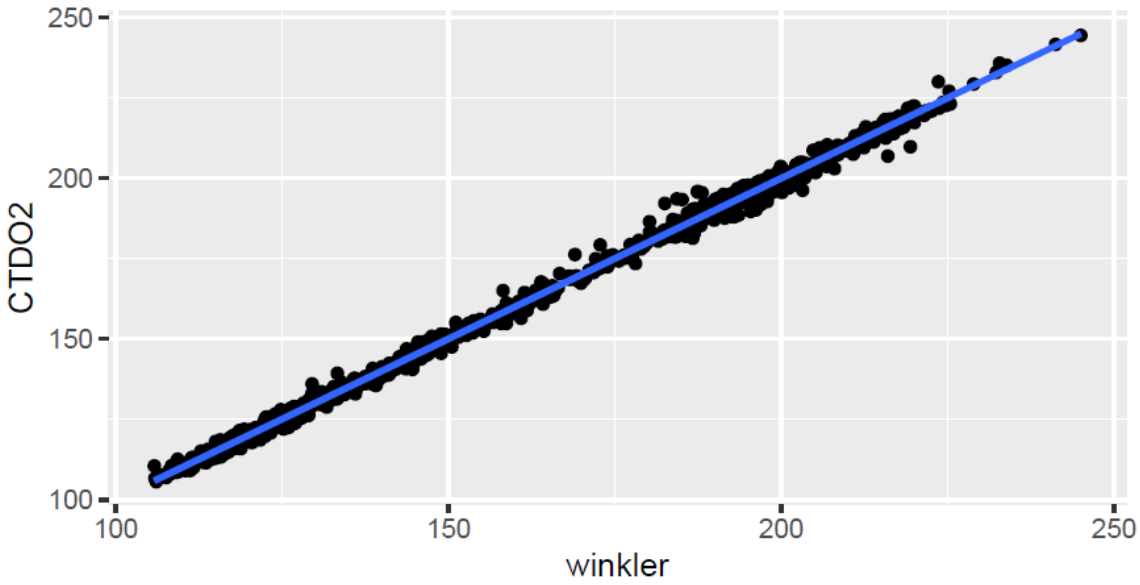

Figure 62: Regression of Winkler O_2 titrations against CTD O_2 measurements during the GOMECC-4 cruise.

Samples were drawn from all casts and all Niskin bottles into volumetrically calibrated 125 ml iodine titration flasks using Tygon tubing with a silicone adaptor that fit over the petcock to avoid contamination. Bottles were rinsed three times and filled from the bottom, overflowing three volumes while taking care not to entrain any bubbles. The draw temperature was taken using a digital thermometer with a flexible thermistor probe that was inserted into the flask while the sample was being drawn during the overflow period. These temperatures were used to calculate µmol kg-1 concentrations, and a diagnostic check of Niskin bottle integrity. One-ml of MnCl2 and one-ml of NaOH/NaI were added immediately after drawing the sample was concluded using a Repipetor, the flasks were then stoppered and shaken well. DIW was added to the neck of each flask to create a water seal. The flasks were stored in the lab in plastic totes at room temperature for at least 1 hour before analysis. Twenty-four samples plus duplicates were drawn from each

station except the shallow costal stations where fewer samples were drawn depending on the depth or as directed by the chief scientist. Total number of hydrocast samples collected was 1880. A total of 140 sets of duplicates were run. The preliminary median difference between replicates was 0.3 umol kg-1 for stations 1-30. The total number of samples flagged after initial shipboard reduction of quality control: Questionable (QF=3, 1445): Not reported (QC=9, 1758).

Dissolved oxygen analyses were performed with an automated oxygen titrator using amperometric end-point detection (Langdon 2010). The titration of the samples and the data logging and graphical display was performed on a PC running a LabView program written by Ulises Rivero of AOML. The titrations were performed in a climate controlled lab at 18.5°C-20°C. Thiosulfate was dispensed by a 2 ml Gilmont 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. Four to three replicate 10 ml iodate standards were run xx times during the cruise. The reagent blank determined 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 and end of the cruise.

3.4.2. Issues

A set of standards with a standard deviation of over 20 was used to run several samples before the mistake was identified. Corrections were done to those samples after the cruise so they could be used. The case containing the samples collected for station 57 was not run in time and the DIW at the neck of the flasks evaporated, rendering those samples useless. A case of discrete underway samples was likewise forgotten, and those samples were lost as well. Otherwise the system behaved well during the cruise.

3.5. Nutrient Measurements

Analyst on board: Ian Smith (AOML/CIMAS)
Science lead: Jia-Zhong Zhang (AOML/NOAA)

3.5.1 Equipment and Techniques

Dissolved nutrients (phosphate, silicate, nitrate, nitrite, and ammonia) were measured using an automated continuous flow analytical system with segmented flow and colorimetric detection. The five-channel auto-analyzer used was produced by SEAL Analytical.

The major components of the nutrient system consisted of an autoXY-2 auto-sampler, two AA3 high precision peristaltic pumps, five Digital Colorimeter detectors, and custom software for digitally logging and processing the chromatograms. In addition, glass coils were used for mixing the nutrients with their appropriate reagents to produce the proper reaction for analysis. Phosphate and ammonium samples were analyzed at 37°C. Silicate, nitrate, and nitrite were analyzed at room temperature.

Nutrient samples were collected from the Bullister bottles in 50 ml acid-washed sample bottles after three seawater rinses. Sample analysis typically began within 1 hour of sample collection after the samples had warmed to room temperature.

Detailed methodologies are described by Gordon *et al.* (1993).

3.5.2 Analytical Methods

There were 1808 samples taken at both discrete depths and from the ship's underway system and were analyzed for phosphate (PO_4^{-3}), nitrate (NO_3^-), nitrite (NO_2^-), orthosilicic acid (H_4SiO_4), and total ammonia (NH_3 and NH_4^+). Nitrite was determined by diazotizing the sample with sulfanilamide and coupling with N-1 naphthyl ethylenediamine dihydrochloride to form an azo dye. The color produced is measured at 540 nm. Samples for nitrate analysis were passed through a cadmium column, which reduced nitrate to nitrite, and the resulting nitrite concentration (i.e., the sum of nitrate + nitrite which is signified as N+N) was then determined as described above. Nitrate concentrations were determined from the difference between N+N and nitrite (Zhang *et al.,* 1997). Phosphate was determined by reacting the sample with molybdic acid to form phosphomolybdic acid. This complex was subsequently reduced with hydrazine, and the absorbance of the resulting phosphomolybdous acid was measured at 820 nm (Zhang *et al.*, 2000). Silicic acid was analyzed by adding an acidic solution of ammonium molybdate to seawater to produce silicomolybic acid (Zhang and Berberian, 1997). 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.,* 1993) were employed to reduce the toxicity of our waste stream. Ammonia in solution reacts with alkaline phenol and NaDTT (Zhang *et al.,* 1997) at 37ºC to form indophenol blue in the presence of sodium nitroferricyanide as a catalyst. The absorbance of indophenol blue at 660 nm is linearly proportional to the concentration of ammonia in the sample.

3.5.3 Standards and Sampling

A mixed stock standard consisting of silicic acid, phosphate, and nitrate was prepared by dissolving high purity standard materials (KNO_3, KH_2PO_4, and Na_2SiF_6) in deionized water using a two-step dilution for phosphate and nitrate. This standard was stored at room temperature. A nitrite stock standard was prepared about every 15 days by dissolving $NaNO_2$ in distilled water, and this standard was stored in the refrigerator. A ammonia stock standard was prepared about every 15 days by dissolving $(NH_4)_2SO_4$ in distilled water, and this standard was stored in the refrigerator. A daily nitrite and ammonia mixed standard was prepared diluting 12 ml of nitrite stock and 5 ml of ammonia stock into a 250 ml with DIW.

Working standards were freshly made every day by diluting the stock solutions in low nutrient seawater. The 4 working standard (C1-C4) were made by the addition of the 0 ml (C1), 5 ml (C2), 10 ml (C3), and 15 ml (C4) respectively of daily mixed standard (containing nitrite and ammonia) and a secondary mixed standard (containing silicic acid, nitrate, and phosphate) into a (4) 500 ml calibrated volumetric flasks of Low Nutrient Seawater (LNSW). DIW was added to each of the 4 working standards to 30 ml (C1), 20 ml (C2), 10 ml (C3), and 0 ml (C4) to correct for matrix differences between working standards.

Nutrient concentrations were reported in micromoles per kg. Lab temperatures were also recorded for each analytical run. Pump tubing was replaced twice during the cruise.

Nutrient samples were drawn into 50 ml HDPE sample bottles that had been stored in 10% HCl. The bottles were rinsed three to four times with sample before filling. Samples were then brought to room temperature prior to analysis. Samples were analyzed from deep water to the surface. Deionized Water (DIW) was used as a wash and base line carrier. LNSW was used as the medium for the working standards.

3.6. Dissolved Inorganic Carbon (DIC)

Analysts on board: Charles Featherstone (NOAA/AOML) and Eva Jundt (Texas A&M Corpus Christi)

Science leads: Dr. Leticia Barbero (AOML/CIMAS) and Dr. Rik Wanninkhof (NOAA/AOML)

3.6.1 Sample collection:

Samples for DIC measurements were drawn (according to procedures outlined in the PICES Publication, *Guide to Best Practices for Ocean CO2 Measurements*) from Niskin bottles into 294 ml borosilicate glass bottles using silicone tubing. The flasks were rinsed once and filled from the bottom with care not to entrain any bubbles, overflowing by at least one-half volume. The sample tube was pinched off and withdrawn, creating a 6 ml headspace, followed by 0.20 ml of saturated $HgCl_2$ solution which was added as a preservative. The sample bottles were then sealed with glass stoppers lightly covered with Apiezon-L grease and were stored at room temperature for a maximum of 12 hours.

3.6.2 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 (CM5017 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.*

3.6.3. DIC Analysis:

In coulometric analysis of DIC, all carbonate species are converted to CO2 (gas) by addition of excess hydrogen ion (acid) to the seawater sample, and the evolved CO2 gas is swept into the titration cell of the coulometer with pure air or compressed nitrogen, where it reacts quantitatively with a proprietary reagent based on ethanolamine to generate hydrogen ions. In this process, the solution changes from blue to colorless, triggering a current through the cell and

causing coulometrical generation of OH- ions at the anode. The OH- ions react with the H+, and the solution turns blue again. A beam of light is shone through the solution, and a photometric detector at the opposite side of the cell senses the change in transmission. Once the percent transmission reaches its original value, the coulometric titration is stopped, and the amount of CO_2 that enters the cell is determined by integrating the total change during the titration.

a) DIC Calculation:

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

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

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 (µmol/kg) using density obtained from the CTD's salinity. The DIC values were corrected for dilution due to the addition of 0.120 ml of saturated HgCl2 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 1.00068. 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 1.60 µmol/kg for AOML 3 and 1.50 µmol/kg for AOML 4.

The coulometer cell solution was replaced after 24 – 28 mg of carbon was titrated, typically after 9 – 12 hours of continuous use. The blanks ranged from 12 -58.

b) <u>Calibration, Accuracy, and Precision</u>:

The stability of each coulometer cell solution was confirmed three different ways.

1. Gas loops were run at the beginning of each cell
2. CRM's supplied by Dr. A. Dickson of SIO, were analyzed at the beginning of the cell before sample analysis.
3. Duplicate samples from the same niskin, were measured near the beginning; middle and end of each cell.

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

The accuracy of the DICE measurement is determined with the use of standards (Certified Reference Materials (CRMs), consisting of filtered and UV irradiated seawater) supplied by Dr.

A. Dickson of Scripps Institution of Oceanography (SIO). The CRM accuracy is determined manometrically on land in San Diego and the DIC data reported to the data base have been corrected to the following CRM batches 188, 192 and 194. The CRM certified values for batch 188 is 2099.26 µmol/kg, batch 192 is 2070.83 µmol/kg and batch 194 is 2025.17 µmol/kg.

The precision of the two DICE systems can be demonstrated via the replicate samples. Approximately 10.6% of the niskins sampled were duplicates taken as a check of our precision. These replicate samples were interspersed throughout the station analysis for quality assurance and integrity of the coulometer cell solutions. The average absolute difference of these replicates was 1.44 (AOML 3) and 1.26 (AOML 4) µmol/kg - No major systematic differences between the replicates were observed.

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

c) Calibration data during this cruise:

UNIT	AVG Gas Cal Factor	Pipette	AVG CRM Correction	Std Dev	AVG Difference Dupes
AOML 3	1.003916	27.990 ml	1.60, N= 37	1.35	1.44
AOML 4	1.003393	29.387 ml	1.50, N = 38	1.00	1.26

d) Instrument Repairs

Valve 7 on AOML 4 was replaced at the beginning of the cruise. AOML 3 and AOML 4 functioned well for the remainder of the cruise.

e) Underway DIC Samples

Underway samples were collected from the flow thru system in the hydrolab during transit between lines of stations. Discrete DIC samples were collected approximately every 2 hours with duplicates every fifth sample. A total of 144 discrete DIC samples including duplicates were collected while underway. The average difference for replicates of underway DIC samples was 1.47 µmol/kg (AOML 3) and 1.07 µmol/kg (AOML 4), and an average STDEV of 0.91 (AOML 3) and 0.76 (AOML 4).

f) Summary:

The overall performance of the analytical equipment was good during the cruise.

Including the duplicates, a total of 1896 samples were analyzed from 141 CTD casts for dissolved inorganic carbon (DIC), which equates to a DIC value for 100% 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.

3.7. Discrete *p*CO_2 Measurements

Analysts on board: Alicia Uribe (UABC), Leticia Barbero (AOML/ CIMAS)
Science leads: Rik Wanninkhof (AOML/NOAA), Leticia Barbero (AOML/ CIMAS)

3.7.1 Sampling:

Samples were drawn from 11-L Bullister 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 warmed and to provide space for the stopper and tubing of the analytical system. Saturated mercuric chloride solution (0.2 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 8 hours prior to analysis.

The analyses for pCO2 were done with the discrete samples at 20°C. A primary water bath was kept within 0.03°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 12 bottles, which took approximately 3.5 hours, including the six standard gases. When 12 bottles were moved into the primary water bath for analyses, the next 12 bottles were moved into the secondary water bath. No sample bottle spent less than 2 hours in the secondary water bath prior to being moved to the analytical water bath.

Duplicate samples from the same Niskin-style bottles were drawn to check the precision of the sampling and analysis. Discrete samples were collected from the underway (UW) flowing seawater line aboard the ship. The results for the UW samples compared well with the results for the autonomous UW pCO2 instrument.

Over 1900 samples were drawn from 141 CTD casts. From the UW seawater line, 126 samples were drawn. 153 of duplicate bottles were drawn at numerous depths. The average relative standard error was 0.21%, while the median relative error was 0.15%.

3.7.2. Analyzer Description:

The principles of the discrete pCO2 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 Membrana, Inc.), which contain bundles of hydrophobic micro-porous tubes in polycarbonate shells ($2.5 \times 2.5 \times 0.5$ 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 32 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 12 sample bottles, the miniature contactors, a water circulation pump, a copper coil connected to a refrigerated circulating water bath, an immersion heater, a 12-position sample distribution valve, two thermistors, and two miniature pumps. The immersion heater works in opposition to the cooler water passing through the copper coil. One thermistor is immersed in the water bath, while the second thermistor is in a sample flow cell after the second contactor. The difference between the two thermistor readings was consistently less than 0.02°C during sample analyses. In a separate enclosure are the 8-port gas distribution valve, the infrared analyzer, a barometer, and other electronic components. The gas distribution valve is connected to the gas pump and to six standard gas cylinders.

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

A custom program developed using LabView™ controls the system and graphically displays the CO_2 concentration, as well as the temperatures, pressures, and gas flow during the 15-minute equilibration (Figure 63). The analytical system was running well enough that the equilibration period was shortened to 12 minutes for the second half of the cruise. The CO_2 in the gas phase changes greatly within the first minute of a new sample and then goes through nearly two more oscillations. The oscillations dampen quickly as the concentration asymptotically approaches equilibrium. The flows are stopped, and the program records an average of ten readings from the infrared analyzer along with other sensor readings. The data files from the discrete pCO_2 program are reformatted so that a Matlab™ program designed for processing data from the continuous pCO_2 systems can be used to calculate the fugacity of the discrete samples at 20°C. The details of the data reduction are described in Pierrot *et al.* (2009).

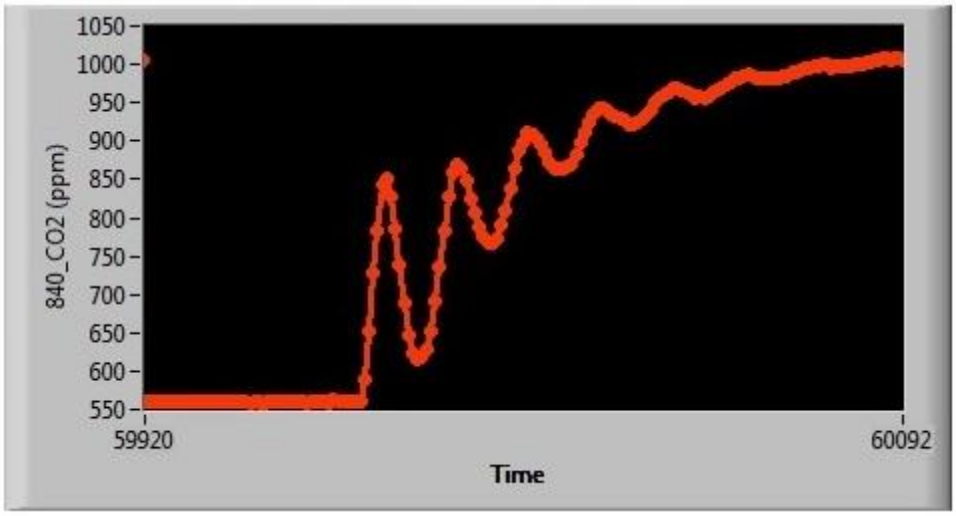

Figure 63: CO_2 oscillations at the start of the first sample in a set of twelve.

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. Alicia Uribe and Leticia Barbero collected and analyzed all the samples.

Standard Gas Cylinders	
Cylinder Number	**ppm CO_2**
JB03282	288.46
JB03268	384.14
CB11243	591.61
CA05980	792.51
CA05984	1036.95
CA05940	1533.70

Figure 64 shows preliminary surface discrete pCO_2 values obtained from samples collected underway and from the surface bottle of each CTD cast.

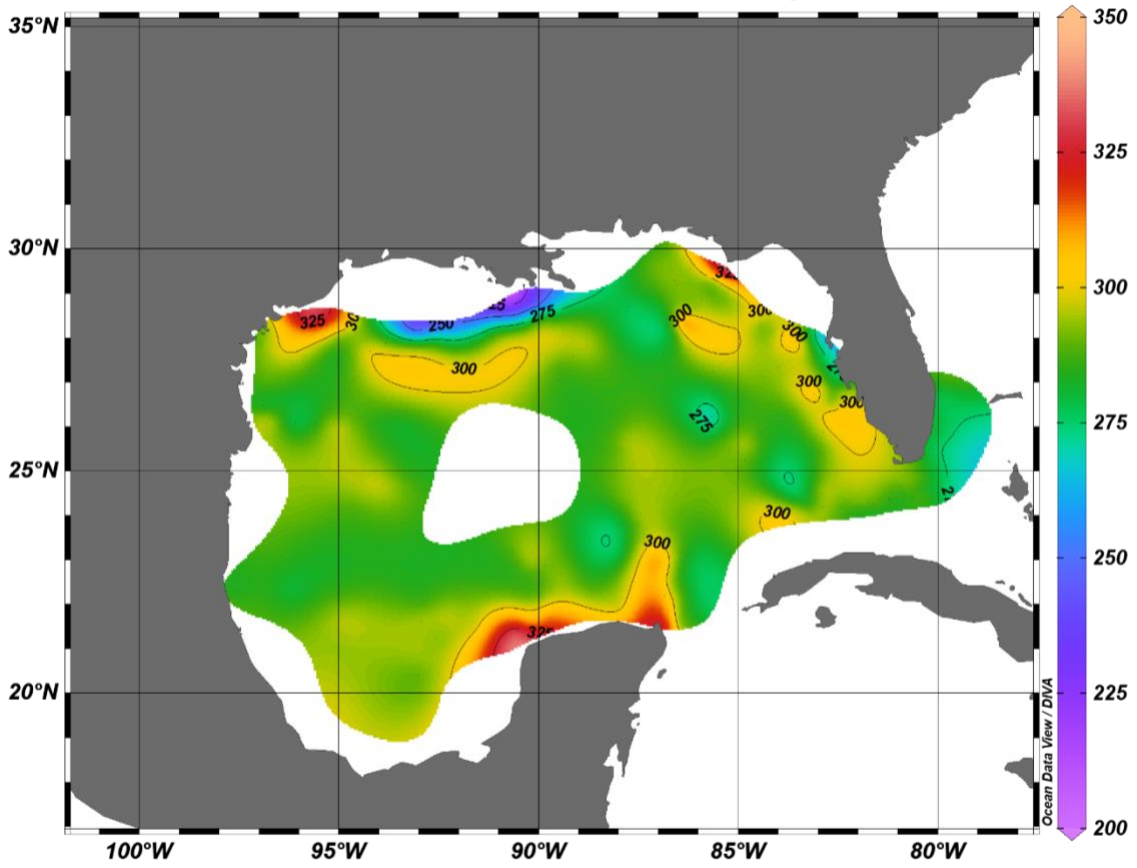

Figure 64: Preliminary surface discrete pCO_2 measurements collected from underway samples and from the surface bottle at each CTD cast during GOMECC-4.

3.8. Total Alkalinity Measurements

Analysts on board: Gabriela Cervantes, Mariana Cupul (UABC)
Science lead: Leticia Barbero (AOML/CIMAS)

3.8.1. Alkalinity Definition:

The total alkalinity of a seawater sample is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant K ≤ 10–4.5 at 25°C and zero ionic strength) over proton donors (acids with K > 10–4.5) in 1 kilogram of sample (Dickson, 1981).

By Dickson's definition, the total alkalinity, (TA), is expressed as:

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [H_3SiO_4^-] + [NH_3] + [HS^-] - [H^+] - [HSO_4^-] - [HF] - [H_3PO_4] - [HNO_2]$$

3.8.2. Alkalinity Measurement System:

Two titration systems were used: "System 1" used a Metrohm 765 Dosimat Titrator and an Orion 720A pH meter controlled by a personal computer (Millero *et al.,* 1993). "System 2" used a Metrohm 665 Dosimat Titrator and an Orion 2-Star pH meter. The cells consisted of a 400 ml water-jacketed glass beaker. A plexiglass reference electrode (Orion 900200) and a glass pH electrode (Orion 8101BNWP) were used to measure the e.m.f during the titration. The samples were delivered in the cells using a water-jacketed pipette, the calibrated volume of which was 185.63 ml at 22°C. A small air pump, which was also used to empty the cells after the titrations were complete, was used to pressurize and push the sample to fill the pipette. The filling and emptying of the pipette is controlled by a series of pinch valves controlled by manual electric switches. The tubing was first rinsed with small volumes of sample, then the pipette was rinsed with a full volume. The pipette was then filled again, and the volume delivered to one of the cells. Both the pipette and the cells were kept at 22 ± 0.1°C with a Neslab constant-temperature bath. The acid titrant, a 0.100347 mol/kg^{-1} HCl solution in ~0.6 molal NaCl solution, was made by Dr. Dickson of Scripps Institution of Oceanography (SIO) and stored in 1-L glass bottles, which were used to refill the acid bottles of the Dosimat when the level fell below the half mark.

The volume of HCl delivered to the cell is traditionally assumed to have a small uncertainty (Dickson, 1981) and is equated with the digital output of the titrator. Certified standard Reference Material (CRM) Batch 194, 192 and 188 prepared by Dr. Dickson were used at sea to monitor the performance of the titrators. Roughly two CRMs a day were used to calibrate the instruments, once at the beginning of the day and once at the end. A total of 28 and 81 CRMs were run on System 1 and System 2, respectively. All TA data were corrected using the average of the before and after measured CRM values for each cell, unless one CRM measurement presented issues, in which case only the good measurement was used for the correction.

The progress of the titration is controlled by a computer program written in National Instrument's Labwindows/CVI 4.1, and the total alkalinity is computed from the titrant volume, concentration, and e.m.f. measurements using a non-linear least-squares approach that corrects for the reactions with sulfate and fluoride ions (Dickson *et al.,* 2007).

3.8.3. Sampling:

Samples for total alkalinity measurements were taken at all GOMECC-4 stations (1-141). Two Bullister bottles at roughly each station were sampled twice for duplicate measurements. Seawater samples were drawn from the Bullister bottles on the CTD rosette with a 40-cm length of silicon tubing fitted directly over the petcock of the Bullister bottle and the other end was inserted into the bottom of a 500-ml Corning glass-stoppered sample bottle. The sample bottle was rinsed three times with approximately 300 ml of seawater. The sample bottle was slowly filled from the bottom and allowed to overflow for one volume. Once filled, the sample was poisoned with a saturated solution of mercuric chloride and the bottles were kept in a constant water bath at 22°C for at least an hour before analysis.

3.8.4. Quality Control:

Dickson laboratory Certified Reference Material (CRM) Batch 194, 192 and 188 were used to determine the accuracy of the total alkalinity analyses. The total alkalinity certified value for each batch is:

Batch 194: 2169.83 ± 0.77 µmol/kg

	# of CRMs measured	Average Measured Value (µmol/kg)	Standard Deviation (µmol/kg)
System 1	5	2170.26	± 2.53
System 2	16	2171.75	± 1.45

Batch 192: 2213.68 ± 0.77 µmol/kg

	# of CRMs measured	Average Measured Value (µmol/kg)	Standard Deviation (µmol/kg)
System 1	18	2215.60	± 2.15
System 2	45	2216.38	± 2.08

Batch 188: 2264.96 ± 0.77 µmol/kg

	# of CRMs measured	Average Measured Value (µmol/kg)	Standard Deviation (µmol/kg)
System 1	6	2265.51	±2.92
System 2	20	2268.95	± 3.25

On most stations, duplicates were drawn on one Bullister bottle. The standard deviation for the duplicates measured on GOMECC-4 (for both systems) is 3.21:

Duplicate: 147

Underway: 126

Total samples analyzed: 2031

Figure 65 shows preliminary surface alkalinity values obtained from samples collected underway and from the surface bottle of each CTD cast.

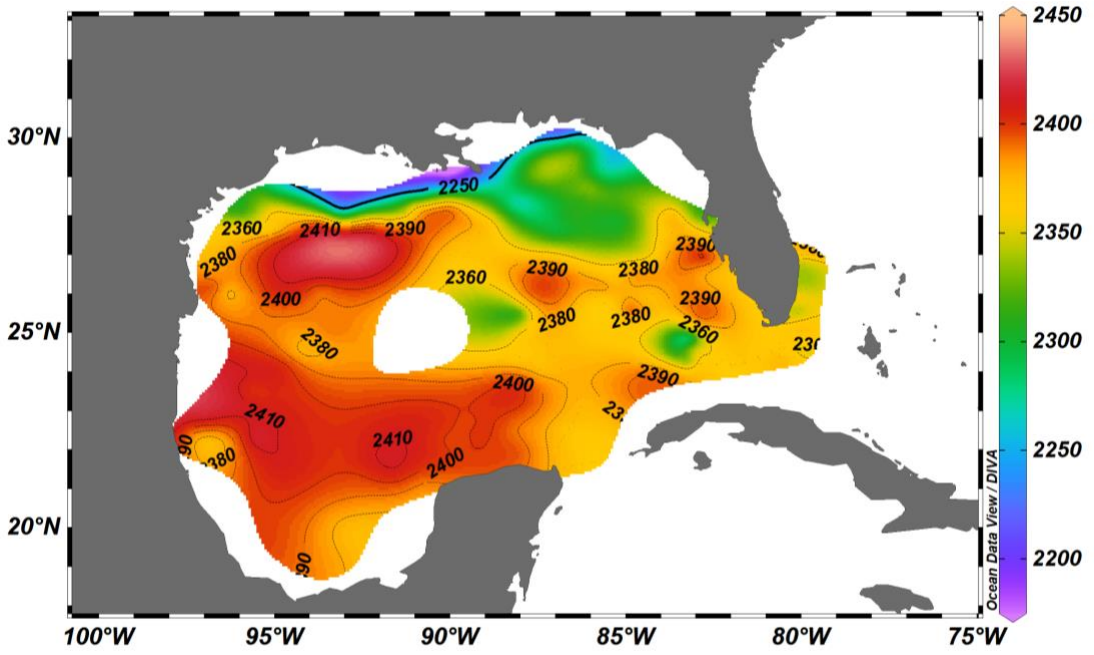

Figure 65: Preliminary surface total alkalinity measurements collected from underway samples and from the surface bottle at each CTD cast during GOMECC-4.

3.9. Discrete pH Analyses

Analysts on board: Loraine Martell Bonet, Macarena Martín Mayor, and Juan Millán Otoya (USF)

Science lead: Robert H. Byrne (USF)

3.9.1 Sampling:

Samples were collected for pH analysis immediately following O_2 in the rosette sampling sequence. Seawater samples were collected from the Niskin bottles directly in 10-cm glass cylindrical optical cells (~30 mL volume) using a section of silicone tubing (~15 cm long). One end of the silicone tubing was first attached to the nipple of the Niskin bottle. The nipple was pushed in to initiate flow, and the silicone tubing was squeezed to eliminate air bubbles. The other end of the silicone tubing was attached to the optical cell, which was agitated to eliminate any residual bubbles. After ~15 seconds of sample flow, the cell was capped at one end. The silicone tubing was then detached from the optical cell and, with the water still flowing, the other cap was rinsed and used to seal the optical cell.

Samples collected this way are not exposed to the atmosphere, and each cell is flushed with at least three cell volumes of seawater. The samples were collected, taken into the lab, and rinsed with tap water to eliminate salt on the outside of the cells. The cells were dried thoroughly, and the optical 60 windows were cleaned with Kimwipes immediately before measurement. Samples were thermostatted at 25 (±0.05)ºC in a custom-made, 36-position cell warmer.

3.9.2. Calculation and Measurement:

The pH_T of each sample was determined on an Agilent 8453 spectrophotometer setup with a custom-made temperature-controlled cell holder. Only the tungsten lamp was turned on. The UV lamp was turned off to prevent photodegradation of organic matter in the samples by UV light. A custom macro program running on Agilent ChemStation was used to guide the measurements and data processing. The macro automated the procedures of sample input, blank and sample scans, quality control, and data archiving. The quality control steps included checking the baseline shift after dye injection and monitoring the standard deviation of multiple scans. Absorbance blanks were taken for each sample and 10 μL of purified m-cresol purple (10 mmol/kg) were added for the analysis. pH_T (total scale) was calculated according to Liu *et al.* (2011):

$$pH_T = -\log(K_2^T e_2) + \log(\frac{R - e_1}{1 - R\frac{e_3}{e_2}})$$

with R being the ratio of absorbances measured at 578 nm (λ_2) and 434 nm (λ_1), corrected for baseline changes using absorbance measured at 730 nm (λ_3): $R = (\lambda_2 A - \lambda_3 A) / (\lambda_1 A - \lambda_3 A)$. The salinity and temperature dependence of $K_2^T e_2$ is given as:

$$-\log(K_2^T e_2) = a + (\frac{b}{T}) + c \ln T - dT$$

where:

a =–246.64209 + 0.315971 S + 2.8855·10^{-4} S^2,
b = 7229.23864 – 7.098137 S – 0.057034 S^2,
c = 44.493382 – 0.052711 S,
d = 0.0781344

and the temperature and salinity dependence of e_1 and $\frac{e_3}{e_2}$ are given by:

e_1 =–0.007762 + 4.5174·10^{-5} T,
$\frac{e_3}{e_2}$ = –0.020813 + 2.60262·10^{-4} T +1.0436·10^{-4} (S–35)

These equations are applicable for samples between temperature ($278.15 \leq T \leq 308.15$) and salinity ($20 \leq S \leq 40$). In all our measurements at sea, T = 298.15.

The pH is calibration-free (no calibrations are needed). Duplicate pH samples, collected from discrete samples taken from Niskin bottles (N = 203), displayed a standard deviation of 0.001.

3.9.3. Perturbation Determination for pH:

Small changes in sample pH (measurement perturbations; Clayton and Byrne, 1993) created by the addition of titrant to samples were quantified using samples collected from profiles. For each perturbation determination, ΔpH was defined as ΔpH = pHfinal – pHinitial, where

pHinitial is the total scale pH taken after a single titrant addition and pHfinal is the total scale pH after a second titrant addition.

An equation developed using this perturbation data was used to correct pH measurements:
$pH^0 = -0.0042 \cdot pH - 0.0326$
where pH is the raw pHT measurement and pH0 is the perturbation-corrected pHT measurement.

3.9.4. Quality Control:

All spectrophotometric pH and $[CO_3^{2-}]_T$ (see next section) measurements were tentatively flagged if the baseline shifted more than 0.002 absorbance units. A series of five spectra were averaged for each determination, and samples were rerun if the overall standard deviations were higher than 0.0004 for pH measurements. This process was repeated until the standard deviation of multiple readings was within 0.0004 for pH. Absorbance values were saved so that the quality criteria can be evaluated in the future.

A total of 1901 pH samples and 1901 carbonate ion samples were collected from the 141 stations, and 252 pH and 252 carbonate ion samples were collected from 126 underway points. In the pH data set, perturbation-corrected pH_T measurements were reported along with their associated quality-control flags. Both pH_T and $[CO_3^{2-}]_T$ were reported at the measurement temperature of 25ºC.

3.10. Discrete Carbonate Ion Analyses

Analysts on board: Loraine Martell Bonet, Macarena Martín Mayor, and Juan Millán Otoya (USF)

Science lead: Robert H. Byrne (USF)

3.10.1. Sampling:

The carbonate ion $[CO_3^{2-}]_T$ samples were collected in 10-cm quartz cylindrical optical cells in the same manner as the pH samples. Samples were collected after pH in the rosette sampling sequence.

3.10.2. Calculation and Measurement:

The carbonate ion concentration of each sample was determined on an Agilent 8453 spectrometer setup with a custom-made temperature-controlled cell holder. A custom macro program was used to guide the measurements and data processing in a similar manner as was done for pH measurements.

The UV lamp was turned on for carbonate ion analysis. A UV blank was taken for each sample and 20 μL of 0.022 M $PbClO_4$ were added (Acros Organics, 99% purity). Absorbances (A) were measured at two wavelengths on the Pb(II) absorbance peak ($_1\lambda$ = 234 nm and $_2\lambda$ = 250 nm) and at a non-absorbing wavelength ($_3\lambda$ = 350 nm). Absorbance values were used to calculate absorbance ratios: $R = (\lambda_2A - \lambda_3A) / (\lambda_1A - \lambda_3A)$ (Byrne and Yao, 2008).

The ratios were corrected for spectrophotometer wavelength offsets using the equation given in Sharp *et al.* (2017): $R^0 = R - 0.265 \cdot \Delta\lambda_{241.1}$. Corrected absorbance ratios (R^0) are calculated using an instrument-specific wavelength offset at 241.1 nm ($\Delta\lambda_{241.1}$), which was determined using SRM 2034 from the National Institute of Standards and Technology.

Carbonate ion concentrations ($[CO_3^{2-}]_T$) were then calculated using the equation:

$$-\log[CO_3^{2-}]_T = \log\left\{\frac{CO_3\beta_1}{e_2}\right\} + \log\left\{\frac{R^0 - e_1}{1 - R^0 \cdot \frac{e_3}{e_2}}\right\}$$

where $CO_3\beta_1$ is the formation constant for $PbCO3^0$ and the e_1 terms are molar absorptivity ratios. The following equation is equivalent to equation 8 of Sharp *et al.* (2017). The fitting parameters given for measurements at 25ºC are:

$$\log\left\{\frac{CO_3\beta_1}{e_2}\right\} = 6.87057 - 0.142142\ \mathrm{S} + 0.00190892\ S^2$$

$$e_1 = 0.787458 - 00339648\ S + 0.000583574\ S^2$$

$$\frac{e_3}{e_2} = 2.52288 - 0.0383205\ S$$

where S is salinity. Duplicate carbonate ion samples, collected from discrete samples taken from Niskin bottles (N = 203), displayed a standard deviation of 2 μmol/kg.

3.10.3. Quality Control:

All spectrophotometric pH and $[CO_3^{2-}]_T$ measurements were tentatively flagged if the baseline shifted more than 0.002 absorbance units. A series of five spectra were averaged for each determination, and samples were rerun if the overall standard deviations were higher than 0.001 for $\log[CO_3^{2-}]_T$ measurements. This process was repeated until the standard deviation of multiple readings was within 0.001 for carbonate. Absorbance values were saved so that the quality criteria can be evaluated in the future.

A total of 1901 pH samples and 1901 carbonate ion samples were collected from the 141 stations, and 252 pH and 252 carbonate ion samples were collected from 126 underway points.. In the $[CO_3^{2-}]_T$ data set, calculated carbonate ion concentrations and measured (uncorrected) absorbance ratios were reported, along with their associated quality-control flags. Both pH_T and $[CO_3^{2-}]_T$ were reported at the measurement temperature of 25ºC.

4. Biological measurements

4.1. Plankton Community Dynamics/Trophic Interactions across Continental Margins

Analysts on board: Miranda Irby (NCSU), Hans Prevost (ULL)
Science leads: Astrid Schnetzer (NCSU), Beth Stauffer (ULL)

4.1.1. Objectives:

1. Characterize plankton communities (from picoplankton to metazooplankton) along spatial gradients of eutrophication-driven acidification, hypoxia, and harmful algal bloom prevalence.
2. Quantify changes in carbon flow to higher trophic levels by conducting on-deck microzooplankton and copepod grazing experiments along gradients of ocean acidification, eutrophication, harmful algal blooms, and hypoxia.
3. Identify phytoplankton and/or zooplankton indicator species/assemblages most impacted by environmental stressors and aim to determine sentinel sites/regions within the Gulf of Mexico best suited to track future ocean acidification impacts.

4.1.2. Methods:

CTD sampling was conducted at two or three depths, depending on whether a chlorophyll max was present within the water column, selecting an offshore, intermediate, and nearshore site. Water from the CTD was concentrated onto GF/Fs for DNA-based analyses (<200 µm and <20 µm size fractioned) and chlorophyll-a measurements (whole seawater and <20 µm size fractioned). The filters were stored at-20ºC. Whole seawater was preserved in 5% acid Lugol's for microscopy and 1% formalin for flow cytometry analyses. Zooplankton surface tows were preserved in ethanol and selected copepods, which were used in grazing experiments, were preserved in formalin. DNA and Lugol's samples will be transported to North Carolina State University and flow cytometry and chlorophyll-a samples to University of Louisiana at Lafayette for further processing and analysis.

4.1.3. CTD Sampling:

Whole seawater was collected from the CTD from surface, chlorophyll max (if applicable) and the lower water column, and at selected sites also from depths around 2500 m (for molecular analyses). For molecular analysis, 3 liters of whole seawater was passed through a <200 µm in-line mesh to exclude most zooplankton grazers and select for the microplankton communities. For chlorophyll-a, Lugol's analysis and flow cytometry, 2 liters of whole seawater was taken from each depth and preserved as detailed above.

4.1.4. Molecular and Chlorophyll-a Sampling:

Replicate samples for molecular analysis were filtered onto GF/F filters using varying volumes for different depths. For surface and chlorophyll max depths, 400 mL of <20 µm and <200 µm size-fractioned seawater was concentrated onto GF/F's in quadruplicate. For the lower

water column and depths around 2500m, the volume was increased to 800 to ensure sufficient biomass in duplicates. 150-200 mL of whole seawater from all depths was filtered onto GF/F's in duplicate for chlorophyll-a analysis. Whole seawater was additionally screened through a <20 µm mesh to allow for the chlorophyll-a quantification of nanoeukaryotes in the <20 µm size classes. Filters were stored in aluminum foil and transferred to the ship's -20ºC freezer for the duration of the cruise. They will be shipped back to the laboratories on dry ice for processing and analysis.

4.1.5. Microzooplankton Dilution and Copepod Feeding Experiment:

Zooplankton surface tows were conducted at 11 different stations. The zooplankton samples were split multiple times using a Folsom plankton splitter to preserve 200 mL in ethanol for community analyses, select live copepods from part of the tow for the grazing experiments and concentrate additional portions onto pre-weighed 200 µm mesh fin duplicates for wet weight measurements. Screens were stored frozen at -20°C. The grazers for the experiments were picked using a dissecting microscope.

Microzooplanton incubation experiments were set up according to the methods outlined in Landry et al. (1995). 20 L of surface water was either collected from the CTD of a depth of 2 m or with a bucket from the surface. The whole seawater was screened through a 200 µm mesh to exclude larger zooplankton grazers. 5 L of the <200 µm seawater was then filtered through a 0.2 µm capsule filter to create particle-free seawater to use for the dilution experiments. These dilutions are outlined in Dilution and nutrient addition experiment details. After dilutions were complete, nutrients were added to the bottles (nitrate ($NaNO_3$) and phosphate (Na_2HPO_4) with concentrations of 10 mM and 1 mM, respectively). In addition to the microzooplankton grazing treatments, we also examined copepod feeding by adding additional bottles that had 20-30 copepods added back into the bottles holding <200 µm water in triplicate (

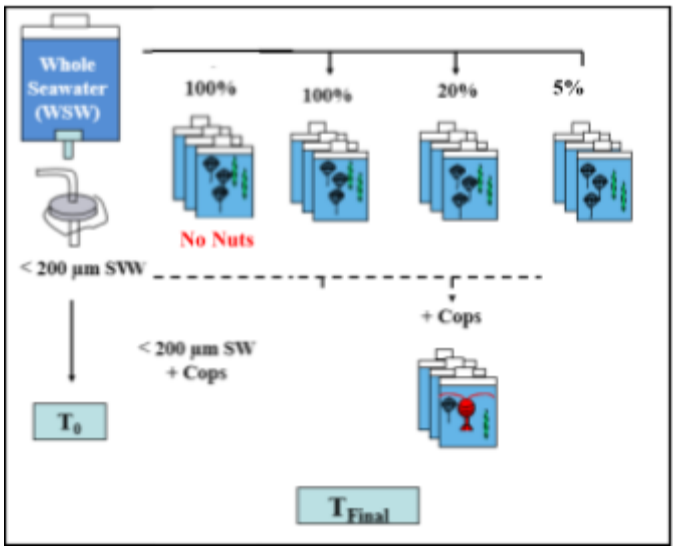

Figure 66).

Bottles were then incubated for around 24 hours in an on-deck incubator that had a continuous flow of ambient seawater to replicate oceanic conditions. The bottles were shrouded in

a neutral density screen to block out around 50% of light to simulate irradiances at 2m below sea level. PAR levels and temperatures were taken throughout the duration of the experiments. Samples were obtained at the beginning of the experiment (T_0) and at the end of the experiment (T_f) to examine changes in protist communities in response to changed microzooplankton and/or copepod grazer numbers. Changes were examined for chlorophyll, pico-and nanoplankton assemblages (flow cytometry), and microplankton communities (Lugol's samples). Copepods were removed from the bottles during T_f sampling and were preserved in formalin for taxonomic identificationFilters and flow cytometry samples were stored in the ships -20ºC freezer. Lugol's samples were stored in dark boxes until shipment to their destinations. Formalin samples are to be analyzed using an EasyCyte Guava flow cytometer to calculate picocyanobacteria, picoeukaryote, and nanoplankton abundances in the Stauffer Lab at UL Lafayette. The Lugol's samples are to be counted for autotrophic and hetero-/mixotrophic microplankton in the Schnetzer lab at NCSU. Community shifts and biomass changes within the bottles compared to with and without grazers were determined to quantify and characterize changes in carbon flow and plankton trophic interactions along different gradients of ocean acidification, hypoxia, and eutrophication within the Gulf of Mexico.

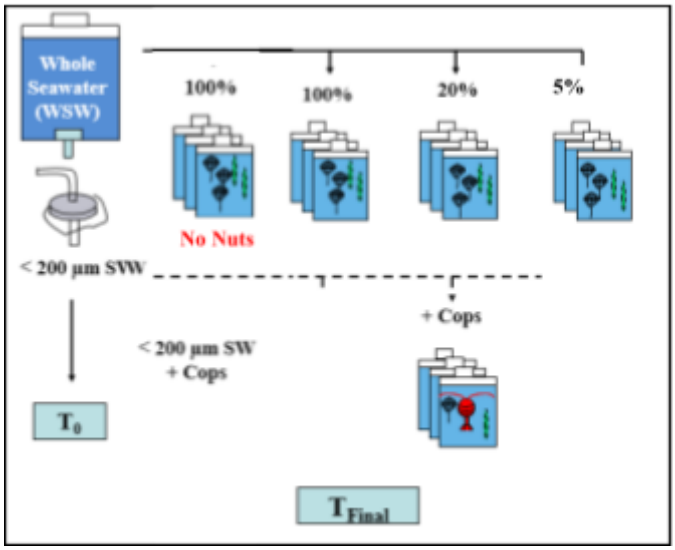

Figure 66: Depiction of the dilution and copepod feeding experimental design

Bottle #s	Dilutions	<200 SW	FSW	N stock solution (10 mM)	P stock solution (1 mM)
1-3	5% x 3	50 mL	950 mL	0.50 ml	0.50 ml
4-6	20% x 3	200 mL	800 mL	0.50 ml	0.50 ml
7-9	100% x 3	1000 mL	0 mL	0.50 ml	0.50 ml
10-12	100% + cop x 3	1000 mL	0 mL	0.50 ml	0.50 ml

13-15	100% No Nuts x3	1000 mL	0 mL	--	--

Table 5: Dilution and nutrient addition experiment details

4.2. Environmental DNA

Analyst on board: Sean Anderson (NOAA-NGI)
Science leads: Luke Thompson (NOAA-NGI), Sean Anderson (NOAA-NGI)

This project is new to GOMECC-4 and involves the collection and filtration of environmental DNA (eDNA) samples across the Gulf of Mexico. There are several objectives of this project that are designed to implement eDNA sampling to better understand the impact of OA on marine life (bacteria to fish), develop models to predict indicator species and their responses to future climate scenarios, and inform ecosystem management strategies.

Objective I:
Conduct a multi-trophic-level analysis of the effects of OA on ecosystem biodiversity, using data from eDNA sequencing.

Objective II:

Use eDNA sequence data with physical and chemical parameters to develop models of ecosystem biodiversity and ultimately identify indicator taxa of OA in the Gulf region.

To address these objectives, we performed eDNA collection and filtration spatially in the Gulf of Mexico, sampling along horizontal and vertical transects to capture a wide gradient in OA-related measurements like pH, DIC, temperature, and total alkalinity. Overall, we collected 485 eDNA samples, which will represent a substantial contribution to eDNA biodiversity monitoring in this ecosystem. eDNA samples will be processed and analyzed in FY22.

4.2.1 Sampling Scheme:

Seawater samples were collected for eDNA filtration at 3-4 sites per transect line, representing a nearshore, offshore, and intermediate site. At each site, samples were collected at three discrete depths, one at the surface layer, chlorophyll maximum, and depth closest to the seafloor. With this sampling design, biodiversity was targeted over horizontal and vertical scales across the Gulf, encompassing a broad range of OA measurements. When possible, eDNA sampling was coordinated with other biological sampling (e.g., ichthyoplankton net tows, grazing experiments, and microscopy) for methods comparison.

4.2.2 Sample Collection and Filtration:

Whole seawater was collected from the CTD rosette using 2.7-L sterile Whirl-Pak bags. Bags were filled with ~2 L of seawater and triplicate bags were collected from each of the three depths per site (9 total bags per site). Samples were immediately filtered through 0.2 µm Sterivex filters (Millipore) using a peristaltic pump. Filters were sealed, labeled, and frozen on the ship at -80°C. Filtration equipment and benchtop space were sanitized after each site using a 2-5% bleach solution.

4.*2.3 Lab Processing, Bioinformatics, and Data Analysis:*

DNA samples will be processed at the 'omics lab at NOAA's Atlantic Meteorological and Oceanographic Laboratory using recently developed protocols (Anderson and Thompson 2021). DNA will be extracted from filters using an automated KingFIsher Flex instrument (ThermoFisher), which can process up to 96 samples per run. Next-generation sequencing preparation of eDNA samples will be performed using an automated Opentrons OT-2 instrument, which automates pipetting and PCR plate preparation and will increase sample throughput. PCR plates will be sequenced separately using different primers to capture a range of marine life, from bacteria (16S rRNA), metazoans (mitochondrial COI), and fish and other vertebrates (12S rRNA). Sequencing will be performed on an Illumina MiSeq at the Genomics Core at Michigan State University.

Bioinformatics processing of eDNA will be facilitated with access to high-performance computing resources available through NOAA and at a partner cooperative institute (Mississippi State University). eDNA sequence data will be processed separately for each targeted group of organisms using a newly developed NOAA-led bioinformatics toolkit, called Tourmaline (https://doi.org/10.1101/2021.09.15.460495). Tourmaline wraps popular amplicon processing software to infer amplicon sequence variants (ASVs), which represent unique DNA sequences (Callahan et al. 2016). Taxonomy of ASVs will be estimated using a classification software called Mitohelper, recently developed by the 'omics lab at AOML (Lim and Thompson 2021). ASV counts and taxonomy will be exported to R and analyzed to reveal biodiversity (species richness), community composition, and relative abundance of different marine organisms. Generalized additive models (GAMs) will be used to estimate relationships between diversity and relative abundance vs. OA-related parameters. Models will target taxonomic groups that are most vulnerable to OA and may represent ecosystem indicators, including foraminifera, pteropods, and other mollusks. Other groups of interest include harmful algae and commercially important fish species.

4.3. Community Structure of Ichthyoplankton

Analysts on board: Gonzalo Daudén-Bengoa (CICESE), Alexis Wilson (University of Southern Mississippi)

Science leads: Sharon Herzka (CICESE), Frank Hernandez (USM)

4.3.1 Introduction:

In the last 200 years oceans have absorbed nearly 30 per cent of the released carbon dioxide (CO_2) into the atmosphere (Sabine *et al.*, 2004). The absorbed CO_2 reacts with water to form carbonic acid, which leads to a decline in sea water's pH. This process, known as ocean acidification, causes shifts in the carbonate-bicarbonate balance (Stocker, 2014).

The increasing concentrations of CO_2 in marine ecosystems will negatively impact the populations of zooplankton and ichthyoplankton(Bednaršek *et al.*, 2012; Rossi *et al.*, 2015). Among the whole life cycle of fishes, the early life stages are the most sensitive to acidification due to their limited development, impacting the olfactory system and otoliths (internal calcareous structures that are part of the auditory and balance systems) development (Munday *et al.*, 2011), tissue damage (Frommel *et al.*, 2012) and a decrease in prey availability that have calcareous shell, such as ostracods and pteropods (Bednaršek *et al.*, 2012). Therefore, these changes during the early life stages of fish may have a negative impact in larvae survival and hence an impact in settlement and adult population size (Rossi *et al.*, 2015).

Studies focused on defining the composition and distribution of ichthyoplankton in the Gulf of Mexico (GoM) have been mainly focused in US Economic Exclusive Zone (EEZ), from studies in the continental shelf (Richards *et al.*, 1993; Muhling *et al.*, 2012), to the deep water region (Lindo-Atichati *et al.*, 2012; Meinert *et al.*, 2020), and studies in the Mexican EEZ have increased in the past decade (Batta-Lona *et al.*, 2019; Daudén-Bengoa *et al.*, 2020; Compaire *et al.*, 2021). Additionally, several studies have focused on the larval transport from the Caribbean sea and Cuba through the Yucatan Channel (Paris *et al.*, 2005; Muhling *et al.*, 2013). However, studies encompassing the whole GoM are more scarce (Murawski *et al.*, 2018) and to our knowledge fish larvae studies concerning the GoM have not been assessed yet. Therefore, this baseline information will provide in the long term insight for the evaluation of changes in species distribution and abundance, the relation with oceanographic variables and the effect of shifts in environmental conditions and anthropogenic impacts, such as the effect of ocean acidification on marine fish populations within the gulf.

The objective of this study is to define basin wide the distribution and abundance of larvae of fish species along the continental shelf and the beginning of the deep water region (< 1000 m) of the whole GoM including the Yucatan Channel Mexican waters, and US waters Florida Straits and Bahamas Channel, based on samples collected during the Gomecc-4 cruise (September 13th to October 21st 2021) onboard the R/V NOAA ship Ronald H. Brown.

4.3.2 Plankton Sampling Protocol:

A total of 51 stations were sampled with bongo net tows during the GOMECC-4 cruise (37% of total sampled stations) in a total of 16 transects covering the whole continental shelf slope and part of the deep waters of the GoM (Figure 67). In all lines, samples were collected at least in one shallow station (approx. 40 m), one station at mid depth (approx. 400 m) and a deep station (approx. 1200 m).

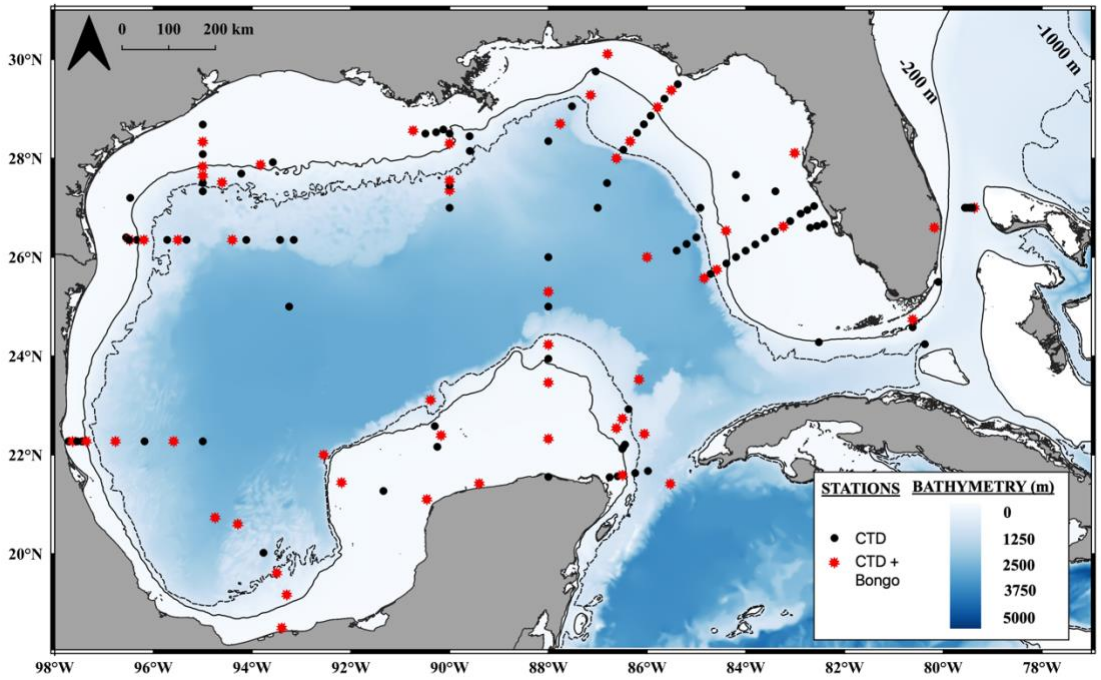

Figure 67: Samples collected during GOMECC-4 cruise (September 13th to October 21st 2021). Black dots (red stars) represent CTD (CTD + bongo) sampled stations. Continuous and dashed line represent 200m and 1000m depth respectively.

According to the bottom depth, maximum bongo depth tows were selected. Stations deeper that 300 m oblique tows were performed at 200m. If depths were shallower, the depth of the tow was adjusted. Zooplankton samples were collected with two pairs of bongo nets tows. One small bongo with two nets with a mouth diameter of 20 cm and 150 µm mesh nets and a big bongo with two nets with a diameter mouth of 60 cm and 335 µm mesh nets. To estimate filtration volumes, each net was equipped with General Oceanics flowmeters.

4.3.3.Sample Processing:

From each station a total of 4 net samples were obtained (2 from the small bongo and 2 from the big bongo). These samples were later sorted in 8 subsamples. First big bongo cod end was preserved in 7% buffered formalin for morphology based taxonomy, second big bongo cod end was split with a Folsom splitter where 50% of the sample was preserved in 96% ethanol and refrigerated for metabarcoding of fish larvae and copepods, 25% for copepod gut content preserved in 96% and refrigerated, and the remaining portion of the sample was preserved in sea water and frozen for stable isotope analysis. The same process was done for the two small bongo cod ends.

4.4. Plankton Tow Planktonic Foraminiferal eDNA Project

Analysts on board and Science Leads: Ben Ross (FAMU), Emily Osborne (AOML/NOAA)

Objective: Sequence the DNA of abundant Gulf of Mexico planktonic foraminiferal species commonly used in paleoclimate applications as a foundational step towards utilizing eDNA approaches in the paleoclimate record.

Mini-bongo (mesh size 150 um, 20 minute vertical tows from 200 m to surface) collections from taken at deep/open-GOM stations that are typically rich in planktonic foraminifera (and poor in organic matter) were targeted for this analysis. A total of 8 deep sampling locations distributed across the GOM region will be targeted for this study (Panama City Line, Louisiana Line, Brownsville Line, Tampico Line, Campeche Line, Merida Line, Yucatan Line, Venice Line). Approximately ½ (non-quantitative) of one cod end from the mini-bongo tow was treated with formalin (7%) immediately upon recovery to maximally preserve sample DNA. As soon as possible after formalin preservation, samples were processed in order to minimize foraminiferal shell dissolution in the formalin solution to best visualize shell morphology. Formalin preservative of 7% mixed with filtered GOM surface ocean seawater (pH >8) was used for sample treatment (estimated pH ~7).

For each of the selected sites, approximately 10 planktonic foraminifera were wet picked using a paint brush and binocular dissecting microscope and placed individually in a 1.5 ml sample tube with the 7% formalin-seawater preservative. Samples do not need to be refrigerated. The remaining qualitative ½ of the mini-bongo sample was returned to its sample cup and stored at AOML for future analysis.

Prior to sample foraminiferal selection, a general survey of the sample guided the target species selection of individuals utilized for analysis. The 10 individuals per sample generally reflect, qualitatively, the planktonic foraminiferal population found within the sample. Based on seasonal abundance in the Gulf and utility in paleoclimate application, the following planktic foraminiferal species were commonly found within two samples, surface dwellers: *G. ruber, G. sacculifer, O. universa*, mixed layer/deep dwellers: *N. dutertrei, G. menadrii*, and *G. truncatulinoides*. Upon collection, species identifications and detailed descriptions were recorded to use later in support of eDNA analysis.

DNA sequencing and identification of relevant DNA barcoding sequences for the preserved foraminifera will take place at AOML in 2022. These sequences will be used to identify cryptic speciation among the samples collected as well as to improve the use of environmental DNA methods to accurately characterize Gulf of Mexico planktonic communities. As these species are commonly used in paleoclimate applications, this work will also play a foundational role in adapting eDNA approaches to the study of the paleoclimate record.

5. Underway Measurements

5.1. 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 GOMECC-4, 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_2 system and the surface bottle salinity samples collected at each CTD station. The quality controlled TSG data were incorporated into the underway dataset.

5.2. Underway pCO_2 Analyses

Analyst on shore: Kevin Sullivan (AOML/CIMAS)
Science leads: Rik Wanninkhof (AOML/NOAA), Denis Pierrot (AOML/NOAA)

During the GOMECC-4 cruise, there was an automated underway pCO_2 system from AOML situated in the hydrolab, as it has been since 2007. The design of the instrumental system is based on Wanninkhof and Thoning (1993) and Feely *et al.* (1998), while 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 283 to 539 ppm 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 1.7-2.2 liters/min, which yielded a vigorous spray pattern during this cruise.

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 pCO_2 analytical system operated well throughout the entire cruise.

Standard Gas Cylinders

Cylinder Number	ppm CO_2
CA04957	282.55
CC105863	380.22
CB09696	453.04

CB09032	539.38

Figure 68 shows raw xCO_2 measurements collected during GOMECC-4.

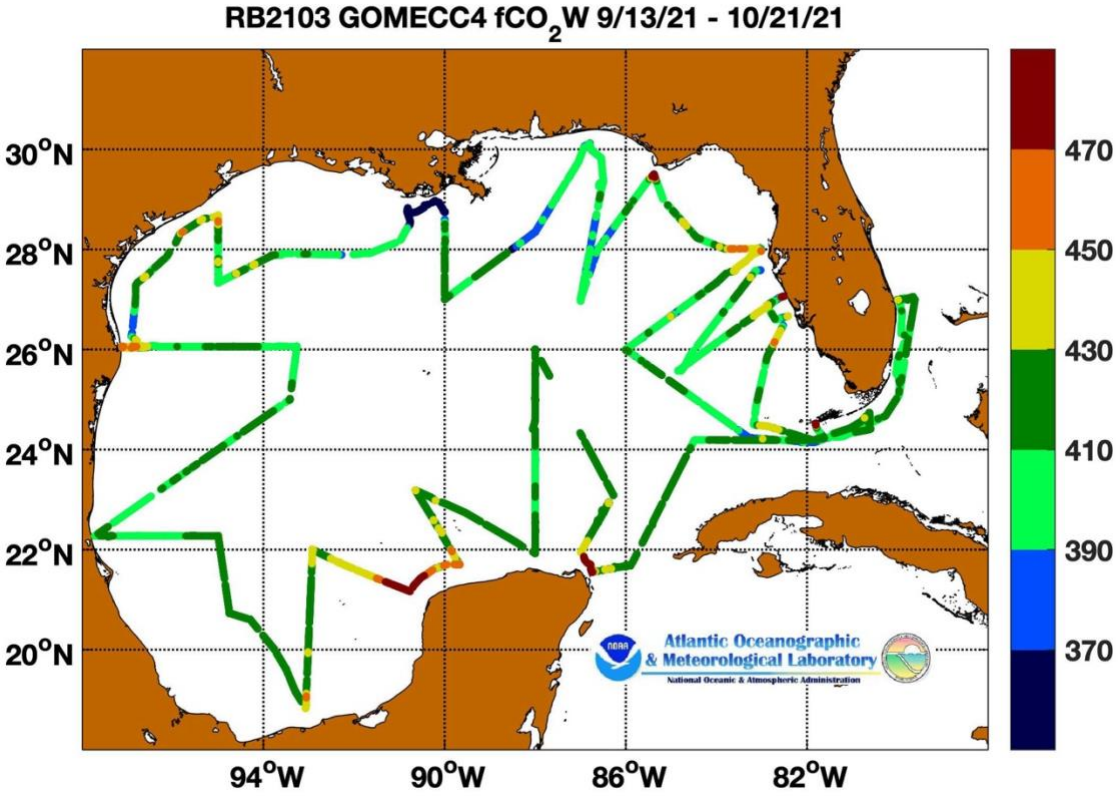

Figure 68: Surface pCO_2 values measured during GOMECC-4.

6. Other activities

6.1. Biogeochemical Argo Float Deployments

Science Lead: Emily Osborne (AOML/NOAA)

Objective: Deployment of four Biogeochemical-Argo (BGC-Argo) floats to autonomously observe seasonal to annual biogeochemical cycles (O2, Nitrate, pH, Chl-a, ocean particulates) across the open-GOM basin.

Historically, the open Gulf region has been chronically under-observed with respect to water column ocean biogeochemistry. The launch of a Gulf of Mexico biogeochemical Argo (BGC-Argo) array during GOMECC-4 will begin to address the observational gap for this large marginal sea. Four Apex BGC-Argo floats mounted with a CTD, oxygen, nitrate, pH and bio-optical sensor were launched (Table 6) during GOMECC-4. Floats were deployed at deep >2500 m GOMECC stations following the protocols established by the SOCCOM BGC-Argo team.

Complimentary high quality seaboard data collections from a coincident CTD cast were collected by GOMECC scientists (routine collections based on the GOMECC science plan) and supplemental surface and chlorophyll maximum water samples were collected and filtered for particulate organic carbon and HPLC pigment analysis for bio-optical sensor comparison. POC and HPCL samples will be shipped to Scripps where POC will be analyzed and HPLC samples will be sent in a batch to NASA for analysis. Resulting data from collected calibration datasets will be hosted on CLIVAR and Carbon Hydrographic Data Office (CCHDO), with the exception of HPLC data which are hosted at NASA SeaBASS.

These floats are capable of simultaneously measuring five biogeochemical variables (oxygen, nitrate, pH, chlorophyll-a, and ocean particulates, in addition to temperature and salinity) over the upper 2000 m of the water column. Following a standard Argo mission (profiling 0-2000 m every 10 days), we anticipate these BGC-Argo floats will operate for upwards of five years. Data from this array have applications to monitoring ocean health in the Gulf, including supporting ocean predictions; fisheries management; informing ocean acidification adaptation and mitigation.

Date	Time (UTC)	Station #	BGC-Argo Float ID	GPS Lat	GPS Lon	Bottom Depth (m)
9/22/21	10:53:33	30	WMO 4903625/ Apex 19821	26 59.12N	86 59.85W	2932.24
9/25/21	9:51:52	37	WMO 4903624/ Apex 19097	27 02.89N	89 58.71W	2302.39
10/4/21	13:43:00	73	WMO 4903623/ Apex 19093	26 03.80N	93 15.95W	3089.24
10/4/21	23:36:30	74	WMO 4903622/ Apex 19073	24 59.70N	93 25.10W	3664.47

Table 6: Summary of GOMECC-4 BGC-Argo deployments.

6.2. Sediment Core Collections

Analysts on board: Emily Osborne (AOML/NOAA), Benjamin Ross (FAMU),
Science lead Lead: Emily Osborne (AOML/NOAA),

Objective: To collect marine sediment cores that can be used to support paleocoeanogrpahic studies of the GOM from the Preindustrial to present, specifically evaluating ocean conditions (temperature, pH, microplastics, HAB cyst concentration) using fossil shells of foraminifera and pteropods bulk sediment analysis.

A series of sediment cores were collected across the US waters within the GOM, specifically at offshore sites along the Tampa, Pensacola, Louisiana, and Galveston Lines (Table 7). Deep stations were targeted for coring in order to sample deep sea mud that are rich in microfossils utilized in paleoceanographic studies. Sediment cores were collected using the

University of South Florida's (USF) Ocean Instruments Multi-Corer 800 (MC-800). The MC-800 is well-suited to recover a total of 8 short (max ~60 cm) sediment cores from deep marine environments simultaneously.

The MC-800 was launched using the ship's A-frame and winch. The package was lowered at a maximum rate of 45 meters per minute (m/min) down to 50 m above the seafloor and the wire angle was closely monitored in the presence of strong currents. At an estimated 50 m above the seafloor the wire was held steady to wait for the instrument to stabilize (~1 minute), before lowering the package at 20-30 mm/min until bottom contact was made. Immediately upon contact, 10-15 m of additional wire were paid out to ensure ship movement would not disturb the instrument on the seafloor. After ~1 minute of allowing the instrument to settle on the seafloor, the MC-800 was lifted and brought to the surface at 30 m/min. Once on deck, sample cores were assessed and measured, removed from the MC-800 body and extruded on deck immediately upon recovery. For each site, 3-4 of the longest and best preserved cores were extruded and sampled at 0.5 cm intervals for the upper 10 cm and 1 cm intervals for the lower remaining portion of the core. Any remaining cores that exhibited good preservation of the sediment-water interface were sampled for the core top (upper 1 cm). Sample bags were transferred to the ship's cold storage for the remainder of the cruise.

Sediment cores will be utilized over the coming years for a range of scientific applications including assessing the signal of OA preserved in marine sediments, the presence and abundance of harmful algal bloom cysts and frustules, and the abundance of micro plastics. Sediment material will also be shared with collaborators, including a partner at Florida A&M affiliated with the NOAA Cooperative Science Center there who will use a sediment core for teaching and research purposes.

Date	Station #	Coring Location	GPS Lat	GPS Lon	Bottom Depth (m)	Approx Core Length (cm)	# Extruded Cores	# Core Top Samples	Total # Samples
9/17/21	9	Tampa Line	25 59.16 N	85 59.86 W	3229	16	3	1	84
9/24/21	34	Pensacola Line	29 03.08 N	87 31.25 W	1499	57	4	1	220
9/25/21	38	Louisiana Line	27 36.84 N	89 59.81 W	1185	56	3	0	154
9/28/21	52	Galveston Line	27 30.99 N	94 36.18 W	941	48	3	2	176

Table 7: Summary of sediment core collections.

6.3. Gulf of Mexico Water isotope Survey

Analysts on board: Emily Osborne (AOML/NOAA), Ben Ross (FAMU), Leticia Barbero (AOML/CIMAS)

Science Leads: Julie Richey (USGS), Emily Osborne (AOML/NOAA),

Objective: Collect water samples across the GOM region to create a spatial map of seawater hydrogen (δ2H) and oxygen (δ18O) that can be applied to regional studies utilizing these isotopic tracers.

The isotopic composition of seawater (δ2H and δ18O) varies as a function of the global hydrologic cycle, and thus is a fundamental tracer of the movement of water through ocean circulation and fluxes of water in/out of the ocean. Organisms that live in the ocean may preserve the isotopic signature of that seawater in marine sediments, where their isotopic composition can then be used to reconstruct physical parameters (e.g., salinity, global ice volume, river runoff, glacial meltwater flux, etc.) in the palaeoceanographic record. The ability to quantitatively reconstruct these parameters in the past relies on modern calibrations using observational data. LeGrande and Schmidt (2006) published δ18Osw-salinity relationships for the tropical Atlantic Ocean and North Atlantic Ocean from a global gridded database of δ18Osw; however, no direct observations of δ18Osw from the Gulf of Mexico were available for the database. These GOM data gaps in our observational network of δ18O of seawater (δ18Osw) force modelers and paleoceanographers to make broad assumptions when conducting quantitative studies at present. To solve this problem, we conducted regional seawater isotope studies with high spatial resolution that will be coiled with a time series of δ18Osw-salinity profiles (0–1100 meters water depth) from a single site in the northern Gulf of Mexico spanning between 2008–2016.

GOMECC-4 represented a valuable platform to collect seawater samples from both nearshore and offshore sites spanning all quadrants of the GOM. Water samples were collected from the CTD niskin(s) following collection of nutrient and salts samples using 20 ml borosilicate glass crimp-top bottles. Sample bottles were triple rinsed with sample water from the rosette and filled to the base of the bottle neck. A crimp-top septum was used to seal sample bottles before storage in a cool dark place. Samples were not poisoned or chemically treated. Samples will be analyzed for oxygen and hydrogen isotopes at the USGS St. Petersburg Coastal and Marine Science Center using a Picarro L2130-i Isotopic Liquid Water Analyzer (Picarro, Inc. Santa Clara, CA).

A total of 124 seawater samples were collected along the 27 deg N, Tampa Line, Panama Line, Pensacola Line, Louisiana Line, Galveston Line, Brownsville Line, Tampico Line, Campeche Line, Merida Line, Yucatan Line, Catoche Line, Cancun Line, Florida Straits Line and the Venice Line. Samples collected were largely from the surface CTD niskin, however at a subset of targeted deep stations profiles were collected using ~12 niskins (alternating bottles on the 24-niskin rosette). For example, profiles were collected proximal to the Mississippi River and Rio

Grande outflow, as well as the Yucatan Channel. This will allow for the evaluation of spatial variability in the δ18Osw-salinity relationship in the Gulf that may arise from relative influences of Mississippi River discharge, loop current incursion and other mesoscale processes.

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