

NATIONAL CORAL REEF MONITORING PROGRAM

Standard Operating Protocol for Spectrophotometric pH Analysis (Climate)

Editors: Coral Reef Conservation Program, NCRMP Atlantic and Pacific
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National Coral Reef Monitoring Program Spectrophotometric pH Analysis SOP

Overview:

Spectrophotometric analysis is the most accurate method when determining the pH of a seawater sample. Through use of indicating meta-cresol purple (mCP) dye specific to the pH range of seawater samples (7.9-8.1), absorbance readings at set wavelengths of 434, 578, and 730nm (Carter et al. 2013, Lui et al. 2011) are used to determine the pH of a seawater sample based upon the shipboard methodology of Carter et al. 2013. Discrete measurement of pH is the most sensitive analysis out of the carbonate suite (DIC, TA, Density), therefore seawater samples must first be run on the specpH machine prior to any other analysis. This analysis requires 60mL of the total seawater sample, and it is not necessary to perform duplicate runs due to the accuracy and precision of the machine (Carter et al. 2013).

Materials:

Agilent Cary 8454 UV-Vis Spectrophotometer
10cm water-jacketed cylindrical quartz cell
Kloehn V6 Pump, P/N 55022
Lauda Alpha RA 12 Refrigerated Water Bath
Fluke 1523 Reference Thermometer with reference probe
Auto_pH_2018.exe Labview Software
2mM pure mCP dye, R=1.65 (USF)
Reference CRM, Tris Buffer in Synthetic Seawater (SIO)

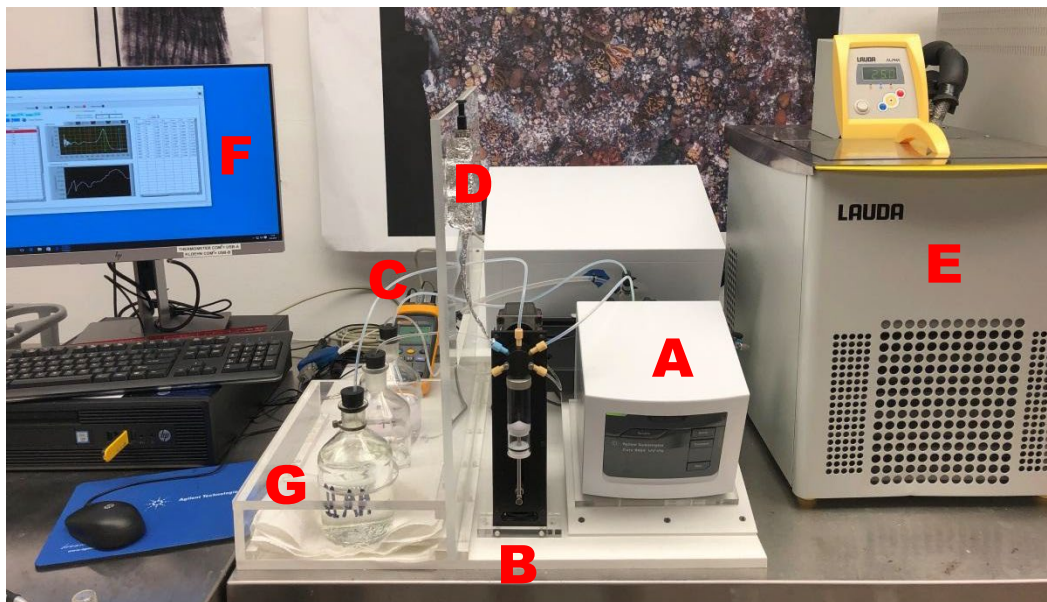


Figure 1. Spectrophotometric (spec) pH analysis set-up consisting of UV-Vis spectrophotometer (A), Kloehn V6 pump (B), Fluke Thermometer (C), mCP dye syringe (D), Lauda Circulating Waterbath (E), Auto pH Labview software program (F), and sample (G).

Setting up the SpecH System:

1. Power on the components of the machine (Fig 1):
 - a. Turn on the Agilent Cary 8454 UV-Vis spectrophotometer. The power button is located on the front face of the machine to the far right near the bottom, located behind the Kloehn pump. The machine takes around 10 minutes to warm up. The power button will be lit orange while the machine is warming up, and will then turn green when the machine is ready to be used.
 - b. Turn on the Kloehn pump via the red switch on the power cord, located underneath the spectrophotometer angled-platform. The pump should make an initial noise when it is turned on properly.
 - c. Turn on the Lauda water bath. Circulation temperature should be set at 25.0°C. Inspect all circulation hoses and junctions to check for any leaks prior to spec analysis.
 - d. Turn on Fluke 1523 Reference Thermometer. The probe is inserted into the outflow of the water coming from the enclosed spectrophotometric cell, this water should maintain a temperature of 25.0°C when the machine is stable. If temperature is too hot or too cold (room temperature could deviate the water bath temperature range), adjust the water bath temperature controls accordingly.
2. Open the *Auto_pH_2018.exe* Labview program on the computer desktop (Fig 2). See [Navigating through the Labview Program](#) below for assistance with the software.

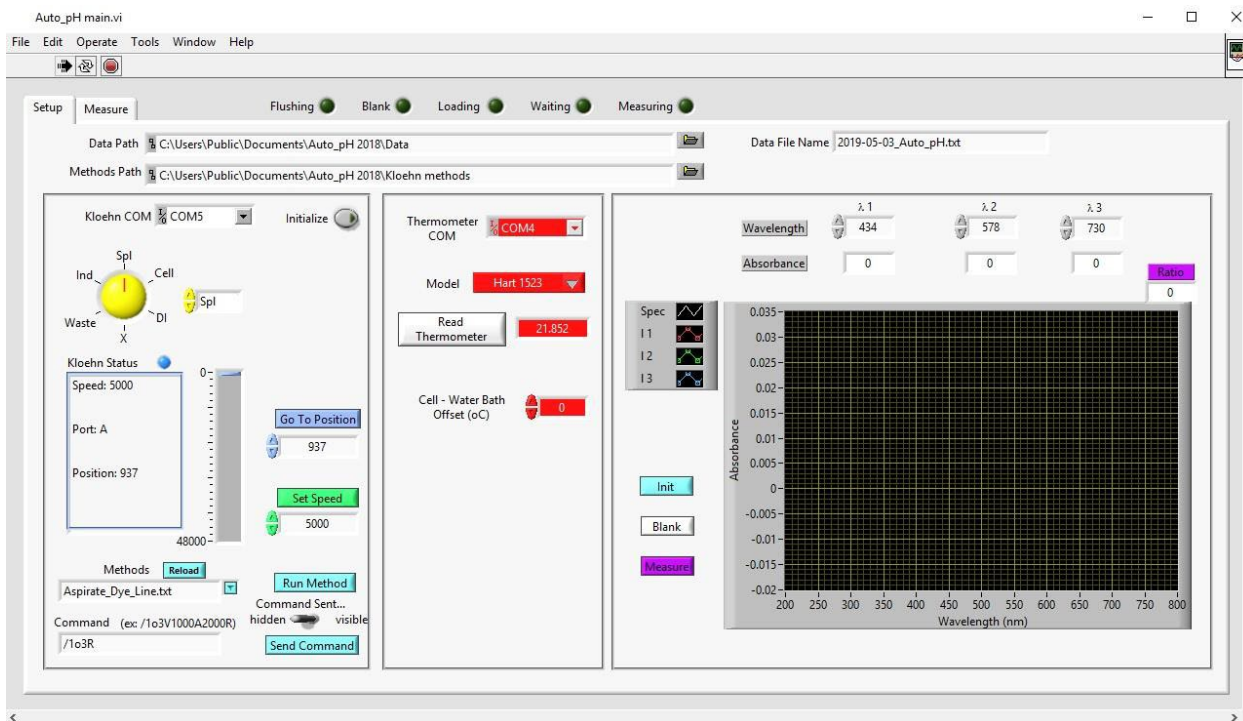


Figure 2. Auto_pH_2018.exe Labview program.

3. In the Setup page, set **Kloehn COM** to **COM5** and press the **Initialize** button. The pump should make a noise if communication is successful. Set **Thermometer COM** to **COM4**, press **Read Thermometer** to check for successful communication. The temperature should show in the box. Set **Cell-Water Bath Offset** to **0.0**.

4. Press the blue **Init** button to initialize the spectrophotometer. Wavelengths and absorbances above the spectrum graph should remain 434nm, 578nm, 730nm, with absorbances of 0. When the button becomes opaque, press **Blank** to zero the spectrophotometer. Check to make sure the spectrum reads 0 absorbance from 350nm on. Noise will be present in the UV region <350nm, that is ok. Any noise or deviation from an absorbance of 0 after 350nm (visible region) is indication that there may be an air bubble, lint, or smudge disrupting the light passing through the cell. Check cell, clean ends with a Kimwipe if necessary, and re-blank until a stable 0 absorbance reading is achieved for 350nm and on.
 5. Fill the syringe with fresh mCP dye.
 - a. Remove foil from tubing and syringe (the dye is photosensitive, which is why it should be shielded from light to prevent degradation)
 - b. Remove syringe from magnetic mount, and unscrew luerlock fitting from tip. Attach secondary free-standing luerlock tip + tube combo, then expel all remaining dye in the syringe into the proper chemical waste container.
 - c. Obtain bottle of mCP dye. Slowly draw up approximately 1-2mL of dye, then draw in some air to clear the tube and create an air bubble inside of the syringe.
 - d. Invert to catch all air bubbles and bring them to the tip of the syringe, then slowly expel the air until dye starts to flow out of the syringe and into the tube.
 - e. Carefully disconnect the secondary luerlock tip + tube combo, then reattach loaded, air-free syringe to the specpH luerlock setup.
 - f. Run the **Aspirate_Dye_Line.txt** method on the Setup page to flush the new dye through the tube and to rid all air bubbles. Repeat as many times as necessary, should take around 3 times to flush all the way through the line.
 - g. Once dye is primed in the tube and no air bubbles are present, replace foil to shield from light.
 6. The system is now ready for use. It is good practice to let the system sit and warm up for around 30 minutes, which in that time you can place the CRM or Tris Buffer in the water bath to reach a temperature of 25°C.
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Standardization:

Even though the spectrophotometric approach is extremely precise and accurate (± 0.005 pH units, Carter et al. 2013) standardization using a certified pH seawater sample is good practice to check the functioning of the system, and to catch drift of the spectrophotometer or any other errors before you start running a sample. Tris Buffer in Synthetic Seawater is the best possible standardization practice (Carter et al. 2013), and should be run once every two weeks or so, pending on specpH usage. Tris Buffer is expensive and comes in small quantity (125mL bottles), therefore it should not be used each time the specpH is up and running. The certified pH of Tris Buffer in Synthetic Seawater with a salinity of 35 at 25°C is **8.0932** (Nemzer and Dickson, 2005).

Reference Seawater CRMs should be used each time the machine is used to run samples. The pH of the current CRM batch can be calculated through the CO2sys software given the certified DIC, TA, and Salinity of the batch. A fresh, unopened CRM should ideally be used for standardization to get the most unbiased result. A previously opened CRM could have had outgassing potential, resulting in a skewed pH that the specpH will pick up. If using a previously opened CRM, take note that the resultant pH may be skewed.

How to Run a Standard:

1. Begin each specpH start up with a CRM or Tris Buffer standardization run to check machine function. Heat standard to 25°C in waterbath.

2. On the Measure page, input the standard name and salinity in the sample list.
 Example: CRM 175 33.458
 Tris 35
 3. Place heated standard in the “Sample” location within the specpH machine. Insert sample tube and rubber stopper into the sample, make sure sample tube is sampling from the middle of the sample rather than the bottom (to avoid drawing up any particulates that may have settled out).
 4. When ready, press **Measure Sample #** button to begin measurement. The program will measure the sample number that is listed in the box, i.e. 1.
 5. The Labview program shows realtime progress of the measurement process through the 5 buttons that illuminate at the top of the program. The 5 steps to the measurement process are:
 - i. **Flushing** = system will draw 20mL of sample into syringe, then flush into cell to rinse the previous sample out.
 - ii. **Blank** = system will draw 20mL of sample into syringe, then expel into syringe. System will wait 150 seconds for temperature to equilibrate, then a blank measurement will be taken.
 - iii. **Loading** = as the blank sample is equilibrating in the cell, the pump will load another 20mL of sample + mCP dye.
 - iv. **Waiting** = the pump will expel the 20mL of sample + mCP dye into the cell and will wait 150 seconds to equilibrate temperature.
 - v. **Measuring** = the system will take a spectrophotometric reading of the sample in the cell.
 6. When the standard is finished being analyzed, the result will show in the Results table.
 7. In the specpH Log Book, record the Sample ID, date of analysis, user initial, input salinity, output temperature, output Rm, and output pH.
 8. Compare the accuracy of the standard output pH to that of literature value. **Standard pH should be within 0.01 pH units of literature value to proceed with sample measurements.** Re-run the standard if not in range, or run a new standard if bias still exists. Troubleshoot machine if necessary.
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Measuring Seawater Samples:

1. Heat seawater sample to 25°C in water bath.
2. Input Sample ID and an arbitrary salinity of 34 into the sample list.

Note: Since pH needs to be run on fresh, unopened seawater samples, the exact salinity has not been determined yet. Arbitrary salinities are used in the specpH process, and then the final reported pH is determined post-analysis once the bottle salinity has been determined.

3. Insert sample tube and rubber stopper into neck of bottle, ensuring sample tube reaches only halfway down the bottle to prevent uptake of any particulate matter that may be at the bottom.
4. Press **Measure Sample #** button to begin measurement. The program will measure the sample number that is listed in the box, i.e. 1.
5. Be observant of any air bubbles that become trapped in the syringe or in the cell. Air bubbles, lint, or particulates can skew measurements. Rid if necessary.
6. When the sample is finished being analyzed, results will show in the Results table.
7. In the specpH Log Book, record the Sample ID, date of analysis, user initial, input salinity, output temperature, output Rm, and output pH. These results will then be used to redetermine the pH of the sample using the final bottle salinity (see “*Calculation of Corrected pH*” below).

Shutting Down Machine:

1. Once all samples have been run, the machine should be flushed with DI water to preserve the quality of the tubing, syringe, and cell.
2. In the Setup page under **Methods**, select **DI_RinseCell.txt** and hit **Run Method** to flush the cell with DI water.
3. Power off all components:
 - a. Turn off Fluke 1523 Reference Thermometer
 - b. Turn off Lauda water bath
 - c. Turn off Kloehn pump via the switch underneath the spectrophotometer angled platform
 - d. Turn off spectrophotometer
4. Exit out of Labview program. Export data onto a USB if desired from the **Data Path** folder instructed in the program.
5. Dispose of mcP/seawater/HgCl2 waste into a proper hazmat waste bin.

Navigating through the Labview Program:

The Labview program has two pages, *Setup* and *Measure*. You can toggle between these pages by selecting the tab at the top right of the Labview page. The Setup page is used to gain communication to the specpH setup, and to initialize the program. The Measure page is used to enter in a sample que, and measure the pH of samples.

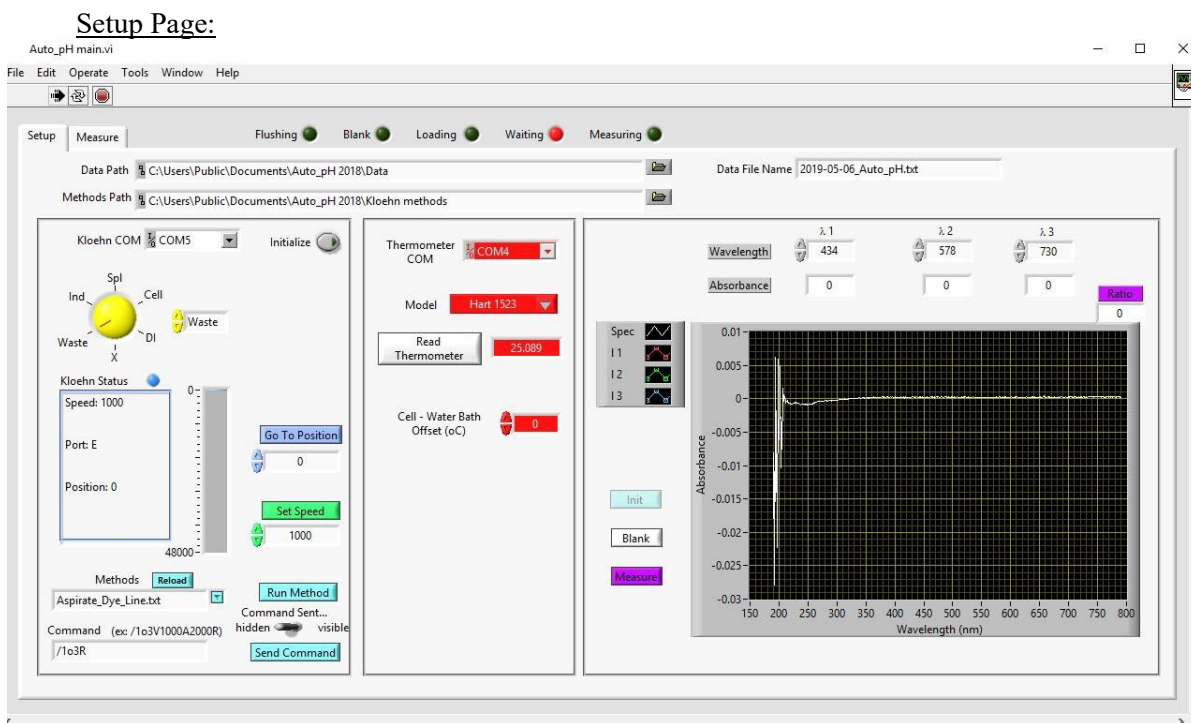


Figure 3. Setup page of Auto pH Labview program showing an active setup.

- **Data File Name** = automatically set to read as Date_Auto_pH.txt. These files will save to the directory established in “Data Path”. Name can be changed if desired.
- **Data Path** = directory where data files will be saved.
- **Methods Path** = directory where the method commands are read from, do not change path this unless command folder is relocated on the computer.

Kloehn Box:

- **Kloehn COM** should be set at **COM5**. Hit the initialize button to check that communication is established. Pump should make a noise if successful.
- The pump is automatically manipulated via preset commands from the program, however it has the option to be manually operated if needed. The yellow dial allows you to change the open port of the pump manually. The Kloehn pump has 46000 steps, and can be manually set.
 - i. **Go to position** = tell Kloehn to move to a desired step (0 is completely closed, 46000 is completely open)
 - ii. **Set Speed** = speed of Kloehn pump as it moves. Automatically set to 5000.
 - iii. **Kloehn Status** = command readout of manual operations to pump, i.e speed, port, and position that was set.
- There are a suite of methods that can be manually initiated to aid in the well-being of the machine, and to keep it clean after seawater use. To initiate a command, select the desired method from the drop down list, then click **Run Method**. Common method commands include:
 - i. *Aspirate_Dye_Line.txt* = flushes out mCP dye line to rid tube of bubbles, or if new dye was loaded into syringe this method helps to prime the line.
 - ii. *DI_RinseCell.txt* = flushes DI water through the cell to rinse out all seawater after sample analysis. Also useful when needing to rid trapped bubbled from inside of cell.

Thermometer Box:

- **Thermometer COM** should be set to **COM4**.
- **Model** = model of the Fluke thermometer, should be set to 1523.
- **Read Thermometer** = gives an instant reading of the thermometer, click this button after setting COM port to ensure successful connection.
- **Cell – Water Bath Offset** = temperature offset that can be manually entered. This is the degree offset between the temperature of the sample and the temperature of the water bath flow, used to correct the output inferred temperature of the sample the Labview program generates. Automatically set to 0.113, can be changed to 0.0 or whatever is justifiable for analysis.

Spectrum Box:

- **Wavelength** = wavelengths of the 3 peaks should be automatically set as 434, 578, and 730nm
- **Absorbance** = desired absorbance wanted for analysis, when blanking the system, an absorbance of 0 should be used.
- **Init** = Initialize the spectrophotometer
- **Blank** = Run a blank reading of the spectrophotometer
- **Measure** = Perform a measurement at the specified wavelengths and absorbances entered above

Measure Page:

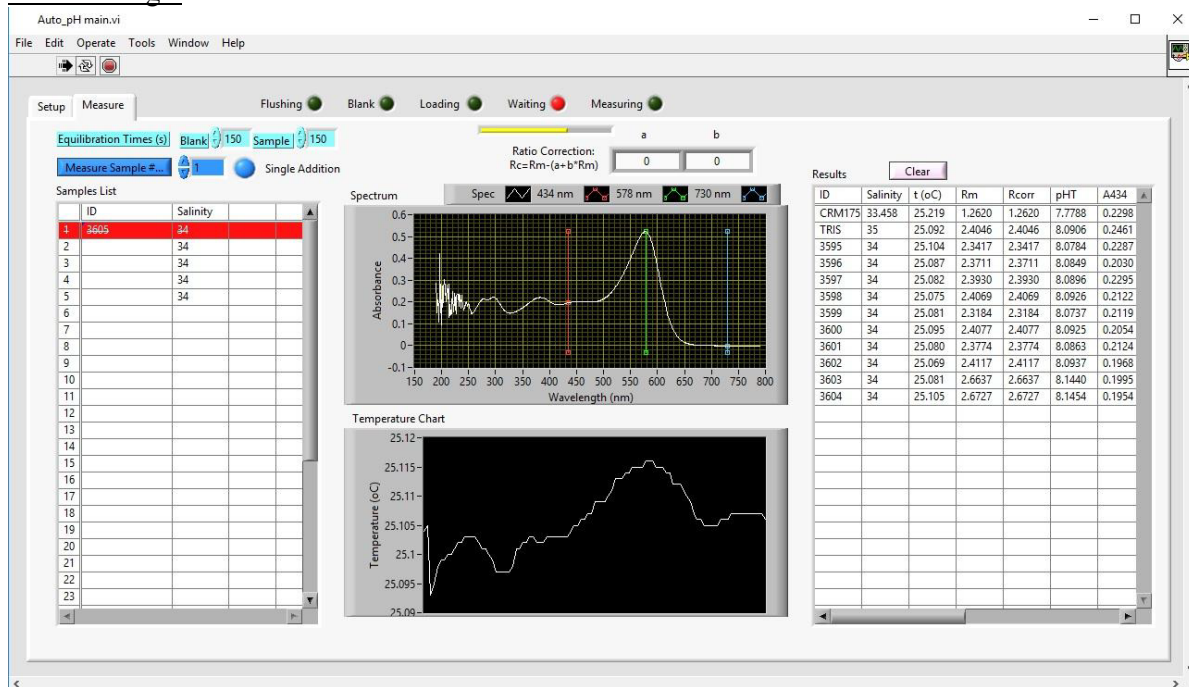


Figure 4. Measure page of Auto pH Labview program showing an active measurement.

- **Equilibration Time(s)** = time in seconds for sample to equilibrate to temperature in the cell. Can adjust as needed pending temperature stability determined by the setup. Automatically set to 150 seconds for both the blank and the sample.
- **Measure Sample #** = Tells program which sample # it will analyze from the que. Automatically set to 1, meaning it will run the sample that is in the first row.
- **Single Addition vs Double Addition** = Automatically set to a single addition analysis, can switch to double by clicking the blue button. Refer to Carter et al. 2013 for double addition methodology.
- **Ratio Correction** = adjust the A and B correction values determined via double addition analysis. Automatically set to 0.
- **Sample List** = Enter in a sample que, or go one by one if desired. Enter in sample ID and and input salinity (use 34 if exact salinity is unknown)
- **Spectrum** = shows the resultant sample spectrum with the set wavelengths highlighted in red, green, and blue
- **Temperature Chart** = shows continual temperature reading throughout the measurement. Temperature should be stable at 25.0°C.
- **Results** = cumulative list of analyzed samples with readouts of:
 - Sample ID
 - Salinity (input salinity)
 - Temperature (sample temperature, corrected from what probe is reading based on correction factor)
 - Rm (measured absorbance ratio)
 - Rcorr (corrected measured absorbance ratio)
 - pHT (calculated pH from Rm, temperature, and salinity values)
 - A434, A578, A730 (absorbance readings of each wavelength)

- Second set of all parameters for double-addition if applicable.

Calculation of Corrected pH:

pH values outputted from Auto pH Labview program are calculated via the following equation (Lui et al. 2011) using the inputted salinity, measured temperature, and absorbance peak measured ratio (Rm):

$$pH = a + b/T + c \ln T - dT + \log((R_m - e_1)/(1 - R_m e_3/e_2))$$

Where for salinity between 20 and 40, and temperature between 278.15 and 308.15K:

$S = \text{salinity}$

$T = \text{temperature in Kelvin}$

$a = -246.64209 + .315971S + 2.8855 \cdot 10^{-4}$

$b = 7229.238464 - 7.098137S - 0.057034S^2$

$c = 44.493382 - 0.052711S$

$d = 0.0781344$

$e_1 = -0.007762 + 4.5174 \cdot 10^{-5}T$

$e_3/e_2 = -0.020813 + 2.60262 \cdot 10^{-4}T + 1.0436 \cdot 10^{-4}$

Have no fear, this complex equation is already set in the Auto pH Labview program and the ACCRETE Bottle Database to formulate pH values; all that is required is to input the correct salinity, temperature, and Rm values.

Correcting pH using Excel file (for samples not in Bottle Database):

1. Open specpHCorrectedData.xlsx (coraldepot\carbonateChemistry\specpHData). All equation coefficients are preset, the calculated rows will need to be dragged down to copy the calculations and populate the cells (Fig 5).

SPEC MEASUREMENTS & CORRECTED pH										EQUATION COEFFICIENTS							
SAMPLE ID	DATE	USER	INPUT SALINITY	TEMP C	Rm	OUTPUT pH	CALC SALINITY (Denistometer)	CORRECTED pH	AVG CORRECTED pH	TEMP K	a	b	c	d	log(K2e2)	e1	e3/e2
example	3/18/2019	LC	35	25	2.4	8.091	34.69958721	8.09113877		298.15	-235.331	6914.264	42.66433	0.078134	7.6483464	0.005707	0.056753
junk1	7/24/2018	LC	35	25.012	2.159	8.0365		8.036537892		298.162	-234.893	6899.79	42.59579	0.078134	7.6465191	0.005707	0.056892
T1	7/24/2018	LC	35	25.031	2.217	8.053822969		8.053822969	32.8	298.181	-235.968	6935.06	42.76446	0.078134	7.6509867	0.005708	0.056563
T2	7/24/2018	LC	35	25.039	2.2911	8.069795284		8.069795284	33	298.189	-235.901	6932.89	42.75392	0.078134	7.650532	0.005708	0.056586
T3	7/24/2018	LC	35	25.042	2.1603	8.040649065		8.040649065	32.9	298.192	-235.934	6933.976	42.75932	0.078134	7.6506694	0.005709	0.056576
T4	7/24/2018	LC	35	25.048	2.2346	8.05721922		8.05721922	33	298.198	-235.901	6932.89	42.75392	0.078134	7.6504179	0.005709	0.056588
T5	7/24/2018	LC	35	25.054	2.2092	8.050972181		8.050972181	33.3	298.204	-235.800	6929.626	42.73811	0.078134	7.6498206	0.005709	0.056621
T6	7/24/2018	LC	35	25.052	2.2541	8.060558994		8.060558994	33.6	298.202	-235.700	6926.352	42.72229	0.078134	7.6493539	0.005709	0.056562
T7	7/24/2018	LC	35	25.055	2.2597	8.061759889		8.061759889	33.6	298.205	-235.700	6926.352	42.72229	0.078134	7.6493156	0.005709	0.056562
T8	7/24/2018	LC	35	25.071	2.2179	8.056724758		8.056724758	33.6	298.221	-235.700	6926.352	42.72229	0.078134	7.6491115	0.00571	0.056656
T9	7/24/2018	LC	35	25.082	2.2346	8.05600449		8.05600449	33.5	298.232	-235.733	6927.445	42.72756	0.078134	7.6491352	0.00571	0.056649
T10	7/24/2018	LC	35	25.08	2.3708	8.085340605		8.085340605	33.7	298.23	-235.666	6925.258	42.71702	0.078134	7.6488346	0.00571	0.056669
T11	7/24/2018	LC	35	25.078	2.225	8.054063936		8.054063936	33.4	298.228	-235.767	6928.536	42.73283	0.078134	7.6493522	0.00571	0.056637
T12	7/24/2018	LC	35	25.071	2.2009	8.04888067		8.04888067	33.3	298.221	-235.800	6929.626	42.73811	0.078134	7.6496094	0.00571	0.056625
T13	7/24/2018	LC	35	25.07	2.2968	8.069863148		8.069863148	33.5	298.22	-235.733	6927.445	42.72756	0.078134	7.6492583	0.00571	0.056646
T14	7/24/2018	LC	35	25.077	2.2603	8.061924539		8.061924539	33.4	298.227	-235.767	6928.536	42.73283	0.078134	7.649365	0.00571	0.056637
T15	7/24/2018	LC	35	25.073	2.1346	8.034005298		8.034005298	33.1	298.223	-235.867	6931.803	42.74865	0.078134	7.6499256	0.00571	0.056605
T16	7/24/2018	LC	35	25.064	2.2213	8.053102843		8.053102843	33.6	298.214	-235.700	6926.352	42.72229	0.078134	7.6495208	0.00571	0.056655
T17	7/24/2018	LC	35	25.085	1.4975	7.861462773		7.861462773	33.4	298.235	-235.767	6928.536	42.73283	0.078134	7.649265	0.00571	0.056639
T18	7/24/2018	LC	35	25.085	1.5574	7.880173021		7.880173021	33.4	298.235	-235.767	6928.536	42.73283	0.078134	7.649265	0.00571	0.056639
T19	7/24/2018	LC	35	25.089	1.5067	7.864171341		7.864171341	33.5	298.239	-235.733	6927.445	42.72756	0.078134	7.649046	0.00571	0.056611
T20	7/24/2018	LC	35	25.094	1.3344	7.806544011		7.806544011	33.5	298.244	-235.733	6927.445	42.72756	0.078134	7.6489822	0.00571	0.056562
T21	7/24/2018	LC	35	25.088	1.6966	7.920904284		7.920904284	33.6	298.238	-235.700	6926.352	42.72229	0.078134	7.6488946	0.00571	0.056611
T22	7/24/2018	LC	35	25.085	1.6977	7.92127945		7.92127945	33.6	298.233	-235.700	6926.352	42.72229	0.078134	7.6489584	0.00571	0.05666
T23	7/24/2018	LC	35	25.09	1.7139	7.92577124		7.92577124	33.6	298.24	-235.700	6926.352	42.72229	0.078134	7.6488691	0.00571	0.056611
T24	7/24/2018	LC	35	25.09	1.7456	7.934621688		7.934621688	33.6	298.24	-235.700	6926.352	42.72229	0.078134	7.6488691	0.00571	0.056611
T25	7/24/2018	LC	35	25.084	2.1953	8.04699538		8.04699538	33.6	298.234	-235.700	6926.352	42.72229	0.078134	7.6489456	0.00571	0.05666
T26	7/24/2018	LC	35	25.094	2.2251	8.053429038		8.053429038	33.7	298.244	-235.666	6925.258	42.71702	0.078134	7.6486559	0.00571	0.056673
T27	7/24/2018	LC	35	25.098	2.2354	8.055860164		8.055860164	33.7	298.248	-235.666	6925.258	42.71702	0.078134	7.6486048	0.00571	0.056674
T28	7/24/2018	LC	35	25.094	2.1613	8.039119423		8.039119423	33.6	298.244	-235.700	6926.352	42.72229	0.078134	7.6488181	0.00571	0.056662
T29	7/24/2018	LC	35	25.113	2.7189	8.15457505		8.15457505	33.7	298.263	-235.666	6925.258	42.71702	0.078134	7.6484134	0.00571	0.056678
T30	7/24/2018	LC	35	25.104	2.5892	8.12980496		8.12980496	33.6	298.254	-235.700	6926.352	42.72229	0.078134	7.6486905	0.00571	0.056665
T31	7/24/2018	LC	35	25.132	3.4861	8.130636545		8.130636545	33.6	298.272	-236.200	6936.363	43.27338	0.078134	7.6484882	0.00571	0.056667

Figure 5. specpHCorrectedData Excel file.

2. Fill in Sample ID, Date, User, Input Salinity, Temp C, Rm, and Output pH columns from the recorded data in the specpH logbook. Copy in the calculated salinity value obtained through the densitometer measurement and subsequent salinity conversion.
3. Populate the “Equation Coefficients” section by dragging down each row to continue the calculation.
4. Last, drag down the “Corrected pH” column to calculate the corrected pH value using the new corrected salinity value and populated equation coefficients.

Correcting pH via “SpecpH” Bottle Database Tab:

1. Open BottleDatabase.accdb (coraldepot\carbonateChemistry). Double click on the “Spec pH” tab to open (Fig 6).

ID_Sample	Date	User	INPUT_Salinity	TEMP_C	Rm	Click to Add
2368	3/20/2019	LC	34.000	25.164		2.3518
2369	3/20/2019	LC	34.000	25.118		2.3708
2370	3/20/2019	LC	34.000	25.175		2.3418
2371	3/20/2019	LC	34.000	25.106		2.3777
2372	3/20/2019	LC	34.000	25.084		2.4103
2373	3/20/2019	LC	34.000	25.139		2.4526
2374	3/20/2019	LC	34.000	25.125		2.3703
2375	3/20/2019	LC	34.000	25.134		2.3594
2376	3/20/2019	LC	34.000	25.139		2.1034
2377	3/20/2019	LC	34.000	25.105		2.3389
2378	3/20/2019	LC	34.000	25.175		2.3379
2379	3/21/2019	LC	34.000	25.140		2.5766
2380	3/25/2019	LC	34.000	25.201		2.4939
2381	3/25/2019	LC	34.000	25.211		2.5867
2382	3/21/2019	LC	34.000	25.170		2.5668
2383	3/21/2019	LC	34.000	25.155		2.5736
2384	3/25/2019	LC	34.000	25.214		2.5635
2385	3/25/2019	LC	34.000	25.227		2.5673
2725	3/18/2019	LC	34.000	25.203		2.0906
2726	3/18/2019	LC	34.000	25.194		2.4396
2727	3/18/2019	LC	34.000	25.201		2.3883
2728	3/18/2019	LC	34.000	25.186		2.3743
2729	3/18/2019	LC	34.000	25.183		2.4227
2730	3/18/2019	LC	34.000	25.196		2.4598
2731	3/18/2019	LC	34.000	25.170		2.4470
2732	3/18/2019	LC	34.000	25.164		2.4139
2733	3/18/2019	LC	34.000	25.166		2.4124
2734	3/18/2019	LC	34.000	25.170		2.4143
2735	3/18/2019	LC	34.000	25.138		2.4333
2736	3/18/2019	LC	34.000	25.143		1.9761
2737	3/18/2019	LC	34.000	25.148		2.0770
2738	3/18/2019	LC	34.000	25.135		2.3340
2739	3/19/2019	LC	34.000	25.153		2.3737
2740	3/19/2019	LC	34.000	25.192		1.9633
2741	3/19/2019	LC	34.000	25.176		1.8235
2742	3/19/2019	LC	34.000	25.179		1.8261
2920	4/5/2019	LC	34.000	25.191		1.8798
2921	4/5/2019	LC	34.000	25.167		1.9578
2924	4/5/2019	LC	34.000	25.238		1.9235
2925	4/5/2019	LC	34.000	25.184		1.8401
2942	4/5/2019	LC	34.000	25.220		2.0753

Figure 6. Spec pH tab within the Bottle Database.

2. Scroll to the bottom of the page. Input sample tag number in “ID_Sample”, and remaining columns respectively as recorded in the specpH logbook. It is critical to enter in the sample ID properly, as this column is linked to other tabs in the workbook and will pull the corresponding information for the specific sample.
3. Right-click the specpH tab, and select Save to save data. You may close the tab once all data has been entered.
4. Open the “Spec pH_CORRECTED” Query tab to observe calculated results. The query pulls from the inputted spec values from the “Spec_pH” tab, as well as values in the “Final_Query” tab. **Final pH can ONLY be calculated once all other Bottle Database tabs are completely populated**, i.e. once a sample has been run on the specpH, DIC machine, TA machine, and densitometer. An error in any of the tabs will result in “#Error” or be observed in the columns, meaning the Query cannot access the calculated salinity value from the “Final_Query” tab. If a problem persists, double check to ensure the “Final_Query” tab does not have any problems with data compilation and calculation.
5. When all carbonate data has been entered into the Bottle Database, the corrected pH can be observed in the “Corrected pH” column at the far right of the “Spec

pH_CORRECTED” Query. Equation coefficients from the subsequent pH equation can also be observed (CONST_a...e3/e2).

References:

Carter, B. R., J. A. Radich, H. L. Doyle, and A. G. Dickson. 2013. An automated system for spectrophotometric seawater pH measurements. *Limnol. Oceanogr.: Methods* 11:16-27

Lui, X., M. C. Patsavas, and R. H. Byrne. 2011. Purification and Characterization of meta-Cresol Purple for Spectrophotometric Seawater pH Measurements. *Environ. Sci. Technol.* 45: 4862-4868

Nemzer, B. V. and A. G. Dickson. 2005. The stability and reproducibility of Tris buffers in synthetic seawater. *Mar. Chem.* 96:237-242

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