Collection and processing of benchmark water samples for DuraFET type pH sensors

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Overview and Background:

Continuously recording pH sensors are field-calibrated with discrete water samples, which are analyzed in the lab for salinity, pH, and total alkalinity. Honeywell Durafet-based pH sensors include SeaFET and ipHat. This protocol describes collection and processing of those benchmark samples.

Methods:

Field Collection: Discrete bottles samples are collected as physically close to the pH sensor as possible and approximately in concurrence with the sensor's voltage measurement by a SCUBA diver using a Niskin bottle, or by hand, as appropriate for the site (moored underwater or near-surface intertidal). Water samples are poisoned with saturated mercuric chloride immediately after collection, sealed, and stored in a cool, dark place until analysis.

Laboratory Processing: Salinity, pH, and total alkalinity in the water samples are measured in the laboratory. Seawater pH was measured using a spectrophotometric method with indicator dye, unpurified m-cresol purple (Sigma-Aldrich, SOP 6b, Dickson et al. 2007). Seawater pH was measured with triplicate samples, and absorbances were measured at 730 nm. 3 mL of seawater was added to a cuvette and measured, and then samples were re-measured after 50 uL m-cresol purple was added. Total alkalinity (AT) was measured using an automated, open-cell potentiometric titration (SOP 3b, Dickson et al. 2007) with a Mettler-Toledo T5 titrator and a DGI111-SC pH probe (Mettler-Toledo). The probe was calibrated using a Tris buffer (A. Dickson Laboratory, Scripps Institution of Oceanography). Titrations were performed using certified acid titrant (~0.1M HCl, 0.6 M NaCl; A. Dickson Laboratory, Scripps Institution of Oceanography). Certified reference materials (CRMs) from A. Dickson Laboratory, Scripps Institution of Oceanography, were used to determine the accuracy and precision of the titrations daily before experimental samples were measured and then again between every approx. 6 samples. Analyzed CRMs were accurate within 10 micromol kg-1. For all analyses on the titrator, 98-102 g of seawater was aliquoted and run. The detailed total alkalinity and water chemistry measurement can refer to the Appendix of this document.

Data Processing: The suite of carbonate chemistry parameters was calculated using the CO2CALC program. Along with temperature and salinity, CO2calc algorithms produce a suite of carbonate system parameters using only two measured variables (of five potential inputs); the remaining three possible inputs are also calculated as algorithm outputs. In our samples, pH, TA

and/or TCO2 are routinely measured, and precedence of use is: 1) measured pH and TA (total alkalinity), 2) measured pH and TCO2 (total dissolved inorganic carbon). Use of TA and TCO2 is not recommended (Hoppe et al, 2012). In situ carbonate system parameters were also calculated using temperature recorded in the field ("adjusted" outputs), per Robbins et al. (2010), with CO-2 constants K1, K2 from Mehrbach et al. 1973 refit by Dickson and Millero, 1987 and pH expressed on the total scale (mol kg-SW-1).

References:

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Appendix 1, Total Alkalinity Protocol

Hofmann Lab – Total Alkalinity Protocol (Gabrielle Titrator, Lenovo Laptop)
DRAFT (Umi, 2017/05/12)

1. Turn on Titrator (front button)
2. Titrant bottle:
   a. Swirl/invert to incorporate condensation
   b. Take off parafilm
   c. Uncork stopper, leave it on top of bottle with air gap
3. Wait until Titrator says "connecting", open laptop, and open LabX
   a. Go to "resources" tab, make sure titrator is "ready" with blue check mark
4. Purging the acid line – do this to get rid of bubbles in line
   a. Discard DI water from cup on titration stand, put it back on
   b. On titrator screen: "manual"-> burette -> purge (rinse)
   c. Lift tube/stopper out of titrant bottle to introduce a large bubble through the system, let it carry all the bubbles out
   d. Flick bubbles in line to dislodge, purge as necessary (Rinse tip ←)
   e. Bubbles on plunger are ok as long as they do not move.
   f. Remove purge cup and discard in waste container.
5. Setting up probe/stand for first sample
   a. Move probe to hole in titration stand labeled "probe", being careful not to bump the tip. Check fluid level in probe.
   b. Undo the rubber stopper on top/side of probe to vent.
   c. Plug in the bubbler, put in titration stand - should read 150-200 on flow meter
   d. Rinse off all probes with DI and dab dry (don't touch tip of electrode)
6. Sample layout
   a. Run 2~3+ filter seawater until reading stabilizes, then run CRM within 5-10 µmol/kg
   b. Run 6-10 samples and finish with CRM.
7. Running a sample:
   a. Tare titration cup, pour sample into cup being careful not to splash onto sides
   b. Aim for 98-102 grams of sample. Recofd measurement whenever it stabilizes first (will decrease slowly with evaporation)
   c. In binder: record unique sample name, mass, and salinity
   d. On titrator screen: Methods -> EQP Rivest 2012 -> Start
   e. Enter mass and name of sample, and run – should take 13-15min
   f. When done, pull sample off, waste, and rinse/dry off electrodes.
8. Calculating TA:
   a. In LabX -> Go to Data and find your sample. Might have to hit "refresh" button. "red" status is OK here.
   b. Go to "measured values", click bottom right arrow to open up the spreadsheet view, then right-click -> copy all as text
   c. Paste into desktop/titrationGoodies/~~Result_TA~~.csv (Result_TA_B134.csv), and save.
   d. Open R script in same directory, hit ctrl+shift+s (or hit source button) to run
   e. When prompted, enter salinity, name of sample, and mass.

i. TA(corr) accounts for dilution by addition of mercuric chloride. Use for any samples that our lab poisoned
ii. TA can be used for CRM, FSW, or any unpoisoned samples (uncommon)
iii. TA is recorded to 2 decimal places.

f. Results will be saved in rTritrationOutput.csv. Do NOT run the R script with this open. Do NOT change this file (unless necessary), and always try to close without saving.

9. Wrapping up titrator:
   a. Rinse off probes
   b. Put rubber plug back on pH probe, put back in buffer.
   c. Unplug bubbler and pull tube out.
   d. Fill titration cup with DI, put on titration stand.
   e. Re-plug titration bottle, wrap tightly with parafilm.
10. Cleaning bottles:
   a. Dump rest of sample in waste container
   b. Wipe off grease with kimwipes
   c. 3 rinses into waste container, try to rinse outside briefly as well
   d. rinse in sink with soap, rinse out all soap residue
   e. remove sharpie with sponge+elbow grease, or ethanol/methanol
   f. bottles go in 10% acid bath for 24h, then air dried on rack above sink
   g. bands/clips get rinsed into waste container, then rinsed in sink and air dried
11. Sources of error:
   a. Titrant evaporated: would be more concentrated, so values would read low
   b. Bubbles introduced: titrator would think you used more acid than you did, so values would read high
   c. Lots of bubbles in line: check stopcock and possibly replace (they get salty over time)
   d. Electrode old: re-run Tris Calibration
12. Have a great day!

Appendix 2, pH Water Chemistry Protocol

pH Water Chemistry Protocol

(Determination of pH using indicator dye m-Cresol purple)
Created by Maddie Housh (9/12/2017)
Hofmann Lab

1. Turn on water bath (should be set to 25C)
2. Turn on spectrophotometer – takes ~5 min. to warm up
   a. Press ENTER key
   b. Press F4 for PC control
3. Open UV Spec
   a. Open previous UV Spec file
      i. Highlight number column and copy
      ii. Delete previous data, then paste numbers back
      iii. Rename file with current date and save
   b. Press "connect to PC"
   c. Set wavelength to 730 nm **("Go to WL")**
   d. Press auto zero (with lid closed and nothing in slot)
4. Select 3 sample bottles you will be measuring pH on
   a. Enter bottle names into excel file (if using field samples)
   b. Transfer 25 mL of water from each bottle into 3 scintillation vials
   c. Cap vials, making sure there are no air bubbles
   d. Put vials into water bath for 20 min.
5. Pour ~100 mL of each sample bottle into plastic cups and measure salinity on each
   a. Enter salinities into excel file
6. Prepare workspace while vials are coming to temperature
   a. Lay out large kimwipe
   b. Gather 3 large pipette tips
   c. Swirl m-Cresol dye
   d. Clean and dry cuvette
   e. Make drying rod
7. Measurement procedure
   a. Pipette 3 mL of seawater sample from vial into clean cuvette
   b. Carefully clean exterior of cell with a kimwipe
   c. Place cuvette in spec and make sure absorbance reads 0.044-0.046
   d. Press key with blue tape to run sample
   e. Pipette 50 uL of m-Cresol dye into cuvette, cap and invert 3 times to mix
   f. Return cuvette to spec and run the sample again
   g. Take temperature of water in cuvette using thermocouple
      i. Record temperature in "comments" column of UV spec file
   h. Dump water from cuvette into waste beaker and rinse with DI squirt bottle 3 times
   i. Dry cuvette with drying rod
   j. Repeat this procedure for each vial

i. NOTE: measured absorbances at 730 nm for each bottle sample should be within 0.01 of each other to ensure accuracy

8. Data entry
    a. Save UV spec document after measurements are complete
    b. Create new sheet in excel file and name with current date
    c. Copy entire UV spec spreadsheet and paste into new sheet on excel file
    d. Sort data A→Z (this separates measurements without dye from measurements with dye)
    e. Copy NO DYE measurements and paste into Sheet 1
    f. Copy DYE measurements and paste into Sheet 1
    g. Copy temperature measurements and paste into Sheet 1
    h. Save excel file
9. Clean up
    a. Empty remaining contents of vials into waste container
    b. Dispose of solid waste
    c. Rinse vials and cups with DI 3x into waste container
    d. Rinse vials and cups with DI in sink 3x and set out to dry
10. ALL DONE!