Quality Assurance Project Plan
For
Shell Hash for Buffering Mudflat Acidification: Interactive Effects of Crush Size and Density on Sediment Porewater Carbonate Chemistry

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1.1 Title and Approvals

Quality Assurance Project Plan for Shell Hash for Buffering Mudflat Acidification: Interactive Effects of Crush Size and Density on Sediment Porewater Carbonate Chemistry

Approvals

12/28/2020

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1.2 Table of Contents

List of Attachments

Attachment 1. Standard Operating Procedure for Spectrophotometric Determination of the Carbonate Chemistry System in Seawater by Nitric Acid Titration.

List of Abbreviations and Acronyms

CBEP	Casco Bay Estuary Partnership
DEI	Downeast Institute
DIC	Dissolved inorganic carbon
pH_T	Total pH
RSD	Relative standard deviation
SOP	Standard Operating Procedure
TA	Total alkalinity
WABRPOA	Washington State Blue Ribbon Panel on Ocean Acidification

1.3 Contacts and Distribution List

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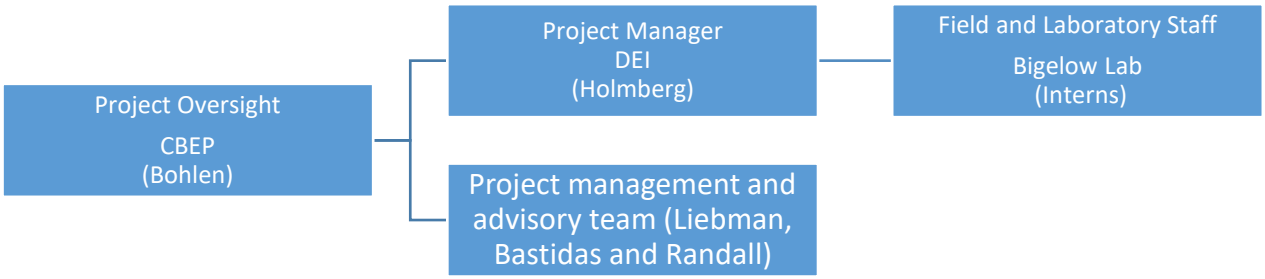
1.4 Project Organization

Casco Bay Estuary Partnership (CBEP) has contracted with Downeast Institute (DEI) to conduct field experiments on the use of oyster shell hash to remediate impacts of coastal acidification on growth of shellfish. This experiment will be conducted in the laboratory at DEI.

Figure 1. Project Organization



1.5 Problem Definition/Background

1.5.1 Origins of project and funding

Maine's Commission to Study the Effects of Coastal and Ocean Acidification issued a report stating: "Spread shells or other forms of calcium carbonate in bivalve areas to remediate impacts of local acidification". In response, the Maine Coastal Program conducted a program to collect oyster shells from participating restaurants in Portland for future spreading on shellfish habitat. This program was called the *Ocean to Plate to Ocean: Eat-Recycle-Restore* project. Sixty cubic yards of oyster shell material is currently stored in Portland awaiting a test to see if sediment could be remediation of coastal acidification. The Maine Coastal Program is in the process of securing a research license for this project. This Pilot Project is funded with a grant from the EPA Climate Ready Estuaries Program.

1.5.2 Science background

Marine ecosystems are under pressure from increasing atmospheric [CO_2] due to anthropogenic carbon emissions, which have given rise to ocean warming and acidification (IPCC 2013). Marine calcifiers are particularly vulnerable due to the often-inhibitory impact of decreasing seawater pH and carbonate ion availability on biogenic calcification (Cyronak et al. 2016, de Putron et al. 2011). Although marine sediment porewater is naturally acidified and undersaturated with respect to aragonite relative to the overlying seawater, there is concern that increasing acidification and undersaturation of the overlying seawater will push porewater below its natural minima, which may result in calcifier biodiversity shifts in the sediment (Green et al. 2009, Widdicombe et al. 2009). Several studies have tested the hypotheses that buffering marine sediments with crushed bivalve shell recycled from the seafood industry, i.e. shell hash, will result in desirable increases in sediment porewater alkalinity, pH, and saturation state of

aragonite (Ω_{Ar}), and/or concomitant benefits to bivalve abundance (Green et al. 2009,2013; Ruesink et al. 2014; Clements et al. 2016; Greiner et al. 2018; Dethier et al. 2019; Drylie et al. 2019; Beal et al. 2020); in addition, some coastal communities have recommended the spreading of shell hash on mudflats to benefit bivalve recruitment and survival (WABRPOA 2012, Bentley & Schneider 2015). Results are mixed, however, with about half of studies reporting no impact of shell hash addition – perhaps due in part to the lack of standardization of shell crush size and density treatments and differing methodology for porewater carbonate chemistry analysis.

1.5.3 Project Objectives

The goal of the present study is to determine which (if any) combination of various shell crush size and density treatments has the greatest impact on sediment porewater carbonate chemistry in the laboratory in order to inform future, larger-scale studies testing ocean acidification mitigation strategies in the field.

1.6 Project/Task Description and Schedule

The experiment will begin in December of 2020 and run for two weeks.

Table 1: Project schedule.

	Sampling Activities	Laboratory Measurements
Week 0	Set up Experiment Collect sediment from mudflat Mix oyster shell with sediment	
Weeks 1 and 2	Run experiment, collect overlying water in mid-point of tank for temperature, salinity, pH and TA	Seawater temperature, salinity, pH_T, TA
End of Week 2	End experiment, collect sediment and measure porewater	Porewater temperature, salinity, carbonate chemistry (pH_T, TA, DIC, pCO_2, and Ω_{Ar})
Week 3	Initial analysis of data	
Week 4	Begin writing up results	
Week 5	Draft project final report	

1.7 Data Quality Objectives and Criteria for Measurement Data

1.7.1 Data Quality Objectives

Our project objectives are to evaluate whether oyster shell hash crush size or density alters sediment porewater carbonate chemistry, as measured by pH_T, TA (total alkalinity), DIC (dissolved inorganic carbon), pCO_2, and especially Ω_{Ar} (which is the estimated saturation state of aragonite and is a calculated

value). To determine if there is a difference between the treatments, we expect to see a difference greater than 1% in porewater Ω_{Ar}.[1]

1.7.2 Data quality Indicators

Here are the data quality indicators and performance goals for each analytical measurement in the experimental setup for both the continuous overlying seawater system (Table 2a) and for porewater measurements (Table 2b).

In addition to the analytical performance goals, the following indicators are incorporated into the study design:

Precision: The study design includes five replicates per treatment.

Comparability: Samples will be taken before and after the two-week experiment.

Representativeness: Sediment will be measured from the mudflat 24 hours post-collection (prior to the start of the experimental trial).

Completeness: At least two replicates are needed to compute statistical tests.

*Table 2a. Analytical methods and performance goals for sensitivity (detection limit), range, accuracy and precision for **continuous overlying water measurements** mounted on a float in running seawater setup. RSD is relative standard deviation.*

Parameter	Purpose	Equipment	Detection Limit and Range	Accuracy (RSD)	Precision (RSD)
pH	Calculate full carbonate chemistry parameters; Ensure stable pH during experiment	Oakton EW-35805-67 refillable, glass body, double junction pH electrode	0.01, -2 – 16 pH units	0.13%	0.04%
temperature	Ensure stable temperature during experiment;	temperature probe (Neptune Systems PRBTMPJR)	0.10, 0 – 100 °C	3.33%	0.67%

[1] This is the minimum detectable difference in omega using this method after accounting for uncertainty. Between-replicate variation is likely to be higher, and of course a meaningful treatment effect would be one that exceeds that variation.

	Calculate carbonate chemistry parameters				
conductivity (salinity)	Ensure stable salinity during experiment; Calculate carbonate chemistry parameters	conductivity probe (Neptune Systems)	0.10, 0 – 45 ppt	1%	0.31%
total alkalinity	Calculate full carbonate chemistry parameters	automatic titration colorimeter (Neptune Systems Trident)	10,1748 – 5242 µmol/kg	0.72%	0.72%

*Table 3b. Analytical methods and performance goals for sensitivity (detection limit), range, accuracy and precision for **experimental design** porewater measurements. RSD is relative standard deviation.*

Parameter	Purpose	Equipment	Detection Limit and Range	Accuracy (RSD)	Precision (RSD)
pH	Calculate full carbonate chemistry parameters	UV/Vis spectrophotometer (Agilent Cary 60) equipped with a Peltier-thermostatted cell holder (Agilent G6870A)	0.001, 6.91 – 8.77 pH units	0.01%	0.01%
temperature	Calculate carbonate chemistry parameters	handheld meter and probe (YSI EcoSense pH100A, 605375)	0.10, -10 – 120 °C	3.33%	0.67%
conductivity (salinity)	Calculate carbonate chemistry parameters	handheld meter and probe (Oakton SALT 6+, EC-CONSEN91B).	0.1, 1 – 50 ppt	1%	0.31%
nitrate	Calculate full carbonate	UV/Vis spectrophotometer	0.1, 335 – 526 µM	0.20%	0.20%

(total alkalinity is calculated from the nitrate measurement)	chemistry parameters	(Agilent Cary 60) equipped with a Peltier-thermostatted cell holder (Agilent G6870A)			

1.8 Special Training Requirements/Certification

Doctor Robert Holmberg is a postdoc at DEI, he received his Ph.D. in 2019 and has established the ocean acidification laboratory at DEI.

1.9 Documents and Records

Electronic data and any paper data sheets produced from the study will be maintained by Dr. Holmberg for a period of no less than five years. A copy of the QAPP will be shared with all project partners.

2. DATA GENERATION AND ACQUISITION

2.1 Experimental Design and Sampling Methods

The experiment will consist of 3 shell crush size treatments (0.5, 2.5, 5 mm particle diameter), 3 shell density treatments (5, 10, 15 g shell/100 cm^2 sediment), and a control treatment in which no shell is added in a full factorial, 5x-replicated design. Samples of *Crassostrea spp.* shell discarded from the seafood industry (collected by the Ocean to Plate to Ocean project) will be cleaned, cured, and ground to the particle size prescribed by each of the 3 levels of the shell crush factor using an industrial shell grinder (Shellfish Equipment, Inc.). Crushed shell will be sieved through a series of graded mesh sieves to achieve greater uniformity of particle size, and confirmed with microscopic analysis. 55 benthic sediment cores will be collected at low tide from a mudflat in Beals, Maine, USA using a glass bowl (15.24 cm diameter, 6.99 cm depth, 0.95 L) modified with a hole drilled in the center of its base. Each sediment core will be transferred right-side-up into one of 55 glass bowls (identical to the corer except drilled with multiple, smaller drainage holes) and sealed with plastic covers for transport to the lab. Upon arrival at the lab, bowls will be uncovered, 50 of the bowls will be randomly assigned treatments, and shell will be added and raked into the upper 0.5 cm of sediment as necessary (i.e. 5 with no shell as control and 5 bowls with the crush shell size and density at each of the 9 levels of treatment). **The remaining 5 bowls will not be assigned treatments and reserved for sampling initial carbonate chemistry conditions.** All bowls will be interspersed randomly on the floor of an indoor flow-through seawater basin in an arrangement of 5 rows and 11 columns. Untreated seawater pumped in continuously from Black Duck Cove (Beals, Maine, USA) will submerge the experimental units to a depth of 0.75 m. After submersion for 24 hours, the experimental trial will begin and run for an additional 2 weeks, during which the basin will be fully drained of seawater for 3 hours and refilled twice daily to

simulate tidal cycling. An actuating ball valve controlled by a timer will be fitted to the seawater input pipe to automate refilling.

2.2 Sampling Location

The study will be carried out at the Downeast Institute, which has running seawater and other controlled conditions, as described here: https://downeastinstitute.org/our-facility/for-scientists/ocean-acidification-laboratory/.

2.3 Sampling methods

2.3.1 Overlying seawater carbonate chemistry datalogging and analysis

During the experimental trial, overlying seawater pH (total hydrogen ion scale, pH_T), temperature, and salinity will be monitored continuously and logged once per minute using a refillable, glass body, double junction pH electrode (Oakton EW-35805-67), temperature probe (Neptune Systems PRBTMPJR), and conductivity probe (Neptune Systems) mounted on a float within the flow-through seawater basin and interfaced with an aquarium controller system (Neptune Systems Apex). The pH electrode will be calibrated daily using tris and 2-aminopyridine artificial seawater pH buffers (Dickson et al. 2007) composed to match ambient seawater salinity (~32 ppt) and pH-calibrated at near-ambient seawater temperature (15 °C). All pH_T measurements will be temperature compensated. Overlying seawater total alkalinity (A_T) will be monitored and logged intermittently every 6 hours using an automatic titration colorimeter (Neptune Systems Trident) interfaced with the same aquarium controller system. Upon conclusion of the experimental trial, the remaining carbonate chemistry parameters (dissolved inorganic carbon, DIC; partial pressure of CO_2, pCO_2; saturation state of aragonite, Ω_{Ar}) will be calculated from mean pH_T, mean temperature, mean salinity, and A_T for each 6-hour interval using CO2SYS v2.1 (Pierrot et al. 2006).

2.3.2 Sediment porewater carbonate chemistry sampling

After 24 hours submersion, the non-experimental bowls will be sampled for porewater carbonate chemistry. Prior to sample collection, the in situ temperature of the upper 0.5 cm of sediment in each unit will be recorded using a handheld meter and probe (YSI EcoSense pH100A, 605375). One sediment plug will be collected from each unit using fabricated PVC corers (0.5 cm depth, 12.74 cm inner diameter, approximately 63.74 cm3 sediment). Each sediment plug will be scraped from the corer into a 50 ml centrifuge tube and centrifuged at 1,500 rpm for 5 minutes to separate porewater from sediment. Prior to removal, the salinity of the supernatant from each sample will be recorded using a handheld meter and probe (Oakton SALT 6+, EC-CONSEN91B). The supernatant will be removed from its tube and filtered using disposable 0.45 µm syringe filters, and 3 ml of sample will be distributed into each of 4 rectangular quartz cuvettes (1 cm pathlength).

2.4 Sample Handling and Custody

Plugs from each experimental unit will be sampled and centrifuged on the same day as collection and kept at room temperature. Carbonate chemistry analyses will be conducted on the same day within 6 hours of sampling.

2.5 Analytical Methods

2.5.1 Carbonate chemistry / acidification for overlying water

Overlying seawater analysis is described in section 2.3.1 above.

2.5.2 Carbonate chemistry / acidification for porewater

Porewater pHT and remaining carbonate chemistry parameters (AT, DIC, pCO2, Ω_{Ar}) will be calculated in duplicate at in situ conditions according to Cuyler & Byrne 2018 (see Attachment 1 Standard Operating Protocol) using a UV/Vis spectrophotometer (Agilent Cary 60) equipped with a Peltier-thermostatted cell holder (Agilent G6870A). Modifications to that method will include application of a mathematical correction for sulfonephthalein indicator dye impurities (Douglas & Byrne 2017) instead of using purified dyes, replacement with the most up-to-date model for the characterization of m-cresol purple dye in seawater (Müller & Rehder 2018), and scaling down the cell pathlength from 10 cm to 1 cm (approximately 1/10th the volume of sample). Cresol red dye (Patsavas et al. 2013) will be used in place of m-cresol purple where seawater pHT approaches the lower end of the indicating range of m-cresol purple (pHT < ~7.70) at ambient conditions (this may apply to all samples). Upon conclusion of the experimental trial, experimental units will be removed from the basin in groups of 4 and sampled for porewater carbonate chemistry using the same method.

2.6 Quality Control

All data will be reviewed for errors before entry into electronic records. Data will be inspected to look for extreme values, outliers, and duplication that may indicate recording errors.

Continuous data (e.g. Neptune dataloggers) will be graphed to look for spurious values or instrumental drift and verified against discrete wet chemistry sampling (see methods in Table 2b) for total dissolved inorganic carbon and pH at weekly intervals throughout the experiment, respectively. Dataloggers are all recently (within 1 year) calibrated by manufacturer and calibrations and any offsets between replicated instruments or sensors are quantified prior to use.

2.7 Instrument/Equipment Calibration, Testing, and Maintenance

Dataloggers will be operated according to manufacturer's recommendations.

UV/Vis spectrophotometer wavelength and absorbance calibrations will be verified using NIST reference materials (SRM 2034, SRM 930d respectively). pH, alkalinity, and DIC measurements/calculations will be verified using certified reference materials from Andrew Dickson's lab.

2.8 Data Management

Original data (paper data sheets and electronic records) will be maintained by Downeast Institute. DEI will also store copies of selected derived data products (spreadsheets, databases, or files formatted for statistical analyses). DEI will store final data analysis products such as output from statistical models, graphics and tables, and make them available to project partners upon request for review.

3. ASSESSMENT, OVERSIGHT AND REPORTING

3.1 Assessment and Oversight

3.1.1 Corrective Actions

Dr. Holmberg will routinely check the status of the experimental setup, e.g. water flow, dataloggers and take corrective actions to ensure water flow is sufficient to meet the research design's goals.

3.1.2 Deviations from QAPP

Dr. Holmberg will conduct periodic assessments of the project for deviations from the QAPP, such as inspection and maintenance of data loggers and laboratory instruments.

3.2 Reporting

3.2.1 Data presentation and statistical methods

Overlying seawater carbonate chemistry will be summarized in a time-series plot, but otherwise excluded from the analysis as all experimental units were subject to the same conditions. All statistical analyses will be conducted using R (R Core Team 2018).

In order to test for a difference in porewater carbonate chemistry between initial conditions and post-experimental trial controls, a repeated-measures MANOVA will be fit using the MANOVA.RM package in R (Friedrich et al. 2020). The model will include sediment porewater pH_T, A_T, DIC, pCO_2, and Ω_{Ar} as response variables and pre-/post-experimental trial as a 2-level explanatory factor.

Relationships between shell crush size, shell density, and sediment porewater carbonate chemistry parameters of interest will be modeled with redundancy analysis (RDA), computed using the vegan package in R (Oksanen et al. 2019). Response data will be standardized to obtain analyses on correlation response matrices, and scores will be computed using scaling 2 (response-focused scaling; Borcard et al. 2018). Sediment porewater pH_T, A_T, TCO_2, pCO_2, Ω_{Ar} will be entered in a response matrix Y, while shell crush size and shell density will be entered in an explanatory matrix X. Permutation tests using 999 permutations will be computed to determine whether explanatory matrix (X) predicts Y overall (global permutation test), and whether shell crush size or shell density individually predict Y in variation partitioning. Adjusted R^2 values will be extracted for the model as well as for each variable using variation partitioning. A triplot illustrating the correlative relationships amongst carbonate chemistry parameters (vectors) and shell crush size, density, and the interaction of crush size and density (vectors with arrowheads), and the values of observations (rings) will be generated (Borcard et al. 2018).

3.2.2 Data reports

All usable data will be included in summary data report shared with all project partners. A final report will be submitted to CBEP. Upon acceptance, CBEP will post the final report on its web page, as part of CBEP's searchable archive.

4. DATA REVIEW AND USABILITY

4.1 Data Review and Validation

Data from the dataloggers and instrumental analyses will be checked to determine whether they are within acceptable ranges and will be flagged if they depart from these ranges. They will also be checked for drifts from calibration and precision goals.

4.2 Data Usability

Data will be evaluated against the project goals and the Data Quality Objectives. The project manager will evaluate whether the DQI goals were met.

5. REFERENCES

Beal, B. F., Coffin, C. R., Randall, S. F., Goodenow, C. A., Pepperman, K. E., & Ellis, B. W. (2020). Interactive Effects of Shell Hash and Predator Exclusion on 0-Year Class Recruits of Two Infaunal Intertidal Bivalve Species in Maine, USA. *Journal of Experimental Marine Biology and Ecology*, *530–531*.

Bentley, C., & Schneider, D. (2015). *Report of the Commission to Study the Effects of Coastal and Ocean Acidification and Its Existing and Potential Effects on Species That Are Commercially Harvested and Grown along the Maine Coast*.

Borcard, D., Gillet, F., & Legendre, P. (2018). *Numerical Ecology with R, Second Edition*, 2nd ed. Springer.

Clements, J. C., Woodard, K. D., & Hunt, H. L. (2016). Porewater Acidification Alters the Burrowing Behavior and Post-Settlement Dispersal of Juvenile Soft-Shell Clams (*Mya arenaria*). *Journal of Experimental Marine Biology and Ecology*, *477*, 103–111.

Cuyler, E. E., & Byrne, R. H. (2018). Spectrophotometric Calibration Procedures to Enable Calibration-Free Measurements of Seawater Calcium Carbonate Saturation States. *Analytica Chimica Acta*, *1020*, 95–103.

Cyronak, T., Schulz, K. G., & Jokiel, P. L. (2016). The Omega Myth: What Really Drives Lower Calcification Rates in an Acidifying Ocean. *ICES Journal of Marine Science*, *73*, 558–562.

Dickson, A. G., Sabine, C. L., & Christian, J. R. (2007). *Guide to Best Practices for Ocean CO_2 Measurements*.

Douglas, N. K., & Byrne, R. H. (2017). Achieving Accurate Spectrophotometric pH Measurements Using Unpurified Meta-Cresol Purple. *Marine Chemistry*, *190*, 66–72.

Dethier, M. N., Kobelt, J., Yiu, D., Wentzel, L., & Ruesink, J. L. (2019). Context-Dependence of Abiotic and Biotic Factors Influencing Performance of Juvenile Clams. *Estuarine, Coastal and Shelf Science*, *219*, 201–209.

Drylie, T. P., Needham, H. R., Lohrer, A. M., Hartland, A., & Pilditch, C. A. (2019). Calcium Carbonate Alters the Functional Response of Coastal Sediments to Eutrophication-Induced Acidification. *Scientific Reports*, *9*, 1–13.

Friedrich, S., Konietschke, F., & Pauly, M. (2020). MANOVA.RM: Resampling-Based Analysis of Multivariate Data and Repeated Measures Designs. 2020.

Green, M. A., Waldbusser, G. G., Reilly, S. L., Emerson, K., & O'Donnell, S. (2009). Death by Dissolution: Sediment Saturation State as a Mortality Factor for Juvenile Bivalves. *Limnology and Oceanography*, *54*, 1037–1047.

Green, M. A., Waldbusser, G. G., Hubazc, L., Cathcart, E., & Hall, J. (2013). Carbonate Mineral Saturation State as the Recruitment Cue for Settling Bivalves in Marine Muds. *Estuaries and Coasts*, *36*, 18–27.

Greiner, C. M., Klinger, T., Ruesink, J. L., Barber, J. S., & Horwith, M. (2018). Habitat Effects of Macrophytes and Shell on Carbonate Chemistry and Juvenile Clam Recruitment, Survival, and Growth. *Journal of Experimental Marine Biology and Ecology*, *509*, 8–15.

IPCC. (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., et al., eds.

Müller, J. D., & Rehder, G. (2018). Metrology of pH Measurements in Brackish Waters-Part 2: Experimental Characterization of Purified Meta-Cresol Purple for Spectrophotometric pH_T Measurements. *Frontiers in Marine Science*, *5*, 1–9.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2019). Vegan: Community Ecology Package. 2019.

Patsavas, M. C., Byrne, R. H., & Liu, X. (2013). Physical-Chemical Characterization of Purified Cresol Red for Spectrophotometric pH Measurements in Seawater. *Marine Chemistry*, *155*, 158–164.

Pierrot, D., Lewis, E., & Wallace, D. W. R. (2006). MS Excel Program Developed for CO_2 System Calculations. *ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee*.

de Putron, S. J., McCorkle, D. C., Cohen, A. L., & Dillon, A. B. (2011). The Impact of Seawater Saturation State and Bicarbonate Ion Concentration on Calcification by New Recruits of Two Atlantic Corals. *Coral Reefs*.

Ruesink, J. L., Freshley, N., Herrold, S., Trimble, A. C., & Patten, K. (2014). Influence of Substratum on Non-Native Clam Recruitment in Willapa Bay, Washington, USA. *Journal of Experimental Marine Biology and Ecology*, *459*, 23–30.

Washington State Blue Ribbon Panel on Ocean Acidification (WABRPOA). (2012). *Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response.*

Widdicombe, S., Dashfield, S. L., McNeill, C. L., Needham, H. R., Beesley, A., McEvoy, A., … Berge, J. A. (2009). Effects of CO_2 Induced Seawater Acidification on Infaunal Diversity and Sediment Nutrient Fluxes. *Marine Ecology Progress Series*, *379*, 59–75.

ATTACHMENT 1

SOP: Spectrophotometric Determination of the Carbonate Chemistry System in Seawater by Nitric Acid Titration

By Robert J Holmberg

Downeast Institute

Updated: 11/13/20

CONTENTS

1. BACKGROUND

This SOP follows the procedure outlined in Cuyler & Byrne 2018: “Spectrophotometric calibration procedures to enable calibration-free measurements of seawater calcium carbonate saturation states”, in which the complete carbonate chemistry system in a sample of seawater is calculated from spectrophotometric pH measurements of paired subsamples - one of which has been titrated with a single nitric acid addition - and a spectrophotometric nitrate measurement of the titrated subsample. The procedure is effectively a streamlined titration made possible because nitrate absorbs in the UV range, allowing the quantity of titrant added to be measured simultaneously with sample pH; the need to measure titrant gravimetrically or volumetrically is eliminated. Notably, it enables rapid, accurate, calibration-free calculation of the saturation state of aragonite (Ω_{Ar}) using a single analytical instrument (the ubiquitous UV/Vis spectrophotometer).

The following modifications to Cuyler & Byrne's procedure are implemented:

1) The latest comprehensive models for characterizing m-cresol purple (Müller & Rehder 2018) and thymol blue (Hudson-Heck & Byrne 2019), published after Cuyler & Byrne 2018, are built into the accompanying Excel workbook ("Seawater Carbonate System.xlsm").

2) Whereas Cuyler & Byrne used indicator dyes purified by flash chromatography, a mathematical correction for indicator dye impurities (Douglas & Byrne 2017) is here applied so that off-the-shelf, impure dyes may be used instead.

3) "CO2Sys_v2.1.xls" has been modified slightly to remove pop-up messages for compatibility with the macros in "Seawater Carbonate System.xlsm"; it is renamed "CO2Sys_v2.1mod.xls".

4) Whereas Cuyler & Byrne prescribe 10 cm path length optical cells (28.2 ml nominal volume) for all spectrophotometric measurements, the accompanying Excel workbook is designed for compatibility with alternative cell types (e.g. 1 cm, 3.5 ml cells for sediment porewater samples). Adjustments include a correction for path length applied to Cuyler & Byrnes' Equation 15 for calculating the nitric acid dilution factor.

This SOP assumes that indicator dye impurities have been characterized according to Douglas & Byrne 2017, and that the instrument-specific nitrate molar absorptivity has been determined according to Cuyler & Byrne 2018 (see "2018 Cuyler & Byrne Supplemental.docx"). In this SOP, square brackets indicate equipment, supplies, or methodology used at the Downeast Institute.

2. PROCEDURE FOR SPECTROPHOTOMETRIC DETERMINATION OF SEAWATER CARBONATE CHEMISTRY

Reagents:

1) Indicator dye solutions, which may include cresol red, *m*-cresol purple, and/or thymol blue. Cuyler & Byrne recommend 10 mmol cresol red and *m*-cresol purple solutions, and the same concentration is appropriate for thymol blue. Prepare each solution by mixing the powdered dye into 0.7 M NaCl prepared with ultrapure water. Purchase the sodium salt form of each indicator dye, as the dyes in their pure form are insoluble in water. Note: although other procedures involving spectrophotometric pH measurement (including the Douglas & Byrne procedure above) call for buffering dye solutions to seawater pH using NaOH, Cuyler & Byrne do not –

presumably because buffering might interfere with the titration. Thus, unbuffered dye solutions must be specially prepared for this procedure;

2) Nitric acid titrant. Cuyler & Byrne use trace metal-grade HNO_3 diluted to 0.477 M in ultrapure water. Alternative concentrations may be used (the concentration may be specified in the Index sheet of the accompanying Excel workbook).

Equipment and supplies:

1) UV/Vis spectrophotometer [Agilent Cary 60] with thermostatted cell holder [Agilent G6870A];
2) Quartz optical cells with caps. Cuyler & Byrne use 10 cm path length, 28.2 ml, ported cylindrical cells for seawater samples. 1 cm rectangular cells are appropriate for sediment porewater samples.
3) Thermometer to measure *in situ* temperature and verify cell temperature, ideally a digital thermometer interfaced with the UV/Vis software;
4) For convenience, a system for warming optical cells containing seawater samples while they await analysis is recommended. This may consist of a dry bath [BT Lab Systems BT1105 with 1 cm cuvette block], water bath, or other solution;
5) Instrumentation for measuring seawater salinity [Oakton Salt 6+], ideally with the highest possible resolution (though 0.1 PSS may be the highest achievable resolution for sediment porewater samples);
6) If the approximate pH of the seawater sample is unknown, a pH meter and electrode – ideally calibrated to artificial seawater pH standards (Dickson et al. 2007) – is helpful.
7) If analyzing sediment porewater samples, a centrifuge and tubes – ideally capable of centrifuging 50 ml samples or greater – are necessary for separating porewater from sediment.
8) Micropipettes [Gilson Pipetman P10 and P2] and tips appropriate for dispensing indicator dye solutions and nitric acid titrant;
9) Disposable syringes [30 ml], 0.45 µm syringe filters, and drawing tubes for filtering seawater;
10) Disposable serological pipettes [5 ml] and pipette pump for measuring out seawater samples;
11) Intermediate containers for holding filtered seawater [disposable 50 ml centrifuge tubes];
12) Disposable transfer pipettes;
13) Kimwipes;
14) DI water.

Procedure:

1) Power on instrumentation, allowing the UV/Vis lamps to warm up if necessary [not necessary for the Cary 60].
2) Set the thermostatted cell holder and cell warming system to 25 °C.
3) Load the UV/Vis scan software [Cary WinUV Scan] and method ["Seawater Carbonate Chemistry.MSW"]. If there is no saved method, configure the software to scan from 740 nm – 220 nm at 1 nm intervals and a rate of 600 nm/min, measure absorbance, overlay spectra, and apply a baseline correction.
4) Open the "Seawater Carbonate System.xlsm" and "CO2Sys_v2.1mod.xls" workbooks.

5) In the accompanying workbook ("Seawater Carbonate System.xlsm"): Index, enter the cell path length (B3), cell volume (B4), and HNO_3 titrant concentration (B37). In the index, the temperature is fixed and the salinity is used only for calculating the reference second dissociation constant (pK_2e_2) for each indicator dye (to assist the user in selecting a dye with the most appropriate indicating range for a particular seawater sample).
6) In the Seawater Carbonate System sheet, the default format is for "climate level" accuracy. If "weather level" accuracy is adequate, click the "Weather Goal" button.
7) Upon collecting a seawater sample, measure its *in situ* temperature and record in the first sample row of the Seawater Carbonate System sheet (column H). If analyzing a sediment porewater sample, measure the *in situ* temperature directly from the mud at the desired depth. If the "climate level" format is used, the *in situ* temperature of the second replicate will fill in automatically.
8) If analyzing a sediment porewater sample, transfer the core into a centrifuge tube [50 ml], balance the centrifuge with another sample, and centrifuge at 5,000 rpm for 5 mins to separate porewater from sediment.
9) Filter the sample using disposable syringe filters and transfer into an intermediate container [50 ml centrifuge tube].
10) Measure the salinity of the filtered sample and record in the first sample row of the Seawater Carbonate System sheet (column I). If the "climate level" format is used, the salinity of the second replicate will fill in automatically.
11) If the approximate pH of the sample is unknown, measure the pH of the sample using a meter and electrode. In the Seawater Carbonate System sheet, select the default indicator dye with the most appropriate indicating range from the dropdown (A2). Alternatively, dyes may be selected on a per-sample basis (column D).
12) Distribute an equal amount of sample into 2 (if "weather level" accuracy is desired) or 4 (if "climate level" accuracy is desired) optical cells of the appropriate type [1 cm path length rectangular quartz cells] and cap. It is necessary for the sample size to be consistent among cells because the indicator dye impurity correction is dependent on the dye concentration in the cell.
13) Clean the exterior of the first sample cell using a Kimwipe wetted with DI water, dry with another Kimwipe, flick with a finger if necessary to displace bubbles, load into the UV/Vis cell holder, and uncap. Load remaining cells into the cell warming system.
14) When the first sample cell reaches 25 °C, collect a baseline scan.
15) Inject the appropriate amount of indicator dye solution [2.4 µl] into the first sample cell using a micropipette without removing the cell from its holder. Mix by repeatedly sucking up and releasing the sample with a transfer pipette, taking care not to introduce bubbles.
16) Scan the sample and record the absorbances at the non-absorbing wavelength ($\lambda_n A_1$), first absorbing peak ($\lambda_1 A_1$), and second absorbing peak ($\lambda_2 A_1$) for the dye (e.g., 730 nm, 434 nm, 578 nm for *m*-cresol purple) in the Seawater Carbonate System sheet (columns T:V). The workbook will calculate the baseline-corrected absorbances ($\lambda_{1bc} A_1$, $\lambda_{2bc} A_1$) and absorbance ratio (R_1).
17) Inject another, equal aliquot of dye solution into the first sample cell without removing it from its holder. Mix again with the transfer pipette.
18) Scan the sample and again record the absorbances at the non-absorbing wavelength ($\lambda_n A_2$), first absorbing peak ($\lambda_1 A_2$), and second absorbing peak ($\lambda_2 A_2$) for the dye (columns Z:AB). The

workbook will calculate the new baseline-corrected absorbances ($_{\lambda 1bc}A_2$, $_{\lambda 2bc}A_2$), absorbance ratio (R_2), absorbance ratio corrected for the pH change resulting from the addition of indicator dye ($_0R_1$), absorbance ratio further corrected for indicator dye impurities ($_pR_1$), and first sample cell pH_T (pH_{T1}).

19) Remove the first sample cell from the cell holder. Clean and dry a second sample cell, flick with a finger if necessary to displace bubbles, load into the UV/Vis cell holder, and uncap.
20) When the second sample cell reaches 25 °C, collect a baseline scan.
21) Inject the appropriate amount of nitric acid titrant into the second sample cell using a micropipette without removing the cell from its holder. The appropriate amount of titrant is that which depresses the sample pH_T by > 0.6 pH units, and depends on the initial sample pH and titrant concentration. For 0.477 M HNO_3, Cuyler & Byrne use 19 µl for pH_{T1} > 8.0, 14 µl for pH_{T1} < 7.9, and 16.5 µl for pH_{T1} between 7.9 and 8.0. For smaller samples, less titrant is needed to depress the pH_T by the correct interval, and the user should start by adjusting the titrant volume to achieve an equivalent HNO_3 concentration in the cell (e.g. 1.74 µl or less for 3.5 ml sediment porewater samples). If desired, record the volume of titrant added (V_{HNO3}) as a reference (column AM).
22) Scan the second sample cell and record the absorbances at the non-absorbing wavelength for nitrate ($_{385}A$) and the wavelength on the shoulder of the nitrate peak ($_{235}A$) (columns AN:AO). The workbook will calculate the baseline-corrected absorbance ($_{235}A_{bc}$).
23) Repeat steps 15-18 for the second sample cell and record absorbances in the correct places in the workbook (first $_{\lambda n}A_3$, $_{\lambda 1}A_3$, $_{\lambda 2}A_3$ in columns AQ:AS, then $_{\lambda n}A_4$, $_{\lambda 1}A_4$, $_{\lambda 2}A_4$ in columns AW:AY). As before, the workbook will calculate baseline-corrected absorbances ($_{\lambda 1bc}A_3$, $_{\lambda 2bc}A_3$, $_{\lambda 1bc}A_4$, $_{\lambda 2bc}A_4$), absorbance ratios (R_3, R_4), corrected absorbance ratios ($_0R_2$, $_pR_2$), and the second sample cell pH_T (pH_{T2}). In addition, the workbook will calculate the change in nitrate (ΔNO_3^-) between the first and second sample cells.
24) If “climate level” accuracy is desired, repeat steps 13-23 with the third and fourth sample cells.
25) Click the “Calculate With CO2SYS” button. The workbook, together with CO2SYS, will calculate parameters related to seawater borate alkalinity (f_{B1}, f_{B2}), hydroxide alkalinity (f_{W1}, f_{W2}), and carbonate alkalinity (f_{C1}, f_{C2}) for each sample cell, as well as the nitric acid dilution factor (θ). Next, the workbook will calculate seawater dissolved inorganic carbon (C_T) and total alkalinity (A_T); if the “climate level” format is selected, C_T and A_T from both replicates will be averaged. Finally, CO2SYS will calculate all remaining carbonate chemistry parameters for the sample at *in situ* conditions (printed in columns CD:CQ).

3. REFERENCES

Cuyler, E. E., & Byrne, R. H. (2018). Spectrophotometric Calibration Procedures to Enable Calibration-Free Measurements of Seawater Calcium Carbonate Saturation States. *Analytica Chimica Acta*, *1020*, 95–103.

Dickson, A. G., Sabine, C. L., & Christian, J. R. (2007). *Guide to Best Practices for Ocean CO_2 Measurements*.

Douglas, N. K., & Byrne, R. H. (2017). Achieving Accurate Spectrophotometric pH Measurements Using Unpurified Meta-Cresol Purple. *Marine Chemistry*, *190*, 66–72.

Hudson-Heck, E., & Byrne, R.H. (2019). Purification and Characterization of Thymol Blue for Spectrophotometric pH Measurements in Rivers, Estuaries, and Oceans. *Analytica Chimica Acta*, 1090, 91-99.

Müller, J. D., & Rehder, G. (2018). Metrology of pH Measurements in Brackish Waters-Part 2: Experimental Characterization of Purified Meta-Cresol Purple for Spectrophotometric pH_T Measurements. *Frontiers in Marine Science*, *5*, 1–9.