Technical note: An Assessment of Hg^{II} to Preserve Carbonate System Parameters in Organic-Rich Estuarine Waters

Christopher S. Moore [1], Robert H. Byrne [2], Kimberly K. Yates [1]

[1] U.S. Geological Survey, St. Petersburg Coastal and Marine Science Center, 600 4th Street South, St. Petersburg, FL 33701, USA

[2] College of Marine Science, University of South Florida, 140 7th Avenue South, St. Petersburg, FL 33701, USA

Correspondence to: Christopher S. Moore (csmoore@usgs.gov)

Abstract. This work assesses the effectiveness of sample preservation techniques for measurements of pH_T (total scale), total dissolved inorganic carbon (DIC_T), and total alkalinity (A_T) in organic-rich estuarine waters. Using $HgCl_2$-treated and untreated water samples, measurements of these carbonate system parameters were examined over a period of three months. Over this duration, continued respiration of dissolved organic matter (DOM) in untreated samples created large discrepancies in DIC_T concentrations, while DIC_T was effectively constant in treated samples. Changes in A_T were observed for both treated and untreated samples, with treated samples showing the greatest variation. In response to changing A_T/DIC_T ratios, pH_T changes were observed in both treated and untreated samples but were relatively small in treated samples. Improved accuracy of results in organic-rich estuarine waters that reflect the in situ carbonate system characteristics of the samples at the time of collection can be achieved when samples obtained for DIC_T and A_T analysis are collected and stored separately. Accurate analyses of DIC_T can be obtained by filtration and preservation with $HgCl_2$. Accuracy of A_T analyses can be improved by filtration and storage in polypropylene bottles at 4°C without adding $HgCl_2$. Quality of pH_T measurements can be improved by prompt analysis in the field and, if this cannot be accomplished, then samples can be preserved with $HgCl_2$ and measured in the laboratory within one week.

1. Introduction and background

Accurate measurements of carbonate system parameters, including pH_T (total scale), total dissolved inorganic carbon (DIC_T), total alkalinity (A_T) and carbon dioxide fugacity $f(CO_2)$, in coastal and estuarine environments are essential for understanding the mechanisms controlling ecosystem health and its resources (Doney, 2006; Cai et al., 2021). Characterization of the carbonate system is fundamental for a quantitative understanding of buffer intensity, indicating the extent to which pH is maintained within the limited range required for optimal functioning of biological processes (Butler, 1991; Pilson, 2012; Stumm and Morgan, 2012). Measurements of in situ pH_T along with either DIC_T or A_T are essential, as well, for calculating in situ saturation states (Byrne, 2014) relevant to calcareous organisms. Due to the substantial complexity of low salinity (S < 20) environments with high organic matter content, and their susceptibility to rapid changes from freshwater sources, these ecosystems are poorly understood relative to pelagic environments (Hunt et al., 2011; Song et al., 2020).

Carbonate system parameters are typically preserved in the field for subsequent analysis in a suitable laboratory. Following a geochemical investigation of four rivers that discharge into Tampa Bay, Florida USA, where large seasonal variations were observed (Moore, 2022), a quantitative assessment of sample preservation techniques was conducted over a three-month period. Time series measurements of key carbonate system parameters were assessed using both $HgCl_2$-treated and untreated water samples. Although sample preservation techniques have been extensively evaluated in some environments (Wong, 1970; Dickson, 2007), studies in organic rich waters are rare (Mos et al., 2021). In the investigation of Mos et al. (2021) the effectiveness of diverse preservation and storage methods on the total alkalinity (A_T) of aqueous environmental samples (seawater, estuarine water, freshwater, and groundwater) were examined over a period of six months. Our investigation extends the work of Mos et al. (2021) by documenting daily-to-weekly changes in three carbonate system parameters pH_T (i.e., -$\log[H^+]_T$), DIC_T, and A_T for organic-rich estuarine samples preserved with and without $HgCl_2$.

In addition to addressing sample stability issues, these measurements allow assessment of how sample-storage/preservation influence the final concentrations of directly measured parameters (e.g., A_T) and parameters that are calculated from DIC_T and pH_T. Investigations of the internal consistency of carbonate system measurements in high salinity marine waters (direct measurements vs. calculations) have been conducted over several decades. The internal consistency between measured and calculated carbonate system parameters has improved over time due to improvements in both measurement procedures and refinements of the models that are used in carbonate system calculations. For high salinity marine data sets, a variety of investigations have shown that differences between measured A_T and A_T calculated from pH_T and DIC_T are currently on the order of 3 to 4 $\mu mol\ kg^{-1}$. This difference between measured and calculated A_T is similar to uncertainties typically observed for A_T measurements (Millero et al., 1993; Patsavas et al., 2015; Fong and Dickson, 2019). Prior assessments of carbonate system parameters showed that internal consistency (i.e., measurements and calculations of A_T) have been limited to the range of salinity (S) within which sulfonephthalein pH indicators, such as meta–Cresol Purple (mCP), have been carefully calibrated ($20 \leq S \leq 40$). Until the recent publications of Douglas and Byrne (2017) and Müller and Rehder (2018), carbonate system internal consistency evaluations using modern pH measurement techniques in estuarine and fresh waters have not been possible. In addition to providing a general assessment of the use of $HgCl_2$ as a preservative for measurements of carbonate system parameters, our investigation provides perspectives on the influence of Hg^{II} on CO_2 internal consistency calculations under estuarine conditions.

2. Methods and materials

2.1 Sample collection

Hillsborough River water samples were collected on January 26th, 2021, from the boardwalk of the Lowry Park Riverside Trail (28.0138 N, 82.4646 W) (Fig. 1). Water temperature and salinity were measured using a Sea-Bird Scientific Micro Thermosalinograph, and then two 30-L Niskin bottles were used to simultaneously collect surface river water. Water from each Niskin was filtered, promptly analyzed spectrophotometrically to establish initial pH_T and then preserved for analysis of

63 major inorganic nutrients. The filtration was performed under positive pressure using a 12V peristaltic pump (GeoTech) and

64 peristaltic tubing (Masterflex). The tubing was connected to a 142 mm diameter acrylic filter housing (GeoTech) that contained

65 a 0.45 µm cellulose nitrate filter (GeoTech). Water collected for analysis of major inorganic nutrients was additionally filtered

66 using a sterile 0.22 µm pressure filter (Sterivex) attached by Luer lock to a 60 mL syringe and then stored in a 30 mL acid

67 washed high density polyethylene bottle (Nalgene). Once each nutrient sample bottle was sealed, the bottle was stored on ice,

68 transported to the laboratory, and then frozen until analysis (Dore et al. 1996). Next, water samples from each Niskin were

69 simultaneously filtered into a total of 70 borosilicate glass bottles (300 mL total volume) using the methods described by

70 Dickson et al. (2007). Water samples from the first Niskin (i.e., 35 samples) were treated with 100 µL of 6.5% $HgCl_2$

71 (LabChem). Water samples from the second Niskin (i.e., 35 samples) were not treated. Both sets of bottles were promptly

72 sealed with Apiezon M grease for transport. After these samples were collected and processed, additional water from each

73 Niskin was filtered and analyzed spectrophotometrically to determine a final pH_T, and then sampled for analysis of major

74 inorganic nutrients. All water samples were then transported to the U.S. Geological Survey Carbon Laboratory in St Petersburg,

75 Florida where the borosilicate bottles were stored in the dark at room temperature (25°C) and sequentially analyzed, beginning

76 hours after collection, over a period of three months for pH_T (total scale), total dissolved inorganic carbon (DIC_T), and total

77 alkalinity (A_T).

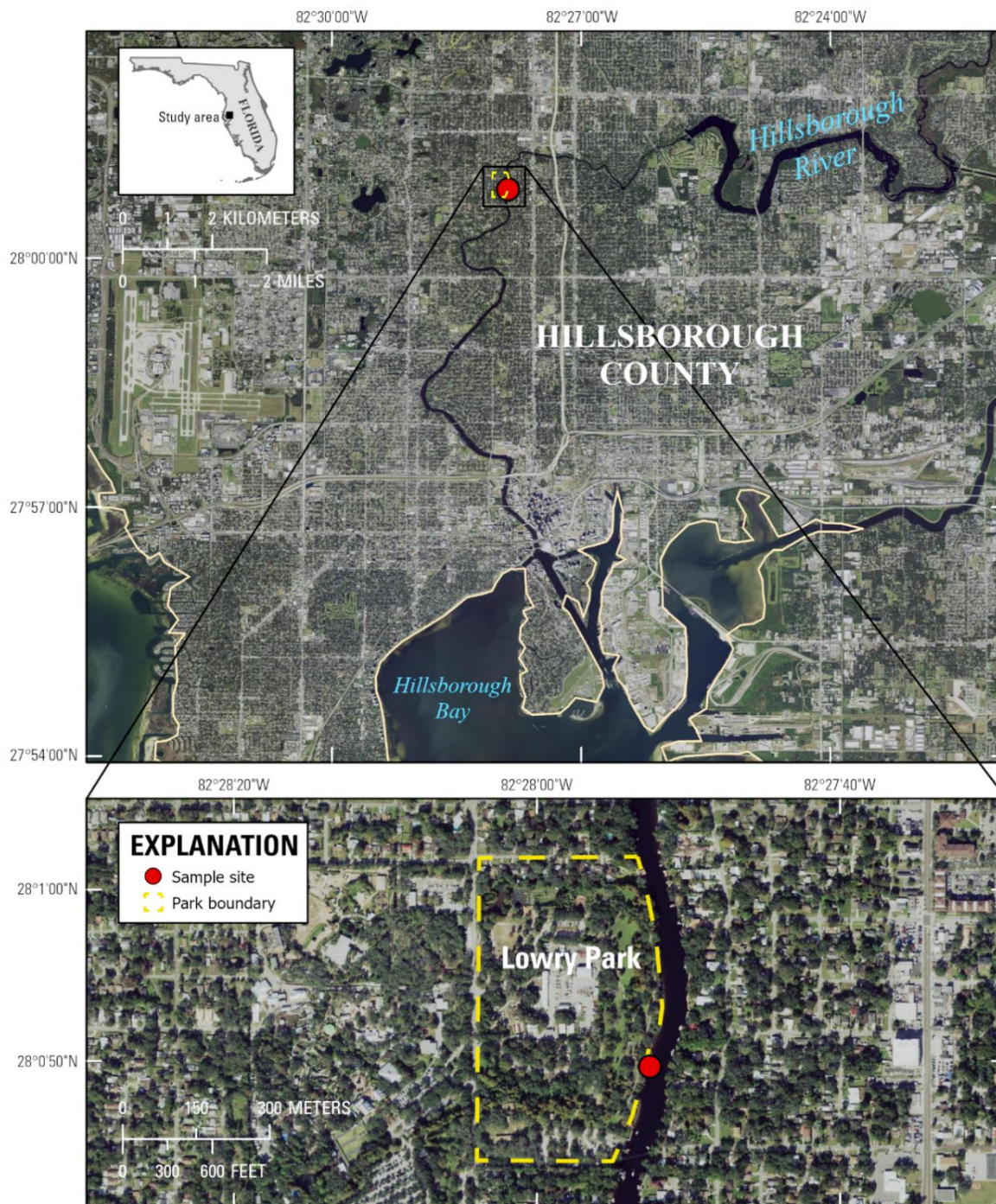



Figure 1. A map showing the location of the sample site on the Hillsborough River in relation to the upper fringe of Hillsborough Bay in Tampa Bay (upper) and to Lowry Park (lower).

2.2 Sample analysis

2.2.1 Field analysis (pH$_T$)

Recent advances in the characterization of spectrophotometric indicator dyes and pK2e2 models for spectrophotometric pH$_T$ and A$_T$ analysis have enabled analysis in waters over a full river-to-sea range of salinity ($0 \leq S \leq 40$) and temperature ($0 \leq T \leq 40$) (pH: Douglas and Byrne, 2017, Rehder and Müller, 2018; A$_T$: Hudson-Heck et al., 2021). Spectrophotometric pH$_T$ field analyses were performed using the methods of Clayton and Byrne (1993). Each filtered pH$_T$ sample was collected in a 10 cm pathlength cylindrical glass cell and placed within an Ocean Optics CUV-UV-10 cuvette holder connected to an Ocean Optics LS-1 Tungsten light source and an Ocean Optics USB2000 spectrometer. Two 10 µL additions of purified M-cresol purple (mCP) indicator dye (Liu et al., 2011) were added with a Gilmont GS-1100 Micrometer Syringe, and pH$_T$ was corrected for any indicator dye perturbation by back correction from the second dye addition (Clayton and Byrne, 1993). Absorbance measurements were made using the Ocean Optics software package OOIBase 32. The measurement temperature for these analyses (approximately 26.5 °C) was determined using a Fluke 51 II Handheld Digital Probe Thermometer. The temperatures provided by this probe were in good agreement with those obtained with a Hewlett Packard 2804A quartz thermometer ($\Delta T \approx 0.125 \pm 0.024$ °C). Sample pH$_T$ was calculated on the total scale using the algorithm of Müller and Rehder (2018). Each pH$_T$ was temperature corrected to 20° C in CO2sys.m (van Heuven et al., 2011) using the K1K2 constants of Waters, Millero, & Woosley (2014), the KSO$_4$ constants of Dickson (1990), and the total boron to salinity ratio (B$_T$/S) of Lee et al. (2010).

2.2.2 Laboratory analysis of pH$_T$, DIC$_T$, and A$_T$ and nutrients

Treated and untreated water samples were analyzed over a period of three months. Samples were first analyzed within a few hours of collection and subsequently analyzed every other day for 7 days. After the first week, samples were then analyzed weekly for the duration of the experiment. The measurement methods used over this three-month period were identical for each type of analysis, and samples were analyzed in the order they were initially collected. On each day of analysis four sample bottles, two treated samples and two untreated samples, were analyzed in the following order: pH$_T$, DIC$_T$ and A$_T$.

Spectrophotometric pH$_T$ analysis in the laboratory was performed using the methods of Clayton and Byrne (1993). Sample water was poured from the borosilicate bottle into two 10 cm pathlength cylindrical glass cells. Each cylindrical cell was placed in a temperature controlled Ocean Optics CUV-UV-10 cuvette holder connected to an Ocean Optics LS-1 Tungsten light source and Ocean Optics USB2000 spectrometer. pH$_T$ analyses in the laboratory were identical to those used for pH$_T$ measurements in the field.

Immediately after the pH$_T$ measurements, water samples for duplicate total dissolved inorganic carbon (DIC$_T$) analyses were withdrawn from the same sample bottle. Using a 60 mL syringe connected to a 3 way Luer lock valve and stopper to minimize gas exchange, two 20 mL samples were drawn from each bottle. Analyses were performed with a CM5017 CO$_2$ carbon coulometer coupled to a CM5330 acidification module (UIC, Inc.) following the methods described by Dickson and Goyet

113 (1994). Sample weights were determined using a Denver Instruments PI-214 analytical balance (± 0.1 mg). Injected sample

114 mass was determined as the difference in syringe weights before and after each sample was injected through a septum into the

115 stripping chamber of the acidification module. Samples were acidified with ~10 mL of 8.5% H_3PO_4. Analytical grade N_2 was

116 used as the carrier gas for CO_2 from acidified samples to the coulometer. The titration endpoint was determined by the

117 coulometer, and DIC_T was calculated using software from UIC, Inc. Accuracy and precision was determined from analysis of

118 certified reference materials (CRM) obtained from Andrew Dickson at the Scripps Institution of Oceanography (Dickson,

119 2010). Repeat measurements (N = 51) of CRM Batch 189 and CRM Batch 183 yielded precisions of ± 1.09 and ± 0.71μmol

120 kg^{-1} respectively (standard deviation of each average daily CRM analysis) and an accuracy of 1.43 ± 1.04 μmol kg^{-1} (average

121 of the absolute value of the difference between each measured CRM and the reported batch concentration).

122 After completion of the DIC_T analyses, measurements of total alkalinity (A_T) were performed using the spectrophotometric

123 methods of Yao and Byrne (1998) and Liu et al. (2015). Water sample mass (~100 g) and masses of added acid were determined

124 gravimetrically using a Denver Instruments PI-214 analytical balance (± 0.1 mg). Water samples were analyzed in an open top

125 square glass cell (Hellma Cells, Inc.) placed within a custom plastic frame connected to an Ocean Optics LS-1 Tungsten light

126 source and Ocean Optics USB2000 spectrometer. Bromocresol purple (BCP) indicator dye (4 mM stock solution) was

127 administered by pipette (100 μL), and the sample was titrated using 0.100 ± 0.0001 N standardized HCl (Lab Chem Inc.) added

128 with a plastic 10 mL syringe fitted with a Teflon syringe needle. Absorbance measurements were made using the Ocean Optics

129 software package OOIBase 32 (Landis, 2005). The pH_T of each solution was measured throughout the titration to an endpoint

130 near pH_T 4.3. The total weight of added acid was determined from the difference in the syringe weight before and after acid

131 addition. At the end of each titration, the solution was purged of CO_2 with a stream of N_2 gas that had been pre saturated with

132 H_2O. After purging, final absorbance measurements were made, and solution temperature was determined using a Fluke 51 II

133 Handheld Digital Probe Thermometer. A_T was calculated using the BCP equations of Hudson-Heck et al. (2021). Accuracy

134 and precision were determined from analysis of Andrew Dickson's certified reference materials (CRM) (Dickson, 2010).

135 Repeat measurements (N = 33) of CRM Batches 183, 186, and 189 yielded precisions of ± 0.93 μmol kg^{-1}, ± 0.76 μmol kg^{-1},

136 and ± 1.39 μmol kg^{-1} respectively, and an accuracy of 0.77 ± 0.93 μmol kg^{-1}.

137 Internal consistency calculations of A_T, from measured pH_T and DIC_T were performed in CO2sys.m (van Heuven et al., 2011)

138 using the K1K2 constants of Waters, Millero, & Woosley (2014), the KSO_4 constants of Dickson (1990), and the B_T/S constant

139 of Lee et al. (2010). Significance tests with 95% confidence levels were performed in Microsoft Excel using the *Data Analysis*

140 *Regression* tool.

141 Nutrient analyses were performed at the University of Tampa. Samples were analyzed for inorganic nitrogen ($NO_3^-+NO_2^-$),

142 inorganic phosphate ($H_2PO_4^- + HPO_4^{2-} + PO_4^{3-}$), ammonia ($NH_3 + NH_4^+$), and silica ($SiO(OH)_3^- + Si(OH)_4$)) using the methods

143 of Gordon et al. (1993).

144

3. Results and discussion

Clear distinctions were observed over three months between untreated and treated ($HgCl_2$) water samples for all three measured carbonate system parameters: pH_T, DIC_T, and A_T. From the first analyses DIC_T(untreated) was greater than DIC_T(treated), and the difference between DIC_T(untreated) and DIC_T(treated) increased throughout the 91 days of analysis (Fig. 2). The DIC_T of $HgCl_2$-treated samples was essentially constant throughout the duration of the experiment (3,095.5 ± 3.2) while the DIC_T of untreated samples increased by approximately 37 µmol kg^{-1} over 91 days. The regression of DIC_T vs time for the untreated samples ($DIC_T = 3{,}100.2 + (0.4088 \pm 0.04)x$) ($p < 0.0001$) (Fig. 2) indicated that DIC_T increased by approximately 0.41 µmol kg^{-1} per day over the course of the experiment (note that anomalous results obtained on day 2 for both untreated and $HgCl_2$-treated samples are not included in the figure but are shown in the associated data release, Moore et al. (2022), https://doi.org/10.5066/P9J9IYFD).

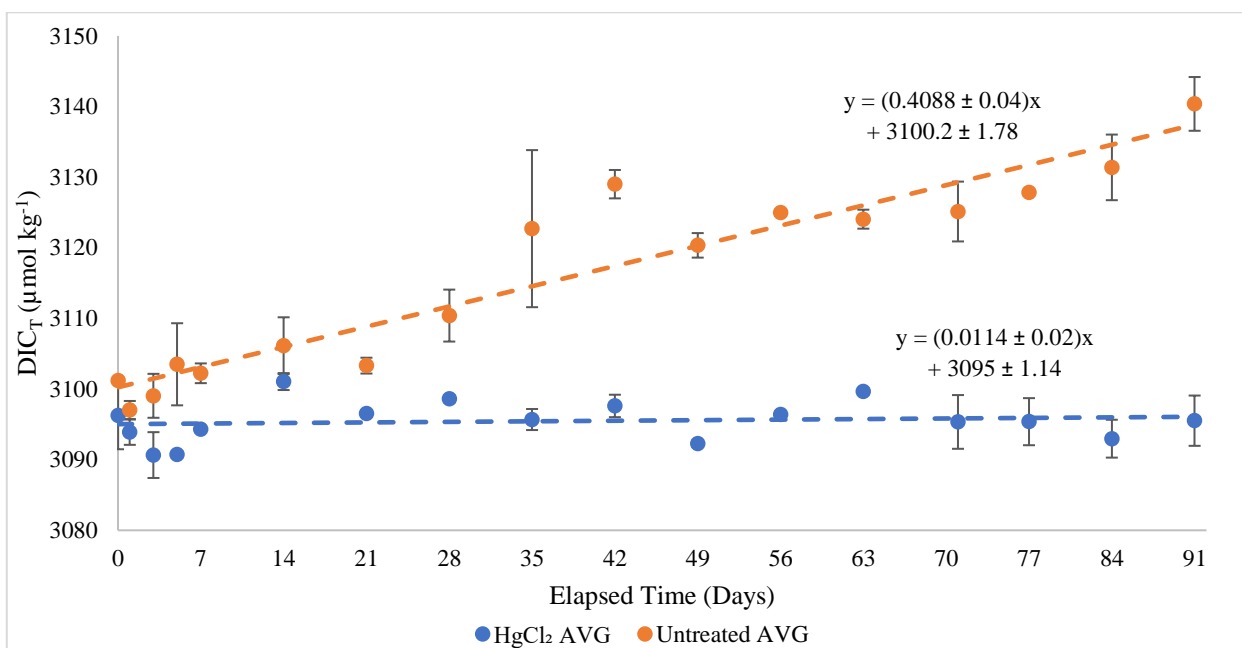



Figure 2. Average (AVG) measured DIC_T in treated ($HgCl_2$) and untreated samples over time where error bars are the standard deviation of the average from each day of analysis. Dotted lines represent a best fit linear trendline for the data.

Respiration of dissolved organic matter (DOM) dramatically increased DIC_T in the untreated samples (Fig. 2). Results for the treated DIC_T samples show that the addition of $HgCl_2$ was effective at curtailing respiration as treated samples were invariant over a period of three months. It is noted that, at the inception of the experiment, there was a small DIC_T difference between the two sample types (treated and untreated) (Fig. 2); the calculated DIC_T intercepts differ by about 5.2 ± 2.4 µmol kg^{-1}. This 5.2 µmol kg^{-1} difference may be a real sampling artifact (i.e., a small difference between samples collected simultaneously at the same location); the difference is similar to the 1 σ uncertainty (± 2.4 µmol kg^{-1}) in the calculated difference between the two intercepts.

165 A_T results also show a large difference between $HgCl_2$-treated and untreated samples (Fig. 3). Unlike DIC_T observations,

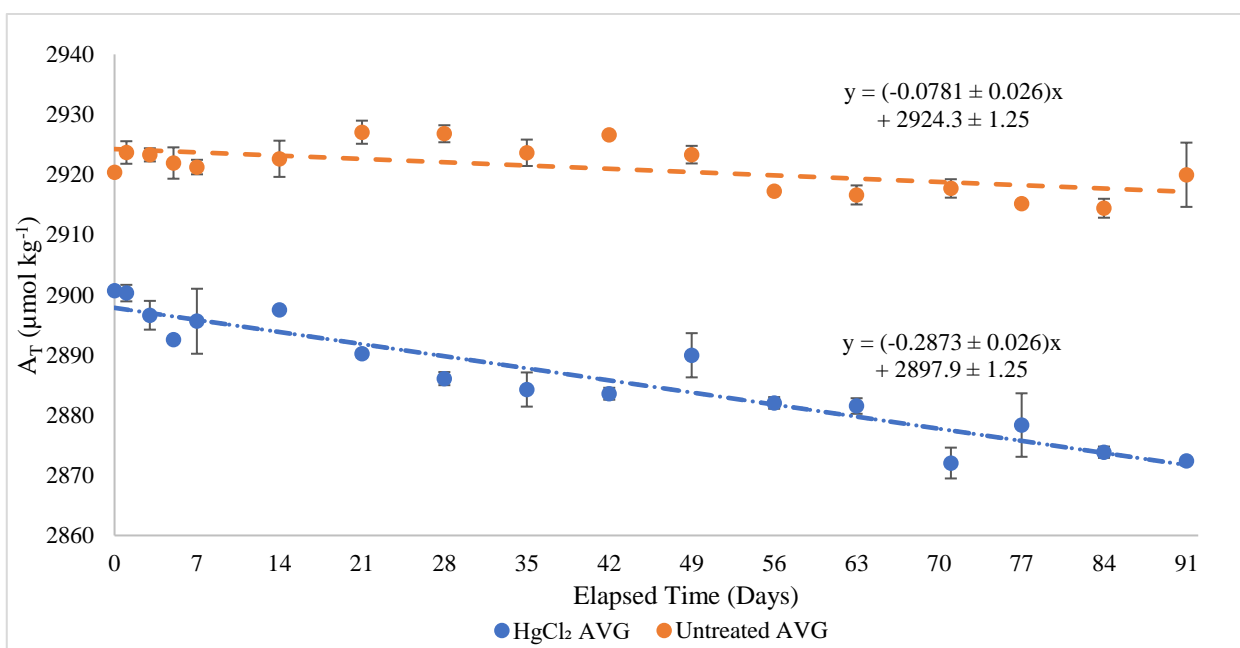
166 however, a large difference between the $HgCl_2$-treated and untreated samples was observed at the inception of the measurement

167 period. Furthermore, in contrast to the near constancy of DIC_T for the $HgCl_2$-treated samples, the A_T of the untreated samples

168 was approximately constant ($p < 0.01$), while the A_T of treated samples decreased by approximately 26 μmol kg^{-1} over the

169 course of the experiment ($p < 0.001$). Overall, at the beginning of the measurement period the A_T of the $HgCl_2$-treated samples

170 was lower than the A_T of the untreated samples by more than 26 μmol kg^{-1}, and this difference grew to more than 45 μmol

171 kg^{-1} at the end of the three-month period of measurements.

172
173 **Figure 3. Average (AVG) measured A_T in treated ($HgCl_2$) and untreated samples over time where error bars are the standard**
174 **deviation of the average from each day of analysis. Dotted lines represent a best fit linear trendline for the data.**

175 Respiration of DOM caused A_T to decrease through time in the untreated samples (Fig. 3). Consistent with the modern revised

176 Redfield equation (P_T:N_T:C_T = 1:16:124) (Takahashi et al., 1985, Broecker et al., 1985; Anderson and Sarmiento, 1994;

177 Körtzinger et al., 2001), respiration of DOM produces an increase in DIC_T that is about 7.3 times larger than the accompanying

178 decrease in A_T. Based on the 37 μmol kg^{-1} increase in DIC_T over 91 days, and the expected factor of 7.3 difference between

179 changes in DIC_T and changes in A_T (i.e., $\Delta DIC_T/\Delta A_T = 7.3$), the expected decrease in A_T is somewhat smaller than the observed

180 decrease. However, considering the uncertainties in slope in Fig. 2 and Fig. 3 for the untreated samples, the expected change

181 in A_T calculated as $\Delta DIC_T/7.3$ (i.e., 5.1 ± 0.5 μmol kg^{-1}) is reasonably consistent with the observed change in A_T (7.1 ± 2.4

182 μmol kg^{-1}).

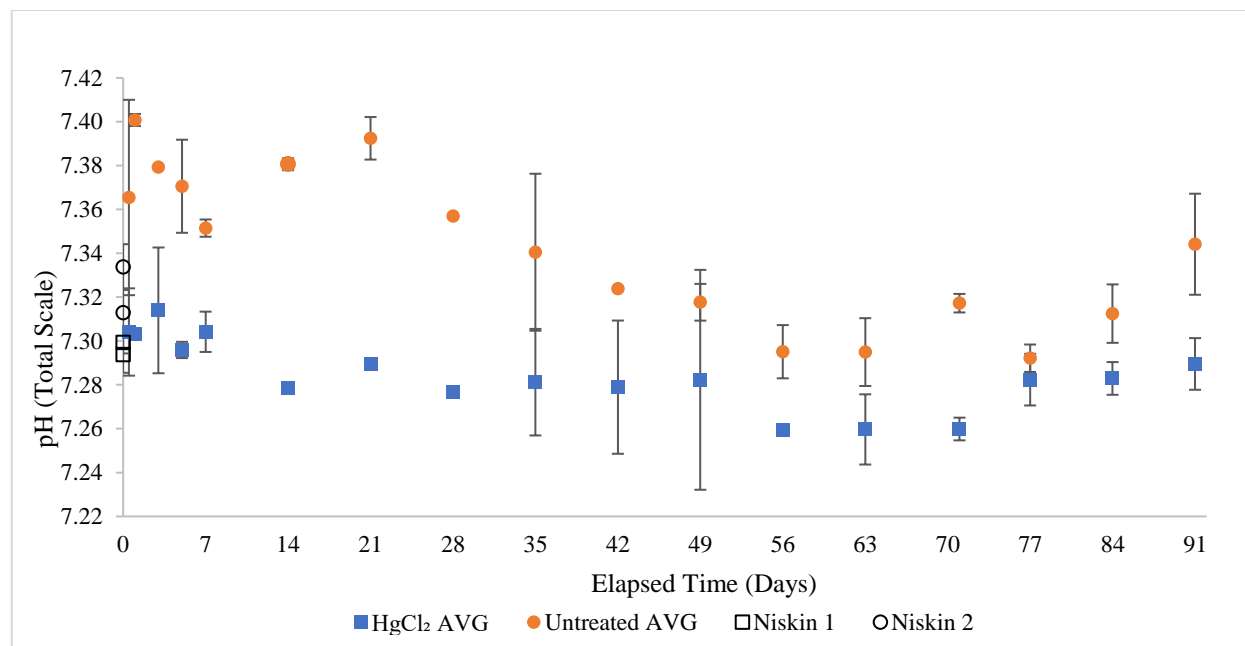
183 Results for pH(20°C)$_T$ of prompt untreated pH$_T$ measurements made in the field (shown as open markers at time zero) and

184 laboratory measurements (solid markers) made after collection of field measurements are shown in Fig. 4. As was observed

185 for DIC_T and A_T, a clear difference was observed between treated and untreated samples. Consistent with the decreasing A_T

186 and the substantially increasing DIC_T for the untreated samples, pH_T (untreated) decreased from an average of 7.377 ± 0.022

187 for days 1 through 21 to 7.317 ± 0.024 for days 28 through 91. The pH_T changes of the treated samples were smaller than those

188 of the untreated samples, averaging 7.302 ± 0.018 for days 1 through 21 and 7.275 ± 0.020 for days 28 through 91.

189
190 **Figure 4. Average (AVG) measured pH_T from the field, notated as Niskin 1 (treated) and Niskin 2 (untreated), and from the**
191 **laboratory, notated as HgCl₂ and Untreated, over time where error bars are the standard deviation of the average from each day of**
192 **analysis. Note the first field measurements occurred several minutes before sample bottle preservation (i.e., the beginning of elapsed**
193 **time axis), while the second and final field measurements occurred after sample bottle preservation. The time between the first and**
194 **second field measurements was approximately 45 minutes. The first laboratory measurements occurred approximately 2.25 hours**
195 **after the final field measurement.**

196 For untreated samples, Fig. 4 shows the expected temporal decrease in pH_T that results from increasing DIC_T and decreasing

197 A_T. For the treated samples, the observed decrease in pH is solely attributable to decreasing A_T through time. The initial offset

198 in pH_T, whereby pH_T(untreated) was greater than pH_T(treated), was expected based on the substantial initial alkalinity

199 difference between the treated and untreated samples.

200 Nutrient results collected from each Niskin before and after borosilicate glass bottle sampling are consistent with the conclusion

201 that the waters sampled by the two Niskin bottles were somewhat different (Table 1). For inorganic nitrogen, phosphate, and

202 ammonia, the nutrient concentrations in Niskin 1 were 1.4 to 2.5 times greater in than in Niskin 2, while silica in Niskin 2 was

203 1.3 times greater than in Niskin 1. Although this is substantial evidence for real distinctions between the initial conditions of

204 the treated and untreated samples (rather than differences created during laboratory analyses), it is the large changes in DIC_T,

205 A_T, and pH_T through time, and the increasingly large differences between treated and untreated samples that is the critically

206 important focus of this investigation (i.e., Fig. 2, 3 and 4).

207 **Table 1. Mean and standard deviation of measured inorganic nutrients sampled from each Niskin bottle at the time of sample**
208 **collection.**

Inorganic Nutrient AVG ($\mu mol\ kg^{-1}$)	Niskin 1 ($HgCl_2$-treated)	Niskin 2 (Untreated)
Nitrogen	4.90 ± 0.16	1.93 ± 0.35
Ammonia	4.19 ± 0.21	2.99 ± 0.5
Phosphate	2.31 ± 0.02	1.32 ± 0.10
Silica	70.03 ± 0.39	90.23 ± 0.42

209

210 Contrary to our original expectations, the treated A_T samples (Fig. 3) showed a larger decrease in A_T than the untreated samples.

211 Our interpretation that this decrease was due to complexation of DOM by Hg^{II} is in accord with the results and interpretation

212 of Mos et al. (2021). Hg^{II} has a strong affinity for organic ligands such as are found in the DOM of natural waters (Andren and

213 Harriss, 1975; Mierle and Ingram, 1991; Varshal et al., 1996; Ravichandran, 2004). The increasing A_T difference between

214 treated and untreated samples over time is plausibly attributable to a slowly increasing extent of DOM complexation by Hg^{II}.

215 After the addition of a comparable concentration (0.03%) of $HgCl_2$ to fresh, estuarine, and groundwater water samples stored

216 in borosilicate glass bottles, Mos et al. (2021) observed a rapid decrease in A_T followed by a subsequent decrease over a period

217 of 1 or 6 months. A decrease in the alkalinity of treated DOM rich samples would be expected if organic bases are complexed

218 by Hg^{II} and a portion of these complexes do not dissociate (i.e., remain untitrated) at the lowest pH in an alkalinity titration.

219 The Hillsborough River headwaters originate in the Green Swamp and Crystal Springs, (Lewis and Estevez, 1988) whose

220 water types are known to be significant sources of dissolved organic groups with substantial and wide ranging affinities for H^+

221 ions (Song et al., 2020). As such, these organics are expected to have substantial affinities for cations such as Hg^{II} that exhibit

222 strong covalent behavior (Martell and Hancock, 2013). Such associations are much weaker in seawater because of strong

223 complexation of Hg^{II} by chloride ions at high salinities.

224 The initial pH_T and A_T differences (treated vs untreated) in the first weeks of the measurements were initially unexpected.

225 However, as shown in Table 1, initial differences in A_T, pH_T, and DIC_T for the treated and untreated water samples were

226 consistent with well defined differences in observed inorganic nutrient concentrations. The most plausible explanation for the

227 initial DIC_T differences in the treated and untreated water samples is that the two water samples had small differences in their

228 characteristics. The most plausible explanation for the large initial A_T and pH_T differences between treated and untreated

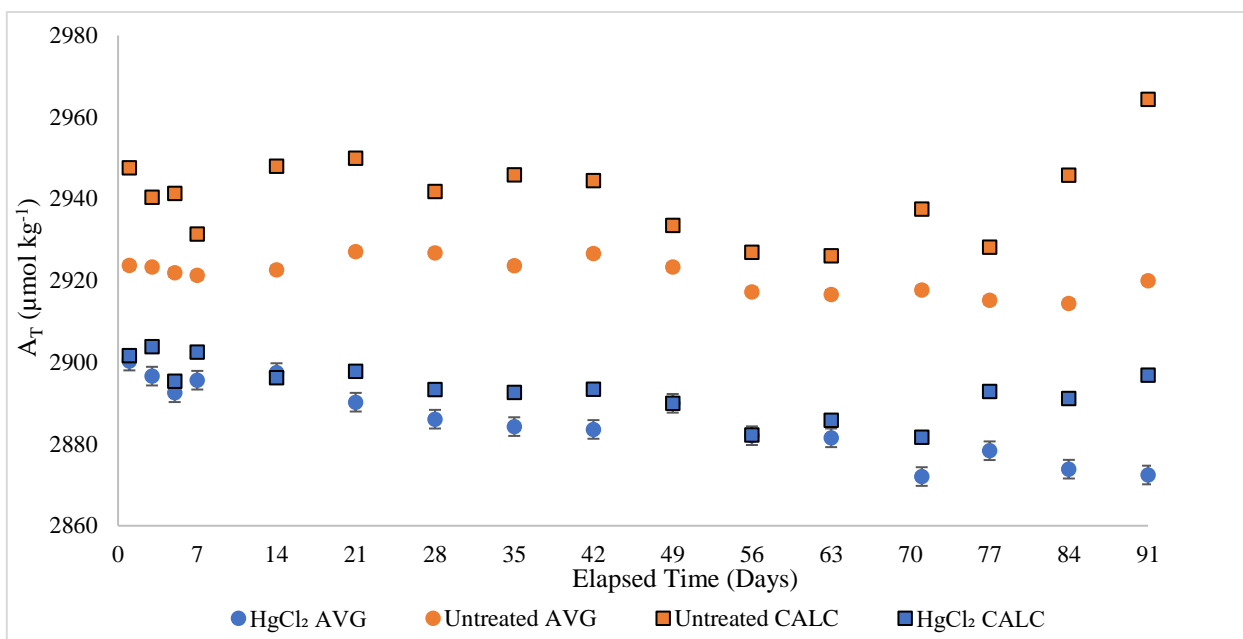
229 samples is initial differences between the collected water samples and is consistent with the observations by Mos et al. (2021)

230 of rapid complexation of organic bases by Hg^{II} that decreased A_T and released H^+ ions. The extent of this complexation then

231 continued over a period of months (Fig. 3).

232   Calculated values of A_T were larger than measured values for both the untreated and treated samples (Fig. 5). For untreated

233   samples the average difference between measured and calculated A_T (A_T(measured) - A_T(calculated)) was -19.5 ± 9.2 µmol kg^{-1}

234   and the average difference for the treated samples was -7.52 ± 6.8 µmol kg^{-1}.

235
236   **Figure 5. Average (AVG) measured versus calculated (CALC) A_T for untreated and treated (HgCl$_2$) samples over elapsed time.**
237   **Error bars are the standard deviation of the average from each day of analysis. Calculated A_T measurements were performed with**
238   **CO2sys.m using measured pH$_T$ and DIC$_T$ for the respective sample type.**

239   In the context of internal consistency measurements for open ocean conditions (high salinity, low DOM), Fig. 5 observations

240   for treated samples (A_T(measured) - A_T(calculated) = -7.52 ± 6.8 µmol kg^{-1}) are comparable to similar calculations (Patsavas

241   et al., 2015) made in open ocean waters. In contrast, the difference between measured and calculated A_T for untreated samples

242   was greater. The reason for the substantial difference in the magnitudes of A_T(measured) minus A_T(calculated) between treated

243   and untreated samples is unclear. Furthermore, while the results shown in Fig. 3 imply that the contributions of organic

244   alkalinity to A_T were substantial, the observation that A_T(calculated) > A_T(measured) for both treated and untreated samples

245   is inconsistent with the fact that CO2sys.m calculations do not (i.e., cannot) account for organic alkalinity contributions to A_T.

246   The observation that measured A_T is not larger than calculated A_T (Fig. 5) points to potential problems with algorithms for

247   CO$_2$-system calculations at low salinities. Resolution of this issue (i.e., differences in measured and calculated A_T) will require

248   further detailed investigation of CO$_2$-system characteristics in organic-rich estuarine waters.

249   **4. Improved approach for sample preservation in organic rich waters**

250   The overarching objective of sound storage and preservation methods is to maintain samples so that they reflect the *in situ*

251   carbonate system characteristics of the samples at the time of collection. The results demonstrate that for DIC$_T$ measurements,

252   poisoning samples with $HgCl_2$ is effective in achieving this objective (Fig. 2). Over a period of three months, DIC_T
253   measurements for the treated samples were essentially invariant while the filtered samples that were not treated exhibit
254   substantial changes. Although it is possible that sample storage at low temperatures, as advocated by Mos et al. (2021), would
255   reduce or even eliminate the respiration derived generation of DIC_T shown in untreated samples, the effectiveness of $HgCl_2$
256   additions is unequivocal. A key point here is that, while Mos et al. (2021) demonstrated that their procedures effectively
257   eliminated changes in A_T, it is much easier to detect changes in DIC_T than for A_T (i.e., $\Delta DIC_T/\Delta A_T = 7.3$), Unless further work
258   demonstrates that filtration in conjunction with low temperature storage eliminates changes in DIC_T, it appears prudent to
259   preserve samples intended for analysis of DIC_T with $HgCl_2$ additions.

260   With respect to preservation of organic-rich samples for A_T analysis, addition of $HgCl_2$ and subsequent long-term storage is
261   ineffective (Fig. 3). Interactions of DOM and Hg^{II} in organic-rich samples appear to increase through time, and this confounds
262   interpretation of A_T results obtained using treated samples. In contrast, for untreated samples, although decreases in A_T are
263   observed that are broadly consistent with the increases in DIC_T (Fig. 2) the changes are small. As such, as shown in the study
264   of Mos et al. (2021), it is possible that storage at lower temperature would further reduce changes in A_T during storage.
265   Therefore, consistent with the results of Mos et al. (2021), improved accuracy of measurements for A_T analysis can be achieved
266   when samples are filtered, stored in polypropylene bottles, and refrigerated at 4°C. Minimizing the time between sample
267   collection and analysis improves accuracy of results (Fig. 3). Notably, preserving DIC_T samples with $HgCl_2$, but not A_T
268   samples, requires that the two types of samples are stored separately.

269   The results shown in Fig. 4 indicate that long-term storage of samples for pH_T analyses affects accuracy of results. Sample
270   pH_T is sensitive to changes in DIC_T and A_T. In the absence of Hg^{II} additions, respiration increases DIC_T (Fig. 2) thereby
271   decreasing the pH_T. For treated samples, changes in A_T can create problems with pH_T measurements. The solution to this issue
272   is prompt pH_T measurements at the time of collection. In contrast to measurements of DIC_T and A_T, spectrophotometric pH_T
273   measurements are uniquely amenable to analysis in the field. As an alternative, if measurements of pH_T in the laboratory are
274   preferred, measurements of pH_T over one week of storage (Fig. 4) were similar to field pH_T measurements obtained
275   immediately before the samples were bottled.

276   Although further investigation of sample preservation and storage could be beneficial, the results obtained in this study support
277   the following improvements for CO_2 system sample collection and analysis of organic-rich waters:

278   •   Accurate analyses of DIC_T can be achieved by filtration and preservation with $HgCl_2$.
279   • A_T analyses can be improved by filtration and storage in polypropylene bottles at 4°C (Mos et al., 2021). Preservation
280   with $HgCl_2$ is not advisable.
281   •   Good quality measurements of pH_T can be obtained by prompt analyses in the field. If this is not feasible, then samples
282   can be preserved with $HgCl_2$ and measured in the laboratory within approximately one week.

283   *Data availability.* The data related to this article is available online at https://doi.org/10.5066/P9J9IYFD, 2022 (Moore et al.,
284   2022). A provisional data release link prior to publication for is available at https://coastal.er.usgs.gov/data-
285   release/provisional/ip139775/.

286   *Author contributions.* CM and RB designed the experiments and CM carried them out. CM and RB prepared the manuscript
287   with contributions from KY. RB and KY reviewed and edited the manuscript.

288   *Competing interests.* The contact author has declared that neither they nor their co-authors have any competing interests.

289   *Acknowledgments.* We thank Andrew Warren and Robert Walker at the Florida Institute of Oceanography (FIO) for providing
290   the Niskin bottles used in this work. We also thank Rob Masserini for providing nutrient analyses. Any use of trade, firm, or
291   product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

293   Anderson, L. A., and Sarmiento, J. L.: Redfield ratios of remineralization determined by nutrient data
294   Analysis, Global Biogeochem. Cy., 8(1), 65–80. https://doi.org/10.1029/93gb03318, 1994.
295   Andren, A. W., and Harriss, R. C.: Observations on the association between Mercury and organic matter
296   dissolved in natural waters, Geochim. Cosmochim. Ac., 1253–1258,
297   https://doi.org/10.1016/0016-7037(75)90132-5, 1975.
298   Broecker, W. S., Takahashi, T., and Takahashi, T.: Sources and flow patterns of deep-ocean waters as
299   deduced from potential temperature, salinity, and initial phosphate concentration, J. Geophys. Res.,
300   90(C4), 6925. https://doi.org/10.1029/jc090ic04p06925, 1985.
301   Byrne, R. H.: Measuring ocean acidification: New technology for a new era of ocean chemistry, Environ. Sci.
302   Technol., 48, 10, 5352–5360. https://doi.org/10.1021/es405819p, 2014.
303   Butler, J. N.: Carbon Dioxide Equilibria and Their Applications, Lewis Publishers, Chelsea, Michigan,
304   https://doi.org/10.1201/9781315138770, 1991.
305   Cai, W. J., Feely, R. A., Testa, J. M., Li, M., Evans, W., Alin, S. R., Xu, Y. Y.,
306   Pelletier, G., Ahmed, A., Greeley, D. J., Newton, J. A., and Bednaršek, N., Natural and
307   anthropogenic drivers of acidification in large estuaries, Annu. Rev. Mar. Sci, 13:23-55 (1),
308   https://doi.org/10.1146/annurev-marine-010419-011004, 2021.
309   Clayton, T. D., Byrne, R. H.: Spectrophotometric seawater pH measurements: Total hydrogen ion concentration
310   scale calibration of m-cresol purple and at-sea results, Deep-Sea Res. Pt. I,
311   40(10), 2115–2129, https://doi.org/10.1016/0967-0637(93)90048-8, 1993.
312   Dickson, A. G.: Standard potential of the reaction: $AgCl(s)+12H_2(g)=Ag(s)+HCl(aq)$, and the standard
313   acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K, J. Chem. Thermodyn., 22(2), 113–

314   127, https://doi.org/10.1016/0021-9614(90)90074-z, 1990.

315   Dickson, A. G.: Standards for ocean measurements, Oceanography, 23(3), 34–47,

316   https://doi.org/10.5670/oceanog.2010.22, 2010.

317   Dickson, A. G., and Goyet, C.: Handbook of methods for the analysis of the various parameters of the

318   carbon dioxide system in sea water, USDOE, Ver. 2, https://doi.org/10.2172/10107773, 1994.

319   Dickson, A. G., Sabine, C. L., and Christian, J. R.: Guide to best practices for ocean CO_2 measurements, PICES

320   Special Publication, OCADS, 3, 191 pp.,

321   https://www.ncei.noaa.gov/access/ocean-carbon-acidification-data-system/oceans/Handbook_2007.html, 2007.

322   Dore, J. E., Houlihan, T., Hebel, D. V., Tien, G., Tupas, L., and Karl, D. M.: Freezing as a method of sample

323   preservation for the analysis of dissolved inorganic nutrients in seawater, Mar. Chem., 53, 173-185,

324   https://doi.org/10.1016/0304-4203(96)00004-7, 1996.

325   Doney, S. C.: The dangers of ocean acidification, Sci. Am., 294(3), 58–65,

326   http://www.jstor.org/stable/26061378, 2006.

327   Douglas, N. K., and Byrne, R. H.: Spectrophotometric pH measurements from river to sea: Calibration of mCP for 0

328   ≤ S ≤ 40 and 278.15 ≤ T ≤ 308.15 K, Mar. Chem., 197, 64–69, https://doi.org/10.1016/j.marchem.2017.10.001,

329   2017.

330   Fong, M. B., and Dickson, A. G.: Insights from go-ship hydrography data into the thermodynamic consistency of

331   CO_2 system measurements in seawater, Mar. Chem., 211, 52–63, https://doi.org/10.1016/j.marchem.2019.03.006,

332   2019.

333   Gordon L. I., Jennings Jr, J. C., Ross, A. A., and Krest, J. M.: A suggested protocol for continuous flow automated analysis

334   of seawater nutrients, WOCE Operation Manual, WHP Office Rept 90-1, WOCE Rept 77 No 68/91, 1-52,

335   http://www.ioccp.org/images/06Nutrients/WOCE_nutrients-manual_1993.pdf, 1993.

336   Hudson-Heck, E., Liu, X., and Byrne, R. H.: Purification and physical–chemical characterization of

337   bromocresol purple for carbon system measurements in freshwaters, estuaries, and oceans, ACS Omega, 6(28),

338   17941–17951, https://doi.org/10.1021/acsomega.1c01579, 2021.

339   Hunt C. W., Salisbury J. E., and Vandemark D.: Contribution of non-carbonate anions to total alkalinity and

340   overestimation of pCO_2 in New England and New Brunswick rivers, Biogeosciences 8:3069–76,

341   https://doi.org/10.5194/bg-8-3069-2011, 2011.

342   Körtzinger, A., Hedges, J. I., and Quay, P. D.: Redfield ratios revisited: Removing the biasing effect of

343   anthropogenic CO_2, Limnol. Oceanogr., 46(4), 964–970, https://doi.org/10.4319/lo.2001.46.4.0964, 2001.

344   Lee, K., Kim, T. W., Byrne, R. H., Millero, F. J., Feely, R. A., and Liu, Y. M.: The universal ratio of boron to

345   chlorinity for the North Pacific and North Atlantic Ocean, Geochimica Et Cosmochimica Acta, 74(6), 1801–1811,

346   https://doi.org/10.1016/j.gca.2009.12.027, 2010.

347   Lewis, R. R., and Estevez, E. D.: The ecology of Tampa Bay, Florida--an estuarine profile, Fish and Wildlife

348   Service U.S. Dept. of the Interior, Washington, D.C., https://www.osti.gov/biblio/6709129, 1988.

349   Liu, X., Patsavas, M. C., and Byrne, R. H.: Purification and characterization of meta-Cresol purple for

350   spectrophotometric seawater pH measurements, Envir. Sci. Tech., 45(11), 4862–4868,

351   https://doi.org/10.1021/es200665d, 2011.

352   Liu, X., Byrne, R. H., Lindemuth, M., Easley, R., and Mathis, J. T.: An automated procedure for laboratory and

353   shipboard spectrophotometric measurements of seawater alkalinity: Continuously monitored single-step acid

354   additions, Mar. Chem., 174, 141–146, https://doi.org/10.1016/j.marchem.2015.06.008, 2015.

355   Martell, A. E., and Hancock, R. D.: Metal Complexes in Aqueous Solutions, Springer, New York,

356   https://doi.org/10.1007/978-1-4899-1486-6, 1996.

357   Mierle, G., and Ingram, R.: The role of humic substances in the mobilization of mercury from watersheds, Water Air

358   Soil Poll., 56(1), 349–357, https://doi.org/10.1007/bf00342282, 1991.

359   Millero, F. J., Zhang, J. Z., Lee, K., and Campbell, D. M.: Titration alkalinity of seawater, Mar. Chem., 44(2-

360   4), 153–165. https://doi.org/10.1016/0304-4203(93)90200-8, 1993.

361   Moore, C. S.: Riverine and estuarine CO_2-system studies on the west coast of Florida, USF Tampa graduate theses and

362   dissertations, https://digitalcommons.usf.edu/etd/9191, 2022.

363   Moore, C. S., Byrne, R. H., and Yates, K. K.: CO_2 system measurements in Hillsborough River,

364   Florida, U.S. Geological Survey data release, https://doi.org/10.5066/P9J9IYFD, 2022.

365   Mos, B., Holloway, C., Kelaher, B. P., Santos, I. R., and Dworjanyn, S. A.: Alkalinity of diverse water samples can

366   be altered by mercury preservation and borosilicate vial storage, Sci. Rep., 11(1),

367   https://doi.org/10.1038/s41598-021-89110-w, 2021.

368   Müller, J. D., and Rehder, G.: Metrology of pH measurements in brackish waters—part 2: Experimental

369   characterization of purified meta-Cresol purple for spectrophotometric pH_T measurements, Front. Mar. Sci.,

370   5. https://doi.org/10.3389/fmars.2018.00177, 2018.

371   Landis, D.: OOIBase32 spectrometer operating software, Dunedin, FL, USA, Ocean Optics, Inc., 2005.

372   Patsavas, M. C., Byrne, R. H., Wanninkhof, R., Feely, R. A., and Cai, W. J.: Internal consistency of marine

373   carbonate system measurements and assessments of aragonite saturation state: Insights from two U.S. coastal

374   cruises, Mar. Chem., 176, 9–20. https://doi.org/10.1016/j.marchem.2015.06.022, 2015.

375   Pilson, M. E. (Second Edition): An Introduction to the Chemistry of the Sea, Cambridge University Press,

376   https://doi.org/10.1017/CBO9781139047203, 2012.

377   Ravichandran, M.: Interactions between mercury and dissolved organic matter - a review, Chemosphere, 55(3), 319–

378   331, https://doi.org/10.1016/j.chemosphere.2003.11.011, 2004.

379   Song, S., Wang, Z. A., Gonneea, M. E., Kroeger, K. D., Chu, S. N., Li, D., and Liang, H.: An important

380   biogeochemical link between organic and inorganic carbon cycling: Effects of organic alkalinity on carbonate

381   chemistry in coastal waters influenced by intertidal salt marshes, Geochimica et Cosmochimica Acta, 27, 123-139.

382 https://doi.org/10.1016/j.gca.2020.02.013, 2020.

383 Stumm, W., and Morgan, J. J. (Third Edition): Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters,

384 John Wiley and Sons Inc., Canada, 2012.

385 Takahashi, T., Broecker, W. S., and Langer, S.: Redfield ratio based on chemical data from isopycnal surfaces, J.

386 Geophys. Res., 90(C4), 6907, https://doi.org/10.1029/jc090ic04p06907, 1985.

387 van Heuven, S., Pierrot, D., Rae, J.W.B., Lewis, E., and Wallace D.W.R.: MATLAB program developed for CO_2

388 system calculations, ORNL/CDIAC-105b. Carbon Dioxide Information Analysis Center, Oak Ridge National

389 Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

390 https://doi.org/10.3334/CDIAC/otg.CO2SYS_MATLAB_v1.1, 2011.

391 Varshal, G. M., Buachidze, N. S., Velyukhanova, T. K., and Chkhetia, D. N.: The role of organic matter in

392 mercury cycle, in: Global and Regional Mercury Cycles: Sources, Fluxes and Mass Balances, NATIO ASI Series,

393 vol 21, edited by: Baeyens, W., Ebinghaus, R., Vasiliev, O., Springer, Dordrecht, 403–414,

394 https://doi.org/10.1007/978-94-009-1780-4_20, 1996.

395 Waters, J., Millero, F. J., and Woosley, R. J.: Corrigendum to "The free proton concentration scale for

396 seawater pH", [MARCHE: 149 (2013) 8–22]. Mar. Chem., 165, 66–67,

397 https://doi.org/10.1016/j.marchem.2014.07.004, 2014.

398 Wong, C. S.: Quantitative analysis of total carbon dioxide in sea water: A new extraction method, Deep-Sea

399 Res., 17:9–17, https://doi.org/10.1016/0011-7471(70)90084-7, 1970.

400 Yao, W., and Byrne, R. H.: Simplified seawater alkalinity analysis, Deep-Sea Res. Pt. I., 45(8), 1383–1392,

401 https://doi.org/10.1016/s0967-0637(98)00018-1, 1998.