Carbonate System Sampling as part of Ocean Acidification impacts on Zooplankton in Northern Oceans.

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METHODS and RESULTS

Hatching Experiments: The details of the preparation and execution of the hatching experiments are being described in reports in preparation (Preziosi, 2012; Preziosi et al., 2012, 2013; Preziosi, Runge, Christensen and Jones, in prep.). In brief, female zooplankton, freshly caught or reared in the lab with plentiful food, were maintained in a healthy condition and their released eggs separated. When the numbers of eggs required in an experiment were sufficient, the eggs were sorted into individual hatching dishes, each containing natural seawater and 30 eggs. Local seawaters were collected, filtered and used to fill several 20 L hatching tanks. Each tank was consisted of a polycarbonate tank and lid, and an aeration tube into which was bubbled a premixed gas, consisting of 20% oxygen, the preselected concentration of CO_2 (ranging from ambient local atmospheric levels to 50000 ppm in the dry gas), and the remainder nitrogen gas (N_2). A siphon mounted within the tank allowed for sampling of the waters without opening the tank. A 1-inch diameter hole in the tank lid, normally stoppered, allowed for immersion of the pH and temperature electrodes occasionally throughout the incubation. After the pH stabilized to its quasi-equilibrium value for the bubbled gas, several hatching dishes containing the eggs were immersed in the tank. The tank was closed and hatching allowed to proceed. At an appropriate time, all hatching dishes were removed for determination of hatching success and the experiment then ended. During the incubation, either once (for incubation times less than 2 days) or twice, at the beginning and near the end of the longer 4-6 day experiments, about 2 liters of tank water were withdrawn for collection of the chemistry samples, as described below. The chemical results were used to determine the tank's pH.

At each sampling event during hatching experiments, about 2 L of the tank waters were siphoned for samples of salinity, titration alkalinity (TA), total carbon dioxide (TCO_2), and nutrients. All bottles for TA, TCO_2, and nutrients had been previously acid cleaned and dried. Nevertheless, all bottles were rinsed four times with tank water prior to filling. Salinity was stored in tightly capped 0.5 L bottles at room temperature for later measurement. TA and TCO_2 samples were drawn in a manner identical to the collection of dissolved oxygen samples. An air bubble of about 1% of the volume of the bottle was left in the top of the bottle and 0.10 ml of a 0.100 mole-Hg/L solution of $HgCl_2$ was added as preservative, yielding a mercury concentration of about 100 μmol/L dissolved in the sample. The sample bottles were tightly capped and stored at room temperature until measurement. Nutrients were collected in plastic vials and frozen at -20°C until analyzed. The temperature of the cold room in which the experiment was being conducted was monitored continuously. All concentrations are reported relative to the weight of the final solution (kg of solution).

In addition to the hatching experiments, we sampled the offshore waters of the Gulf of Maine during a 10-day cruise in autumn of 2012. Seawater was sampled using a vertically profiling CTD with rosette containing 24 30-liter Niskin water sampling bottles. collected. These CTD casts collected waters from the seasurface to within a few meters of the seafloor (depths as great as about 300 m). After the CTD/Rosette had returned from a cast, water from each Niskin was sampled for alkalinity, total carbon dioxide, dissolved oxygen (Carpenter et al., 1965), nutrients, and salinity. Alkalinity and TCO2 were sampled in a manner identical to the collection procedure used for Carpenter oxygen samples. Each alkalinity and total CO2 sample were preserved and stored as described for the hatching experiments. Nutrients and salinities were stored and measured identically to those from the hatching experiments.

Determination of Salinity: Salinity was measured using an Autosal 8400A conductivity salinometer. IAPSO standard seawater was used as standard. Each salinity sample was measured at least 4 times until final 2 measurements were within 0.002 per mil of each other.

Determination of Titration Alkalinity: Titration alkalinity was determined by the open cell titration method (Dickson et al., 2007, SPO-3b). The quantity of sample added to the titration cells was determined gravimetrically. The pH of the samples were determined using a Ross Ultra semi-microelectrode (Orion #8103BNUWP) connected to an op-amp circuit (unity-gain follower) whose output voltage matches the electrode output. This op-amp output was continuously monitored via a 14 bit A-to-D converter and computer. The titration cell was maintained at constant temperature (within +/-0.01°C) using the pumped flow from a thermostatically controlled water bath. The titration solution, a dilute HCl solution dissolved in NaCl, was added with a Gilmont microburet which dispenses a maximum volume of 2.5 ml with volume increments of 0.0001 ml. The temperature of the titration cell was measured routinely throughout all titrations using a Dostmann electronic P-655-PT precision thermometer calibrated accurate to 0.01°C. Over an analysis day, the cell temperature was constant (within 0.02°C), with the temperature being set to a fixed value which was within 0.5°C of the ideal (25°C).

The pH electrode was standardized using two pH standard solutions, one of Tris buffer (2-amino-2-hydroxymethyl-1,3-propanediol) and one of AMP buffer (2-aminopyridine) dissolved in artificial seawater equivalent to salinity of 35. The preparation of these buffers were described in detail in Dickson et al., 2007 (SOP-6a). The slope of the electrode was calculated via equation 1:

$$\text{Slope} = (V_{amp} - V_{tris}) / (pH_{tris} - pH_{amp}), \quad (1)$$

where V_{amp} and V_{tris} were the raw electrode readings in volts and pH_{amp} and pH_{tris} were the calculated pH values of amp and tris buffers in seawater of given salinity and temperature (degrees K, equations 6 and 5 respectively in Dickson et al., 2007, SPO-6a). The slope was normalized to the ideal electrode response at the measurement temperature, giving the slope percent (SP):

$$SP = 100\ Slope / ((R\ T_K / F) \ln(10)) \quad (2)$$

where R is the universal gas constant (8.31447215 J (degrees K)$^{-1}$ mol^{-1}), F is the faraday constant (96485.339924 coulombs mol^{-1}, and T_k is the temperature of the titration cell (degrees K). Temperature in Kelvin is related to temperature in Centigrade by the formula:

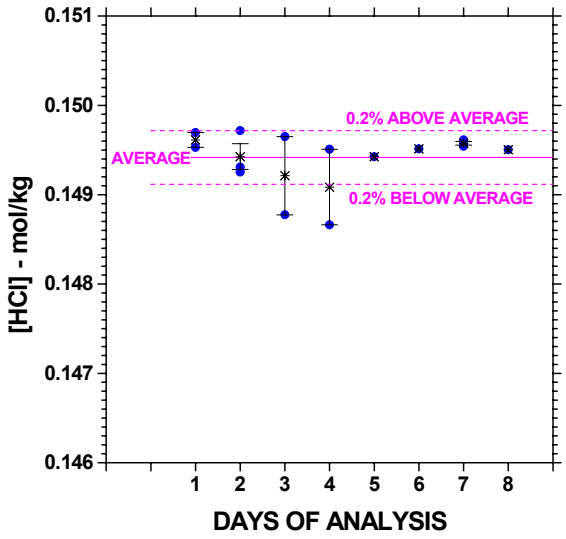
$$T_k = T_c + 273.15 \quad (3)$$

For the titrations occurring over a day of sample analysis, the daily average of all measurements of the slope percent were used in the alkalinity calculations. The electrode had average daily slope percent values ranging between 95 and 98% of the ideal.

In the open cell titration, sufficient quantity of an acid titrant (the HCl-NaCl mix described below) is added via the microburet to bring the pH electrode's reading to just below 160 mv. At this point, a bubbling tube expelling CO_2-free air was inserted to allow the sample's free CO_2 to degas. Subsequently, about 20 increments of acid were added, each with the paired recording of the electrodes voltage output. As a first approximation to the calculating the alkalinity, gran analysis was done (Hanssen and Jagner, 1973). Then, using this result, a chi-squared fit routine was employed to best-fit the ideal titration curves of sulfate, borate, flouride, phosphate, and silicate concentrations to the measured data. Sulfate, borate and fluoride concentrations were estimated from the sample's salinity, while phosphate and silicate concentrations were those measured in the nutrient section. The fitting routine (Dickson et al., 2007) varied both the electrode voltage offset (via the parameter, f, in Dickson et al., 2007, SOB3b) and the sample's alkalinity (for samples being measured for alkalinity) or the HCl titrant's acidity (for standards used to calibrate the HCl-NaCl titrant) until best fit was achieved. Since the microburet dispenses volumes of acid titrant but the equations are solved based on the weight of the solutions, the density of the HCl-NaCl solution was redetermined after each new calculation of the acidity during the fitting procedure following equations presented in Dickson et al. (2007). In contrast, during calculations for alkalinity, the acid strength of the titrant is defined by the daily standardization, so density of the acid titrant was computed at the beginning of the procedure. Lower chi values were obtained when the titration points used in the fitting procedure were those with electrode readings between about 0.205 and 0.230 volts. Chi values of the best fit were generally less than 2 x 10^{-12} $(mol/kg)^2$.

Samples or standards were titrated with a standardized HCl solution in a medium of NaCl and deionized water (DIW). In order to achieve a concentration of 0.450 mol/kg in the titrant solution, the oven-dried NaCl was precisely weighed in large batches which lasted for weeks of sample analysis. The concentration of HCl was determined on each run day by calibration against alkalinity standards. The primary standard was calibrated seawater for alkalinity and total carbon dioxide measurements purchased from the Scripps Institute of Oceanography (SIO, http://andrew.ucsd.edu/co2qc/). Secondary standards were prepared from approximately equal molar concentrations of $Na_2HPO_4 \cdot 7H_2O$ and KH_2PO_4 and made in a NaCl medium. These phosphate alkalinity standards were prepared in a manner which would allow possible carbonate contaminants to degas as CO_2. This was done by dissolving a weighed amount of KH_2PO_4 in DIW. Because the pH of this solution was about 4, TCO_2 in the salt or in the DIW would volatilize. Then the weighed amount of NaCl was added, again allowing any additional TCO_2 to escape. Finally, a weighed amount of $Na_2HPO_4 \cdot 7H_2O$ was added. All DIW additions were done by weight. For most phosphate alkalinity substandards, the final total phosphate concentration was about 4 mmol/kg of solution and the final HPO_4^{-2} concentration was half of the total. The NaCl concentration was near to 0.7 mol/kg of solution. These phosphate alkalinity standards were calibrated against the SIO seawater primary standards. Then, using either the SIO seawater alkalinity standards or the phosphate alkalinity substandards, the acid concentration of the HCl-NaCl titrant was determined on each analysis day prior to measuring the samples. Precision of the calibration of the HCl-NaCl titrant was demonstrated to be within 0.2% for most of the alkalinities determined in this report (**Figure 1**).

Figure 1. The determined acid concentration in a single batch of HCl-NaCl titrant over 8 days in which samples from the different experiments were analyzed. Blue filled circles are individual measurements. Black crosses are the daily mean and upper and lower error bars represent +/- 1 SEM around the mean. For these standardizations, all acid concentrations averaged 0.1493872 mol/kg (solid magenta line) and the standard error of the mean was +/- 0.0000896 mol/kg or 0.05996% of the mean value (SEM%, standard error of the as percentage of the mean value). Daily means were all within about 0.2% of the overall average (dashed magenta lines above and below the overall average line). Daily means were used in each days calculations of alkalinity.



Additional standards, made of Na_2CO_3 in NaCl medium, were used to check the quality of the titrations. Here, a large quantity of NaCl medium of approximately 0.70 mol/kgs was made, with its actual concentration known to 0.01% based on the precision of the weighings involved. Analytical grade, oven-dried sodium carbonate was dissolved in a preweighed amount of medium. The alkalinity was calculated from the carbonate content (ideal alkalinity = 2 x $[CO_3^{-2}]$). The measured alkalinity of these carbonate standards always exceeded by 1-2% the ideal alkalinity based on the weighings. To determine if the NaCl medium contained minor contributions of titratable alkalinity, the alkalinities of a standard curve of phosphate (approximately equal molar amounts of KH_2PO_4 and $Na_2HPO_4 \cdot 7H_2O$) dissolved in the NaCl medium was determined. Regression of the measured alkalinity onto the added $Na_2HPO_4 \cdot 7H_2O$ showed a high correlation (**Figure 2**), with a positive regression intercept of 37.4807 µmol alkalinity/kg at 0 µmol/kg of Na_2HPO_4. A similar regression was found for measured alkalinity versus total phosphate. However, KH_2PO_4, by itself, has little measurable alkalinity because the pK for conversion of $H_2PO_4^-$ to H_3PO_4 is beyond the test range of the titration, so the regression of measured alkalinity onto Na_2HPO_4 had a slightly better correlation coefficient. Using the intercept (37.4807 µ mol/kg) as the alkalinity contribution from the NaCl medium, the actual alkalinities due to sodium carbonate were compared with the ideal alkalinities from the weighings of the individual carbonate standards (**Figure 3**). Here, for all of the carbonate standards, the measured alkalinity averaged 100.2624% of the ideal alkalinity with the SEM% being 0.1028% of the average. The daily mean values of the carbonate standards generally fall within 0.2% of the anticipated values. Since the weight of carbonate used in the standards was about 0.1 gram, the insensitivity of +/- 0.2 mg in the analytical balance would approximately double the overall uncertainty over that just due to the titration procedure (<0.2% from the analysis of acid concentration). Nevertheless, the carbonate standards indicate sufficient accuracy and precision for the measurement of alkalinities in the coastal seawaters.

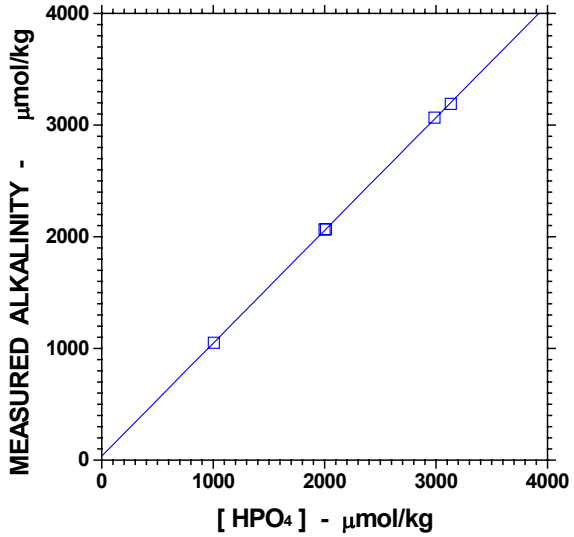


Figure 2. Standard curve of phosphate buffer in 0.7 mol/kg NaCl medium. Blue squares represent individual data. The linear regression (blue line) had an r^2 of 0.999865. The intercept was due to alkalinity of the NaCl solution and had a value of 37.4807 µmol/kg with the standard error of the mean of +/- 5.2534 µmol/kg.

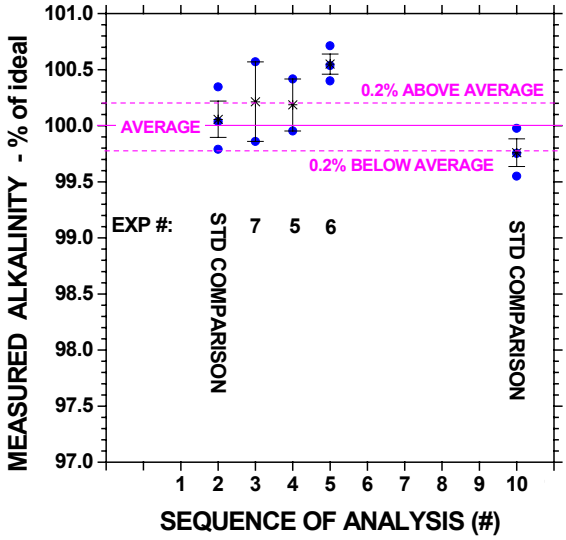


Figure 3. Comparison of alkalinities of Na_2CO_3 standards, corrected for the background alkalinity of the medium, with the ideal alkalinity of the carbonate ions based on weight of the Na_2CO_3. Blue filled circles are individual data. Daily mean values are black crosses and upper and lower error bars represent +/- 1 SEM around the mean. All measurements averaged 100.2624% (measured divided by the ideal) with the SEM% equal to 0.1028%. Daily mean values were generally within 0.2% of 100%.

Determination of Total Carbon Dioxide: Total carbon dioxide (TCO_2) concentrations were determined by acid stripping of a water sample, concentrating the expelled TCO_2 in a cold trap, then injecting it into a Shimadzu Model GC-6A gas chromatograph (gc) with a thermal conductivity detector (Christensen, 2008). Output from the gc was continuously recorded on 14 bit A-to-D converter linked to a computer. The details of the gc system are described in **Figure 4.** In the initial system (used through Hatching Experiment 8), a sample was analyzed by first stabilizing the helium flow with the cold trap in the stripping oven (at 140°C) while connected in the sample detector helium stream. The stripper was filled with 1 ml of 30% H_3PO_4 and the acid loop on the acid injection value was filled with the same acid (0.5 ml). The sample was drawn into the sample loop of the water sample value so that no air bubbles were visibly trapped in the loop. To start a sample run, the trap injection valve was turned so that the heated cold trap was in the stripper circuit. Then the cold trap lowered into a dewar of dry-ice ethanol mix at approximately -80°C. The cold trap was allowed to cool for 2.0 minutes. Then the water sample valve was turned so that the water sample loop was in the stripper circuit. The helium flow then pushed the sample though the intervening tubing and into the stripper containing 1.0 ml of H_3PO_4. At this point, the acid injection valve was turned, putting the acid loop in the stripper circuit, and an additional 0.5 ml of acid was pushed by the helium stream through all the tubing which had been exposed to sample water. This acted to rinse and acidify any sample droplets that may have adhered to the walls of the tubing or valves.

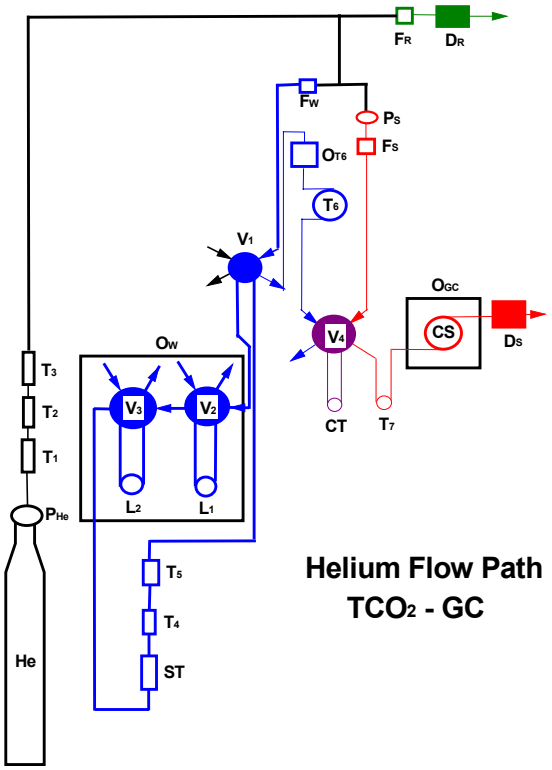


Figure 4. Helium flow path of the GC system for precisely measuring total carbon dioxide in seawater samples (Christensen, 2008). The high purity helium tank was marked with the letters, He, and its pressure regulator was P_{He}. The helium stream was split into 3 separate flow circuits, the detector's reference circuit (colored green), the detector's sample circuit (colored red), and the water stripping circuit (colored blue). An additional pressure regulator was located on the sample detector circuit (Ps). A flow controller was in the beginning of each circuit (F_R, F_S, F_W), and these were set to 18 cc/min. for the reference circuit, 22 cc/min. for the detector circuit, and 100 cc/min. for the water stripping circuit. Traps were labeled with T. Trap 1 (T_1) was a water vapor trap of drierite. Trap 2 (T_2) contained molecular sieve 5a. Trap 3 (T_3) contained ascarite to remove contaminant CO_2 gas. Traps 4 and 5 (T_4, T_5) were liquid water traps following the seawater stripper (ST). Trap 6 (T_6) was a 72 inch Perma Pure dehydration column (MD-070-72S), and just prior to it was an oven (O_{T6}) to heat the incoming flow to 95°C to help volatilize any remaining liquids in the gas stream. Trap 7 (T_7) is a cold trap maintained at -26°C to remove water vapor. The gas chromatograph's oven (O_{GC}) kept the separatory column (CS, 6 ft of 1/8 inch stainless tubing filled with Poropak N and Q separating resins (50% of each, both 80/100 mesh) at 140°C. The two halves of the detector (D_R and D_S) were kept at 150°C, and the detector was run at 160 milliampere sensitivity. Three 6-port Rheodyne teflon valves were in the stripper circuit. The stripper bypass valve (V_1) allowed the stripper circuit to be moved into or out of the stripper helium stream. The acid injection valve (V_2) contained a 0.5 ml loop of tubing for injection of 30% H_3PO_4 through the stripper tubing, to acid rinse the sample loop. The sample injection valve (V_3) allowed a water sample of 1.1130 ml +/- 0.0024 ml (standard error of the mean) to be injected into the stripper circuit. The two values, the acid injection value and the water sample valve, were immersed in a thermostated water bath (O_W) at 25°C. The Valco 6-port 1/16 inch high pressure valve (V_4) allowed the cold trap (CT) to be switched between the water stripper circuit and the detector circuit. The cold trap consisted of 13 inch long segment of stainless steel tubing loosely filled with several thin copper wires coated in high temperature silicone gasket caulking with Poropak N (50/80 mesh) coating its surface. When trapping the CO_2 gas in the water stripping circuit, the cold trap was immersed in a bath of ethanol and dry ice (-78°C). When the trapped CO_2 was to be liberated in the detector circuit, the cold trap was heated to the oven temperature until the analytical run was completed. Tubing of diameter of 1/8 inch was shown as the thicker lines while 1/16 inch diameter tubing was shown as thinner lines. Nylon tubing was used in the water stripping circuit when this tubing was exposed to liquid water or acid.

Stripping proceeded for a total time of three minutes, then the cold trap valve was turned so that the cold trap was back in the sample analysis helium stream, and the trap removed from the dry-ice ethanol bath and rapidly heated to 140°C. The CO_2 captured on the cold trap was released, passed through an addition water vapor trap and through the separation column.

In a typical run, only two peaks were found. Carbon dioxide passed through the separation column first at about 7.2 minutes after starting the run and was completely vented by 8.2 minutes. Water vapor typically started eluting at about 9 minutes, and if the several water traps were operating effectively, the small amount of water vapor was fully eluted before the next sample's CO_2 was injected into the detector circuit. Other major gases of seawater or air (oxygen, nitrogen, argon, etc.) were not trapped in the cold trap, so were vented out of the stripper circuit before the CO_2 trapping had been completed. Thus, these gases do not appear in these chromatograms. The area of the carbon dioxide peak was evaluated based on a linear baseline between the beginning and end of the peak, and in most cases the baseline was visibly flat.

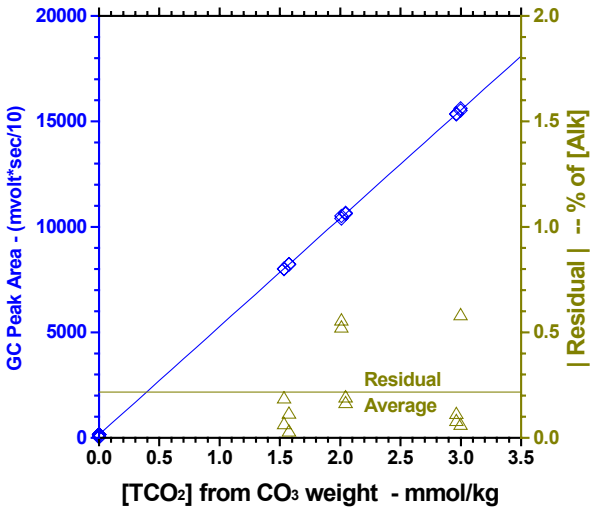


Figure 5. A standard curve of Na_2CO_3 in DIW for the TCO_2 analytical system (blue diamonds). The linear regression (blue line) had a r^2 = .999974 (n=14). The absolute value of the residuals for all non-zero alkalinities (dark yellow triangles) are plotted as the percentage of the regression-estimated alkalinity, and the average was 0.217% of the estimated alkalinity (dark yellow line).

Typically, standardization was performed using Na_2CO_3 standards prepared in DIW which had been equilibrated with ambient air at laboratory temperature (23°C +/- 1°C) for several days. A standard curve consisted of duplicate flasks of three carbonate standards, at about 1500 µmol/kg, 2000 µmol/kg, and 3000 µmol/kg, as well as the equilibrated DIW. Results showed that these curves were linear (**Figure 5**) and that the DIW had a small but measurable concentration of dissolved carbon dioxide. Thus, any carbonate standard would have a total concentration equal to the amount contributed from the Na_2CO_3 and from the DIW. Seawater samples would not have this residual medium CO_2, so a second set of background values were also determined, that of a water sample which had already be stripped and measured and which was still inside the stripper. Given that the stripping had been fully efficient, the stripper would be releasing no more carbon dioxide but would have a similar outflow of water vapor. Thus this "machine blank" was a stripped sample run a second time. Typically, these blanks produced a smaller peak in the chromatogram than found in the DIW blanks, and the peak area was independent of the amount of CO_2 released on the previous run. Repeated measurements showed that the second stripping of a sample typically had about 20 µmol CO_2/kg less than a fresh DIW blank. Assuming that this re-stripped amount represents CO_2 leakage in the GC circuit, and that the re-stripped water released no carbon dioxide, the amount of CO_2 in the DIW would be about 20 µmol/kg, approximately what would be expected for pure water exposed at room temperature to atmospheric levels of CO_2. A second standard, the SIO alkalinity and TCO_2 seawater standard, was compared with these carbonate standards, and the concentrations agreed within the analytical uncertainty of our system. The precision of the

system was evaluated by duplicate measurements from the same sample bottle for 19 seawater samples from these experiments. The average standard error of the mean (expressed as percent of the mean) was 0.26%. Replicate sample bottles had a similar precision so this value would represent the average precision for these samples in this TCO_2 system.

In later hatching experiments (experiment 9 -13) and in samples from the Gulf of Maine cruise, the system was improved by automation of the trapping sequence. In this automation, the cold trap was fixed to a computer controlled elevator which lowered the trap from a heating box (about 150°C) into a bath of dry ice in ethanol (about -80°C) and raised it out of the cold trap and back into the heating box in a timed sequence. In addition, the gas sampling valve (V_4) was also computer controlled which more precisely fixed the duration in which the cold trap was switched between the trapping circuit and the chromatographic circuit. The system was tested with a variety of standards including those made from Na_2CO_3 and SIO seawater standards. Precision was found to improve when the cold trap was allowed to cool in the cold bath for 30 seconds longer than in the initial system prior to the water sample being injected into the stripper. Just prior to running a new sample, the acid sample value (V_2) was positioned so that the acid loop was isolated and contained 0.5 ml fresh H_3PO_4, the water sample valve (V_3) was positioned so that the sample loop was isolated and contained a fresh aliquot of sample water, the stripper had 1.0 ml of fresh H_3PO_4 in it, the stripper circuit, connected via the stripper isolation valve (V_1), was allowed the stripping helium flow to pass through the gas sampling valve, the gas sampling valve (V_4) was turned so that the cold trap was in the chromatographic circuit and was inside its heated box, and the detector output was showing a flat baseline. The timed events were: a) V_4 was turned so the cold trap was in the trapping circuit (0.0 min), b) the cold trap was lowered from the heated box into the cold bath (0.10 min), c) the water sample was injected into the stripper (1.80 min), d) additional H_3PO_4 was injected into the stripper (1.90 min), e) V_4 was turned so that the cold trap was in the chromatographic circuit (4.80 min), f) the cold trap was raised out of the cold bath and into the heated box (4.90 min), and g) the stripper isolation valve (V_1) was turned so that the stripping gases were vented to the atmosphere. For the analysis of a new sample, the previous acid and sample mix in the stripper was removed and new acid readied in the stripper and in the acid stripping value. Also the next sample was drawn into the sample valve. After the CO_2 peak eluted through the detector, the next sample was begun.

This automated version of the TCO_2 system gave increased precision for the analysis. Based on the SIO TCO_2 standards which were used each run day to calibrate the HCl concentration, precision of replicate standards drawn from the same bottle of standard averaged less than 0.06% (expressed as the standard error of the mean divided by the calibrated concentration, **Fig. 6**). An additional assessment of precision was provided from duplicate measurements for individual TCO_2 bottles collected at a variety of sites and depths in the Gulf of Maine. The standard error of the mean (expressed as percent of the mean of the duplicate assays) averaged 0.0947% for 39 separate bottles (**Fig. 7**). Both results indicate that the improved TCO_2 system reduced within bottle variability by more than a factor of two (from about 0.2% for the initial system to less than 0.1% for the improved system).

Dissolved Oxygen: During the cruise across the Gulf of Maine, concentrations of dissolved oxygen were determined by the titration method of Carpenter et al., 1965. Water from the Niskin bottles was drained from each Niskin bottle through Tyson tubing and used to rinse the glass oxygen bottle and then to fill the bottle in the bubble-free manner described by Carpenter et al (1965). These samples were pickled with the first two storage reagents. Over the subsequent 24 hours, the partially pickled bottles were allowed to react and the precipitate settle for several hours, then were shaken to resuspend the precipitate, allowing for more complete reaction of all dissolved oxygen. The iodine content in each bottle was titrated with thiosulfate using a microburet accurate to 0.0001 ml. Standardization of the thiosulfate was done each day using potassium iodate primary standards. The internal volume of all bottles had been determined to 0.05 ml by duplicate gravimetric analyses.

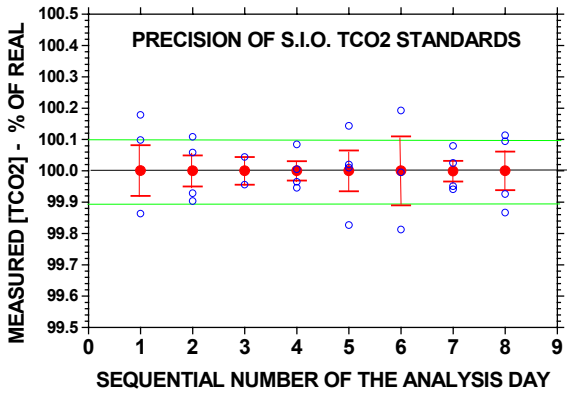


Figure 6. Precision of SIO total carbon dioxide standards for different days of analysis (run days). Open blue circles are the measured TCO2 content divided by the calibrated (listed) concentration for replicate analysis from the same bottle of standard. Red solid circle is the daily mean value (equivalent to the calibrated concentration), and red bars show positive and negative value of the standard error of the mean expressed as percent of the daily mean value. The green lines show the +/- 0.1% level of uncertainty. For the eight analysis days, the standard error of the mean averaged 0.0593%.

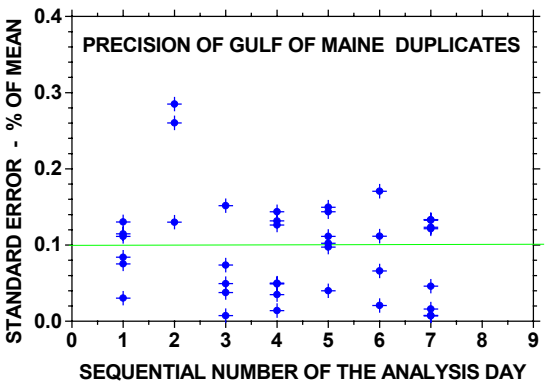


Figure 7. Precision of duplicate subsamples from individual bottles of Gulf of Maine seawater versus different days of analysis (run days). Precision is characterized as the standard error of the mean of the duplicate analyses divided by the mean of the two analyses. The green line shows the level of 0.10% precision. Samples had salinities in the range of 30 - 34.8. For all bottles, the standard error of the mean averaged 0.0947% (n = 39).

Determination of Nutrients: Nitrate and nitrite were determined by the colorimetric method of Armstrong et al. (1967). Ammonium was determined by the colorimetric method of Koroleff (1970) as utilized by Slawyk and MacIsaac (1972). Dissolved inorganic phosphate was determined by the colorimetric method of Drummond and Maher (1995). Dissolved silicate was determined by the method of Armstrong et al. (1967). Based on replicate standards, the precision of the methods averaged +/- 3% of the mean. Concentrations, measured in units of µmol/L, are converted to µmol/kg of solution based on the sample's sigma-t value determined from its salinity and the laboratory temperature at which the nutrients were run (22°C +/- 1°C).

Calculation of Seawater pH: To calculate pH from these data, we used the carbonate equilibration model, CO2SYS (Lewis and Wallace, 1995; DOE, 1994). This employs the equilibration coefficients of Roy et al. (1993) for carbonate coefficients K1 and K2, of Weiss (1974) for carbon dioxide K0, of Dickson (1990b) for borate, of Dickson and Riley (1979) for fluoride, of Dickson (1990a) for sulfate, and of Millero (1995) for phosphate (kp1, kp2, kp3) and silicate. Seawater density at atmospheric pressure was that of UNESCO, 1981.

keywords: seawater carbon dioxide, ocean acidification, CO_2 methods, zooplankton, pH

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