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An Autonomous Instrument for Time Series Analysis of TCO_2 from Oceanographic Moorings

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Abstract

The design and testing of a robotic analyzer for autonomous TCO_2 measurement from oceanographic moorings is described. The analyzer employs a conductimetric method of TCO_2 measurement wherein CO_2 from an acidified sample diffuses across a semi-permeable membrane into a NaOH solution decreasing the conductivity of the base. The instrument is capable of ~850 analyses over a period of at least six months. It is designed to operate to depths of at least 1000m. TCO_2 calibration is based on *in situ* standardization throughout a deployment.

We report both laboratory and *in situ* tests of the analyzer. In the laboratory automated analyses over a period of 38 days at temperatures ranging from 8° to 25° C yielded a TCO_2 accuracy and precision of ±2.7 µmol/kg. *In situ* tests were conducted at the WHOI dock with a deployment of 8 weeks at *in situ* temperatures of 5°-13°C. The accuracy and precision of TCO_2 analyses over the deployment period, based on *in situ* calibration, was ±3.6 µmol/kg.

Laboratory tests of reagent and standard solution stability are also reported. Standards, based on Certified Reference Material were followed for periods of up to 2 years. In all cases TCO_2 increased. Drift of the standards was the equivalent of ~1 to

μmol/kg per 6 months. The conductivity indicator solution was found to be stable for at least 2 months.

1. Introduction

The role of CO_2 in climate and the important part the oceans play in taking up anthropogenic CO_2 has led to great interest in the oceanic CO_2 system and the marine carbon cycle. The oceans are estimated to have taken up about 30% of past anthropogenic CO_2 emissions (Takahashi et al., 1999) and are predicted ultimately, on millennial and longer time scales, to take up some 90% of anthropogenic carbon released to the atmosphere (Archer et al., 1998). The uptake of anthropogenic CO_2 has altered ocean chemistry and processes tied to the carbonate system of the oceans. Orr et al. (2005) report a decrease in the pH of the oceans by 0.1 pH units and predict a decrease of 0.2 to 0.3 over the next century. Decreasing pH lowers $CaCO_3$ mineral saturation, enhancing dissolution in the upper water column and reducing burial of solid $CaCO_3$ (Feely et al., 2004). Decreased pH also inhibits CO_2 uptake by the oceans (Sabine, et al., 2004). Understanding the rates of uptake of CO_2 by the oceans and the processes governing uptake and redistribution within the ocean is critical to assessing the impact of anthropogenic CO_2 on the oceans and in the development of models that seek to predict future impacts.

An important strategy being employed to monitor biogeochemical changes in the oceans is the establishment of time series stations where intensive measurements of a variety of biological, physical and chemical variables are repeatedly made at a fixed location over periods of years. Perhaps the best known of these, where extensive data sets have been collected, are the Hawaii Ocean Time-series (HOT) and Bermuda Atlantic Time-series Study (BATS). Studies at these stations have provided a wealth of insights into temporal biogeochemical variations from almost unbroken data records since 1988. As regards the CO_2 system, data from these studies document a steady increase in TCO_2 (Bates, 2001). Measurements are made both from moorings at these sites and from ships on approximately monthly visits. To date, measurements that fully characterize the TCO_2 system have been limited by the ship schedules. However, it is widely acknowledged that episodic events play a critical role in biogeochemical processes. Monthly cruises, which occupy a location only a small fraction of the time,

can capture neither high frequency nor many episodic events, systematically missing some that are almost certainly important, e.g. severe storms, large scale eddies, internal waves.

Providing a continuous presence at oceanographic time-series sites has led several investigators to develop instruments suited for autonomous measurement of one or more of the parameters required to characterize the CO_2 system (e.g. pH, fCO_2, total alkalinity, TCO_2). These include pCO_2 (DeGrandpre, 1993; DeGrandpre et al., 1995; Merlivat and Brault, 1995), pH (Byrne et al., 1999; Martz et al., 2003, Liu et al., 2006; Seidel et al., 2008) and TCO_2 (Byrne et al., 2002). To our knowledge, within the CO_2 system, only sensors for pH and fCO_2 have been deployed in the ocean for autonomous, unattended operation. This paper describes the development and *in situ* testing of a Robotic Analyzer for the TCO_2 System (RATS).

RATS was designed and built to measure both TCO_2 and pH. The pH instrument is based on the spectrophotometric methods described by Clayton and Byrne (1993) and Zhang and Byrne (1996). The optical cell we employed was a long path length liquid core waveguide (lcw) (Byrne, et al., 1999) permitting absorbance measurements at very low dye concentrations, thereby avoiding perturbation of sample pH by the dye (Chierici, et al., 1999). The lcw used was Teflon AF® tubing. Subsequent to our *in situ* testing Liu, et al. (2006) reported an artifact in pH measurements made with a Teflon AF® lcw. Our measurements of pH are consistent with their findings. RATS can operate with a different optical cell, such as the long path length PEEK cell described by Liu et al. (2006), or can be reprogrammed readily to utilize the approach to spectrophotometric pH measurement taken by Seidel et al. (2008). However, as we have not collected *in situ* data using these alternatives, we discuss here only the TCO_2 instrument.

2. Performance Criteria

The development of the instrument has sought to meet the following criteria:

- Measure TCO_2 over prolonged periods of time with precision of ≤±5 µmol/kg.
- Operate submerged to depths of at least 1000m
- Be capable of *in situ* standardization at user defined intervals
- Operate unattended for up to six months or ~1000 analyses.

The precision sought is less than that achieved in the laboratory but is what we considered realistic for an instrument autonomously operating submerged for periods of many months while still being adequate to address a variety of oceanographic issues. Holding pH constant (RATS TCO_2 analysis is intended to be paired with pH measurement), the TCO_2 error corresponds to ± ~1 μatm in fCO_2 and ± ~5 μmol/kg in alkalinity for seawater of the composition: TCO_2= 1998.2 μmol/kg, TA= 2202.0 μmol/kg, pH= 7.974 (20°C and 1 atm pressure). This resolution can quantify trends from episodic events such as storms and eddies as well as seasonal cycles in most ocean environments. In some regions diurnal cycles also could be resolved. The depth limit is arbitrary and intended to provide access to the "twilight zone". The capacity for *in situ* standardization at regular intervals is important in assessing instrumental performance throughout deployments of many months. The endurance given is somewhat arbitrary and limited by accommodating required reagent volumes rather than power.

The choice of TCO_2 as the measured variable was based upon a number of considerations. First, the TCO_2 / pH pair (and total alkalinity / pH) yields more precise calculations of dissolved carbonate speciation than the fCO_2 /pH pair (Millero et al., 2002). Perhaps most important, changes in TCO_2 in response to photosynthesis and respiration are much larger than those of TA, facilitating accurate determination of variations in these processes that are central to the oceanic carbon cycle. Finally, we deemed implementing an *in situ* method for the determination of TCO_2 simpler and more power efficient than would be the case for alkalinity.

3. Analytical Method

RATS employs the conductimetric method of TCO_2 measurement described by Hall and Aller (1992). In this method CO_2 diffuses through a semi-permeable membrane from an acidified seawater sample into a sodium hydroxide solution. The high fCO_2 of the acidified seawater and low fCO_2 of the base drives quantitative exchange of CO_2 across the membrane. The CO_2 reacts with OH^- in the base to produce $CO_3^=$ and HCO_3^- ions, decreasing conductivity. The limiting equivalent conductivity (cm^2 Int. ohm^{-1} $equiv^{-1}$, 25°C) of OH^- is 198.3, that of HCO_3^- and $CO_3^=$ are much smaller: 44.5 and 69.3, respectively (Robinson and Stokes, 1959). This conductivity contrast forms the basis of conductimetric TCO_2 analysis.

The NaOH concentration of the indicator base used is 7 mM. This concentration was chosen to maximize the response of our CO_2 instrument to samples in the normal range of TCO_2 in seawater. The samples are acidified with 0.024 M H_3PO_4. This relatively low acid concentration has a density well below that of seawater while providing a substantial excess of H^+. The density contrast reduces irreproducible dilution of the acidified sample by mixing in the CO_2 exchange cell.

Figure 1 presents the speciation and conductivity relationships calculated for 7 mM NaOH over a range of TCO_2 concentration. Conductivity is presented as relative specific conductivity (RSC): the conductivity of the base at a given TCO_2 concentration normalized to the conductivity of the unreacted base. This normalizes the cell constant and hence applies to any conductivity cell.

The change in conductivity from the addition of CO_2 exhibited in Figure 1 reflects three reactions:

1. $2OH^- + CO_2 \Rightarrow CO_3^= + H_2O$
2. $OH^- + CO_2 \Rightarrow HCO_3^-$
3. $CO_3^= + CO_2 + H_2O \Rightarrow 2\ HCO_3^-$.

The optimum CO_2 range for measurement is restricted to CO_2 additions (exchange) where reaction 1 dominates (≤ ~2800 µmol/kg TCO_2) and 2 OH^- are consumed per CO_2 added, producing one $CO_3^=$. Once significant amounts of HCO_3^- are produced (above ~ 3000 µmol/kg TCO_2), the sensitivity to CO_2 addition decreases: the change in conductivity per mol of CO_2 added for reaction 2 is only 60% of that for reaction 1, based on limiting equivalent conductance. With further increase in CO_2 (≥ ~3500

μmol/kg) sensitivity becomes very poor as reaction 3 dominates and the conductivity change is < 10% of that for reaction 1. Thus the upper bound of CO_2 addition is set by the need to restrict measurement conditions to the region where the predominant reaction is $OH^- \Rightarrow CO_3^=$. The lower bound of TCO_2 addition is set by the precision of the conductivity measurement (typically a few parts in 10,000) and the objective of achieving a TCO_2 precision of ±5 μmol/kg. The working range, using 7mM NaOH, is sufficient for most oceanographic applications. From Figure 1, a range of CO_2 added from 1600 μmol/kg to 2800 μmol/kg should decrease RSC of the solution from ~0.75 to ~0.58. The precision of measurements outside of this TCO_2 range can be enhanced using a different NaOH concentration.

A schematic of the TCO_2 analyzer is presented in Figure 2. A 10 cc syringe pump is used to move solution in the analyzer. Solution is drawn through the instrument to minimize the number of valves required, permit use of a single pump and minimize power requirements. Rotary distribution valves (RV1 and RV3, Fig. 2) control the flow of solutions. The valve ports remain closed (sealed) except when selected. Sample and standard volume is fixed with a sample loop (500μl). Exchange of CO_2 from acidified sample or standard into the NaOH occurs across a silicone membrane in the exchange cell. The configuration shown is the *in situ* instrument. In the laboratory experiments discussed below, RV3 was a 4-port valve, limiting the number of standards to 2.

TCO_2 analysis consists of seven sequential steps. 1) The sample loop, knotted mixer and acid/sample volume of the exchange cell are flushed with “fresh” acid from the reservoir. 2) The base volume of the exchanger is flushed three times in succession with 120 sec between each base flush. The delays between flushes remove any CO_2 present in the acid in the outer volume of the exchange cell prior to introducing the final base flush into which the sample CO_2 will be exchanged. This procedure also flushes the conductivity cell and tubing between the exchanger and cell with new, unreacted base to serve as the pre-sample peak baseline. 3) The sample loop is then flushed and filled with sample or standard. 4) The sample (standard) is moved through the knotted mixer, with acid both leading and trailing the slug of sample, into the exchange cell. Mixing of the sample with acid is enhanced in the knotted mixer. 5) The acidified sample is held in the exchange cell for 3600 sec to permit nearly complete CO_2 exchange. The long exchange period is based on uptake in the base of ≥99% of the analyte CO_2 at a

temperature of 5°C. At this proportion of complete exchange the rate of further reaction is very slow, rendering the integrated conductivity change highly reproducible, enhancing precision. Exchange is more rapid at higher temperatures, but we have adopted an exchange time suited to low temperatures as the standard exposure time. 6) Following the exchange period, base is drawn through the conductivity cell for 90 sec. while conductivity is measured. This results in a sequence through the cell of unreacted base(=pre-peak baseline), reacted base(=TCO_2 peak), unreacted base(=post-peak baseline). 7) The syringe is emptied. An analysis requires 8.7 minutes plus the exchange time, or as normally run 68.7 minutes. Each analysis consumes 2500 μl of sample/standard, 4760 μl of acid and 3825 μl of NaOH.

4. Instrumental

The analyzer is controlled by a Tattletale™ Model 8 (Onset Computer) process controller. A 12-bit A/D converter is used for temperature and conductivity data acquisition. Temperature and conductivity data are stored as 10-point averages recorded at 10Hz. Conductivity is measured with an Amber Science Model 2055 Conductance Board, a conductivity bridge operating at 1000hz with analog output. Conductivity data are stored and reported here as counts (cts). The resistance of the bridge is set such that the A/D converter yields ~3500 cts (of 4096) for 7 mM NaOH at ~23°C. A battery pack in the controller pressure case is sufficient for ≥1000 TCO_2 analyses. However, reagent volume storage, specifically acid, is limiting currently, permitting ~850 analyses.

The conductivity cell is a custom designed 3-electrode cell. It was developed in consultation with K. D. Lawson of Sea-Bird Electronics, Bellevue, WA. The configuration is analogous to the Sea-Bird CTD conductivity cells in that the electrodes are in series with the center electrode being the “power” electrode and the outer pair common. The guard (common) electrodes isolate the cell from externally generated signals. The electrodes are 0.125” o.d. x 0.060” i.d. (3.2 mm x 1.5mm) Pt tubing 0.80” (20.2mm) long. The electrodes are separated by polysulfone spacers (0.280” (7.1mm) thick with a 0.060” (0.15mm) i.d). The internal volume is 146 μl.

Where possible the analyzer components are contained in pressure-balanced housings to minimize the number of pressure cases required. The valve motors,

conductivity cell, syringe pumps, and thermistor are housed in containers filled with Fluorinert™ FC-40, a low viscosity, low dielectric fluid. Only the controller with battery pack is contained in a pressure case, which is rated for 5000m. The operation of the components housed in pressure-balanced containers with FC-40 has been tested to 1500 psi (~ 1000 m). The limit of the pressure tests was arbitrary and operation to depths approaching 5000m should be possible.

Reagents and standards require containers without fixed volumes. With the exception of the phosphoric acid, solutions used in RATS analyses are kept in Cali-5-Bond™ sample bags (Calibrated Instruments, Inc.). Cali-5-Bond™ is a gas impermeable 5-layer "sandwich" of plastic and aluminum for storage of gases and liquids. Since prolonged storage of the phosphoric acid in the aluminized bags seemed potentially risky, the acid is kept in standard clinical IV bags.

The exchange cell is analogous to that described by Byrne et al. (2002). The cell is, basically, a tube within a tube (inset Figure 2). The inner tube of silicone rubber (0.077" (1.96mm) o.d. x 0.058"(1.47mm) i.d.) contains the NaOH into which CO_2 diffuses. The outer tube is thick-walled polycarbonate (0.25"(6.35mm) o.d. x 0.125"(3.18mm) i.d.) into which the acidified sample is drawn. The length of both tubes is 20cm, giving inner and outer volumes of 339μl and 985μl, respectively. The ~3:1 ratio of outer to inner volume is employed to enhance the amount of CO_2 available per volume of NaOH and hence the resulting conductivity change.

5. RESULTS

Experiments of 5 to 8 weeks duration were carried out in the laboratory and *in situ* to assess the analytical performance of RATS. The laboratory experiments permit assessment of various aspects of calibration, precision, and accuracy under controlled conditions for comparison with *in situ* results that present a variety of additional challenges. The laboratory experiments were followed by a number of *in situ* deployments.

5.1 Laboratory Experiments

To assess TCO_2 analytical performance in the laboratory an experiment of 38 days duration was conducted during which TCO_2 was determined at a series of seven temperatures ranging from 8° to 25°C. The objectives were to assess, over a range of

temperature, 1) instrument stability over the 5-week period, 2) the reliability of large-scale temperature correction, and 3) the accuracy and precision of seawater TCO_2 determinations based on Na_2CO_3 standards. Four solutions were measured at each temperature: two Na_2CO_3 "standards", a "sample" that is a Certified Reference Material seawater (CRM, Batch 37) obtained from Andrew Dickson at Scripps Institution of Oceanography, and a 0.63 M NaCl blank. The TCO_2 concentrations of the Na_2CO_3 standards and the CRM (Batch 37), stored in Cali-5-Bond™ bags, were independently measured by gas phase IR techniques before the start of the experiment. Laboratory TCO_2 analyses used an automated analyzer employing gas stripping of an acidified sample (300 μl) and gas phase IR analysis with a Li-cor™ 6252 analyzer (Li-cor analysis). Li-cor analysis of the CRM seawater yielded the TCO_2 concentration given for Batch 37 (±2 μmol/kg).

During the experiment each solution was analyzed five times at intervals of 5 hours at each temperature, using the automated procedures of the analyzer. All five analyses of a given solution at each temperature were completed prior to beginning analysis of another solution. The NaCl blank was run at each temperature before and after TCO_2 solutions to assess carryover; none was detected.

To achieve temperature control, the laboratory instrument and all solutions used were enclosed in an insulated box containing heat exchange coils and a fan to mix air within the box. The conductivity cell and in-line thermistor cell were immersed in FC-40 to enhance temperature stability. Temperature variation over the 20 hours used for the five successive analyses of each solution typically was ±0.1°C. The temperature range over the ~100 hrs taken for measurements of all the solutions at a given temperature was ~±0.2°C. When the temperature was changed to a new value, 24 hours were allowed for thermal equilibrium to be established in the solutions.

In quantifying TCO_2 concentration we evaluated the precision of both peak height and peak area. Peak height (baseline less conductivity at time points within the peak) was determined on the basis of the average of 5 points on either side of the minimum conductivity value. Peak area is defined as peak height integrated over the interval 8 to 85 sec. Peak width varies slightly with temperature and the 85 sec end-point for peak area determination is set to capture the entirety of peaks at low temperature. At a given temperature and TCO_2 concentration the scatter in peak height is 2 to 3 times that of

peak area. Consequently, the ensuing evaluations of TCO_2 analysis are based on peak area.

5.1.1 Instrument Stability

In comparing TCO_2 analyses made over a range of temperatures for a prolonged period of time, it is essential to establish that observed changes in the baseline (NaOH conductivity) are due solely to temperature variation, i.e. instrumental and NaOH drift are not significant over the time period in which standardization is completed. To test for drift, the baseline conductivity data for all of the runs throughout the 38-day experiment were fitted to the measured temperature with a second order polynomial. The residuals between measured and calculated conductivity exhibit no trend with time, and the root-mean-square of the residuals is 0.84 cts. As a further test for temperature independent drift, we fit the baseline data for the first three temperatures studied (25°, 22°, 17°, days 1-16) to a polynomial and used that regression to calculate expected baseline values from measured temperature for the last interval studied (19.5°, days 34-38). The root-mean-square of the differences between the calculated and measured baseline values for this interval is 1.1 cts. Both approaches to assessing instrumental and NaOH drift yield uncertainties only slightly larger than the uncertainty in determining the baseline in a single analysis (~±0.5 cts). These values correspond to an uncertainty in baseline determination for a given sample over the 38 day period of the experiment of 0.02% to 0.03% at ~20°, indicating that instrumental and NaOH drift were not significant over the 5+ week experiment.

5.1.2 Peak Area Correction

In order to determine the precision and accuracy with which we can determine TCO_2, over the range of temperatures studied, it is necessary to correct the peak area data at each temperature to a common reference temperature. This is done empirically, based on the observed relationship between temperature and the peak area of the standards. The CRM "unknown" was treated similarly to verify that temperature correction based on the Na_2CO_3 solutions is applicable to seawater (the CRM). For each analysis of a solution, the measured peak area is normalized with a correction factor,

$$CorF = PkA_{Tmeas} / PkA_{Tref}.$$

The peak areas at both the measurement (T_{meas}) and reference (T_{ref}) temperatures are calculated for each standard with the polynomial regression of peak area vs. temperature. Note that CorF is a ratio of peak areas, giving the relative change in peak area with temperature. The temperature corrected peak area (PkA_{Tcor}) is given by

$$PkA_{Tcor} = PkA_{meas}/CorF$$

with PkA_{meas} being the peak area measured at temperature T_{meas}.

To apply this approach to measurements of samples with unknown and variable TCO_2, the correction factor (CorF) must be independent of concentration and values derived from the Na_2CO_3 standards must be applicable to seawater samples. This is expected since conductivity changes should only reflect the addition of CO_2 to the NaOH. To test this assumption, the values of the correction factor, CorF, for a reference temperature of 16.5°, are plotted as a function of measured temperature in Figure 3. The figure includes CorF values for all of the analyses of the Na_2CO_3 standards as well as the CRM. Also included are linear regressions of the data for each of the three solutions. The regressions are essentially indistinguishable, consistent with the fact that correction factors (CorF) derived from the Na_2CO_3 standards are identical (i.e. concentration independent), and that the Na_2CO_3 correction factors are identical to those of the CRM. Thus CorF derived from the Na_2CO_3 standards can be applied generally to seawater samples over the concentration and temperature ranges of this experiment.

5.1.3 TCO_2 Calibration and Analysis

The above temperature correction method was applied to the peak areas measured at each temperature to assess the precision and accuracy of TCO_2 determinations of a seawater “unknown” (the CRM), based on the Na_2CO_3 peak area calibration. Peak area values of all three solutions were corrected to a reference temperature of 16.5°C, the mid-point of the range studied, with the Na_2CO_3 correction factor. The TCO_2 concentration of the CRM was calculated with a linear regression of concentration vs. corrected peak area for the Na_2CO_3 standards. The use of a linear regression is not strictly appropriate as conductivity and hence peak area is a non-linear function of CO_2 concentration. However, over the limited concentration range of the Na_2CO_3 standards, departure from linearity is small. We estimate that the error is an overestimate of less than 1 µmol/kg. The average TCO_2 concentration of the CRM

determined over the 38 day experiment at temperatures from 8° to 25°C, based on the Na_2CO_3 calibration, is 2043 ±2.7 µmol/kg. Taking into account a 1 µmol/kg non-linearity correction, a value of 2042 µmol/kg is possible, still within 1σ of the true concentration 2044 µmol/kg).

The measurements made also permit concentration calibration at each of the seven temperatures studied over the course of the experiment. These data demonstrate the short-term (~ 5 day) variability and the consistency of the measurements throughout the period of the experiment. The individual measurements of the TCO_2 concentration of the CRM sample, as well as the averages and standard deviations for each temperature, are presented in Figure 4. The average 1σ for the 7 individual data sets is ±2.2 µmol/kg. Thus the measurement uncertainty is similar for (a) data calibrated and averaged over 38 days at measurement temperatures between 8 and 25°C (± 2.7 µmol/kg) and (b) calibrated over only 5 days at a single temperature (±0.2°C) (±2.2 µmol/kg). Further, the magnitude of the temperature correction to the reference temperature (16.5°) has no effect on the concentration determination (Figure 4).

In summary, the laboratory data indicate that the TCO_2 instrument developed is stable and suited for unattended operation for periods of at least 5 weeks. The TCO_2 reagents and standards are also stable for at least this period of time. The temperature corrections required in comparing analyses over a range of temperature approximating environmental conditions (8-25°) do not introduce any detectable bias in the concentrations of TCO_2 determined. Regardless of whether the data are analyzed as a single group collected over a period of 5 weeks or at individual temperatures over periods of ~5 days, the concentrations and analytical uncertainties are similar. The analyses performed are accurate, yielding an estimated concentration of 2043 µmol/kg for a TCO_2 Certified Reference Material whose stated and confirmed concentration is 2044 µmol/kg. The precision of the measurements over the course of the 38-day experiment was ±2.7 µmol/kg. For comparison, laboratory coulometric TCO_2 analyses typically cite precisions of 1.5-2.0 µmol/kg (Dickson, et al., 2007).

5.2 *In situ* TCO_2 Experiments

The components tested in the lab were incorporated into an instrument suitable for *in situ* deployment in the ocean (Figure 5). As noted above, RV-3, a 4-port valve in the laboratory experiments, was changed to a six-port valve for the *in situ* experiment,

permitting the use of 4 TCO_2 standards for improved calibration. *In situ* tests of performance were carried out at the WHOI dock. The location provides ready access to the instrument, facilities support when needed, and monitoring and control of operations over the Internet. In addition, the location is an environment that challenges the robotic operation of any analytical tool. Currents are strong (several kts), temperature varies rapidly as tides change, and primary production and bio-fouling are high. We deemed the conditions a strong test of the capabilities of the instrument. RATS has been deployed at the dock a number of times over the course of our *in situ* tests. In total, the instrument has been operated submerged for about six months. We report here the results obtained in the most recent deployment, as this was the most thorough test conducted.

Rats was deployed at the WHOI dock from 29 Mar through 23 May 2006, a total of 56 days. The instrument was tethered at ~ 10 m depth for the duration of the experiment. Throughout the test the instrument was connected to the surface by a communications cable. This was done to permit monitoring of operation and downloading of data over the Internet. Previously we tested operation of the instrument with and without the cable and observed no difference in any aspect of operation. Over the course of the experiment, *in situ* temperature ranged from ~4.5° at the outset to ~13.5° at the time of recovery. The diurnal variation of temperature was ~1.2° early in the experiment and ~0.9° over most of the period. Temperature changes of 0.2° to 0.4° in an hour were not uncommon. The depth of the instrument changes with the tide, but the range is quite small: ~0.7 m. Particulate matter in the water was generally high, based on the typical visibility of ~2 m, but was not measured.

The first week of the deployment was used to test operations prior to starting a long automated sequence. The automated analytical sequence consisted of a series of sample and standard analyses that ran for approximately 1 week at a time. The sequence was repeated each week for the remainder of the deployment. This approach was employed to permit downloading of the data files weekly for assessment of performance. At no time was a malfunction detected in the analyzer. Running in this fashion is operationally the same as programming the instrument to run for the entire period of the deployment. Dock water samples were run every 5 hours; standards were run between samples at assigned frequencies based on the number of sample analyses

completed. Each standard was run 3 or four times per sequence (~1 week), a much higher frequency than would be normal for a mooring deployment. This was done to enhance evaluation of instrument stability and calibration. In total 121 standards and 233 samples were analyzed *in situ*.

For calibrating TCO_2 response and assessing accuracy, four standards were analyzed throughout the deployment. Two of these were CRMs modified by the addition of $NaHCO_3$ or HCl and equilibrated with atmospheric CO_2 to extend the TCO_2 concentration range. The other two standards were unmodified CRMs. The two modified CRMs and one unmodified CRM were used as standards for calibration. The second unmodified CRM was treated as a "sample" in order to assess precision and accuracy. The Na_2CO_3 standards were abandoned due to long term drift in TCO_2. As in the case of the laboratory experiment, the standards were and stored in Cali-5-Bond™ bags. The TCO_2 concentrations of the standards were determined before and after the deployment by Li-cor analysis. The pre- and post-deployment concentrations did not differ significantly and the average for each was used for the *in situ* TCO_2 concentration calibration. The Li-cor determined concentrations of the standards are given in Table 1.

5.2.1 Instrument Stability

The stability of the conductivity measurements over the course of the deployment dictates the intervals over which calibration can be used without introducing additional error. To determine if the conductivity measurement changed over the 8-week deployment, either through change in the conductivity cell or change in the conductivity of the NaOH, we have compared baseline measurements made in the laboratory before and after the deployment (a period of ~10 weeks). The temperatures of the pre- and post-deployment measurements are not identical but do overlap. The pre-deployment baseline conductivity and temperature data were fit with a linear regression. The post-deployment baseline data were then compared to values calculated with the pre-deployment regression at the post-deployment temperatures. The residuals of the pre-deployment data, calculated with the pre-deployment linear regression average -.04 ±1.7 cts. The residuals of the post-deployment data calculated with the pre-deployment regression average -2.9 ±6 cts (out of ~3300). The post-deployment baseline residuals exhibit more scatter, but do not differ significantly from the pre-deployment residuals. Since there was no measurable change in the conductivity cell or conductivity of the

base, a TCO_2 calibration over the eight-week deployment is justified. The observed instrument stability indicates that running a complete set of standards (4), at most, once every 7-10 days should be sufficient for calibration purposes. This translates to at least 10-12 sample analyses per standard analysis.

5.2.2 Peak Area Correction

Raw peak area data for the standards was corrected to reference conditions somewhat differently from the procedure described for the laboratory experiments. Throughout the *in situ* deployment we observed that the baseline frequently overshot the pre-peak value at the end of the period used to define the peak. This discrepancy between pre- and post-baseline averaged +2 cts (in 2400 to 2800 cts) and ranged from -2 to +5. Since peak area is defined as $\Sigma(cts_{t=0}\text{-}cts_{t=t})$, in the time period assigned to the peak, the overshoot, on average, reduced peak area slightly relative to an invariant baseline. We do not know the origin of this feature but suspect it is due to very small temperature differences between the conductivity cell and thermistor, originating from rapidly changing external water temperatures. In terms of concentration, the effect is, on average, ~ 0.5 µmol/kg. To account for variations in this baseline difference, peak areas were corrected to both a reference temperature and a reference baseline difference using a multiple linear regression analysis of *in situ* measured peak area and the independent variables temperature and baseline difference (MLR-PkA). Using data from the entire deployment, each standard was regressed separately. The regressions were run twice, first with all of the data and a second time with corrected peak areas that depart from the average by more than 3σ excluded. Each standard was analyzed ~30 times over the 8-week deployment and 1 or 2 points were excluded from each set of data used in the regressions. As in the procedure described for the laboratory experiments, the raw peak areas of the standards were corrected to reference conditions with a correction factor, CorF, such that:

$$CorF = (PkA_{(ref\ T;\ ref\ dBsl)})/(PkA_{(meas\ T;\ meas\ dBsl)}),$$

where PkA= peak area, ref= reference value, meas= measured values and dBsl= pre-post-baseline difference. Both numerator and denominator are calculated with the coefficients of the MLR-PkA for each standard. The reference temperature was 8.5° C, the mid-point of the deployment range, and the reference baseline difference was +2 cts, the average baseline difference.

Since CorF was shown to be independent of concentration in the laboratory experiments, CorF values for unknown samples of variable TCO_2 can be derived from the CorF values of the standards. To obtain this algorithm the CorF values of the standards were regressed against temperature and baseline difference with a multiple linear regression (MLR-CorF). A CorF value for each sample was then calculated from the averaged MLR-CorF coefficients of the three standards. Peak areas were corrected to the reference temperature and baseline difference, with these CorF values.

5.2.3 *In situ* Calibration and Standard Analysis:

Determination of TCO_2 concentration is based on the corrected peak areas of the standards and the pre- and post-deployment average concentration of the standards determined in the laboratory. We have used the standards A, B and C for a calibration curve. Standard D has been treated as a "sample" in assessing performance over the 8-week deployment. The standard curve, based on 28 to 30 analyses each of standards A, B and C, is given in Figure 6. The TCO_2 concentration of standard D calculated from the regression in Figure 6, with peak area corrected as described above for samples, is plotted over the course of the deployment in Figure 7. Excluding the two points indicated on the figure (open symbols) the average concentration is 2034 ±3.6 µmol/kg. This value is not significantly different from the Li-cor average for Standard D (2036 ±4 µmol/kg). There is no significant trend in the concentration with time. In fact, RATS may improve with use; while the average concentration is the same during the periods before and after day 20, the scatter is quite a bit less beyond day 20: ±3.0 vs. ±4.4 µmol/kg. Based on the measured TCO_2 with pH held constant at the value calculated for Std D (CRM Batch 82) at *in situ* conditions (8.5°C, 10m depth), our TCO_2 standard deviation of ±3.6 µmol/kg corresponds to an uncertainty in calculated alkalinity and fCO_2 of ±3.6 µmol/kg and 0.5 µatm, respectively. If an uncertainty in pH of ±0.001 (Seidel et al., 2008) is also considered, the combined uncertainties become ±3.7 µmol/kg and ±1.2 µatm. The above calculation is based on TCO_2/ pH for consistency with the intended *in situ* measurement of this pair of variables. At constant alkalinity and our TCO_2 uncertainty of ±3.6 µmol/kg, the fCO_2 uncertainty would be 6.4 µatm.

5.2.4 *In situ* Sample Analysis:

TCO_2 concentrations for the dock water samples were derived with the same procedure employed for standard D. The TCO_2 concentration of dock water over the

duration of the deployment is shown in Figure 8a. TCO_2 is highly cyclical with a range of ~10 µmol/kg in the colder conditions early in the deployment and up to 40 µmol/kg at the end of the experiment as temperatures warmed and daylight increased. The variations exhibit a roughly 1-day period, suggestive of the diurnal cycle of photosynthesis and respiration, with TCO_2 draw down in mid-day and maxima in the vicinity of midnight. While our sampling frequency is less than ideal for defining diurnal and shorter cycles, we processed the sample data using Lomb's method (Lomb, 1976) to assess the periodicity of TCO_2 concentration. The power spectrum density calculated confirms (Figure 8b) that the only significant frequencies (above .005/hr) are the diurnal cycle (.042/hr or a period of 23.8hr) and tidal cycle (.080/hr or a period of 12.5 hr), with the diurnal cycle being the more significant of the two.

The samples drawn into the system for analysis were not filtered. We avoided filtering because we felt that plugging of a filter in the dock environment was highly likely. There is thus the possibility of entrainment of $CaCO_3$ particles, leading to a TCO_2 concentration above the true dissolved concentration. To assess this possibility we collected dock water samples with a 25 L Niskin on four different occasions. These samples were collected at the depth of the RATS sample tube (within 1 m) simultaneously with the draw of a RATS TCO_2 sample (within ~1 min). A 1L glass bottle was rinsed and filled completely from the Niskin at the dock and immediately returned to the lab. In the lab four samples were drawn from the well-mixed bottle, two were filtered (0.2um pore size) and two were not filtered. TCO_2 was determined in duplicate on each of the four aliquots. The results from these four sets of analyses are presented in Table 2. It is clear from the data that the filtered and unfiltered pairs do not differ significantly. Thus, despite a relatively high particulate load, based on inspection of the bottles, there is no evidence of particulate matter contributing to the TCO_2 concentration measured on the *in situ* samples.

The Niskin samples collected also provide an opportunity to compare RATS analyses with samples collected and analyzed by more traditional laboratory methods. As noted above, the Niskin samples were collected in close proximity to the *in situ* instrument simultaneously with a RATS sample draw for TCO_2 determination. However, such a comparison is likely to be somewhat uncertain, as the waters flowing past the instrument and Niskin are quite heterogeneous and change rapidly (e.g. the diurnal

swings in concentration of ~40 µmol/kg during the period when these comparisons were carried out, Figure 8a). The concentrations determined with RATS at the time of Niskin sampling are included in Table 2. Two of the comparisons agree within error while two exceed the two-sigma uncertainty of the analyses and both positive and negative differences are observed.

The RATS-Niskin difference exhibits a trend from negative to positive over time. While the standard D analyses during this time period (26-55 days) exhibit a positive trend in concentration (Figure 7), variation is not significant relative to the analytical uncertainty. The slope of concentration vs. time for standard D in this period is not statistically significant (slope= 0.16 ±0.20), nor are the concentrations predicted from the fit in this interval significantly different from the deployment average (< 1σ). Finally, there is no trend over the last 15 days. Thus the positive trend in the RATS-Niskin difference, if real, is not instrumental drift but is confined to the samples.

The instrument experienced extensive biofouling over the course of the deployment. The biological activity of the attached growth could have perturbed the immediate environment of the instrument, where samples are drawn. Assuming increased growth with time and rising temperature, as evinced in the enhanced diurnal variation of TCO_2 towards the end of the deployment, increased influence with time is possible. However, as all of the Niskin sampling was conducted in late morning, 4-6 hours after sunrise when photosynthesis is occurring, an enhanced influence of biofouling over time should lead to a RATS-Niskin difference that is increasingly negative, the opposite of what is observed.

We cannot rule out a trend in the RATS-Niskin difference that is due to an artifact of unknown origin. Instrumental drift does not appear to be a factor. The influence of biofouling is not consistent with the observations. Given the uncertainties in the analyses, placement and timing of the Niskin sampling, and dock water heterogeneity, we do not believe that the differences and apparent trend are significant. However, we lack the precisely placed and timed samples to establish this.

6. Reagent Stability

To determine TCO_2 accurately over deployments of 6 months and more, it is important that the reagents and standards be stable throughout. We have assessed the

stability of the solutions we employ for periods of up two years through laboratory experiments. These experiments have been based on storage of the solutions being tested in the Cali-5-Bond™ gas sample bags we use for deployments. The solutions studied included several CRM solutions and CRMs modified with small additions of acid or $NaHCO_3$.

6.1 CRM Stability

The stability of the CRM solutions used for calibration is critical to the accuracy of results and assessment of instrumental performance. These reference solutions have been shown to be stable for periods of years (Dickson, 1997) and are widely accepted TCO_2 reference materials as supplied in sealed glass bottles. However, it is necessary to repackage these solutions in Cali-5-Bond™ bags for *in situ* use. Both the transfer of solution and prolonged storage in the bags provide opportunities for contamination. To assess the stability of CRMs as we store them, a number of bags were rinsed and filled with ~500cc of CRM Batch 61 and periodically analyzed for TCO_2 for up to ~24 months by Li-cor procedures. The results from four of the longest tests are given in Figure 9a. Almost all of the analyses fall at or above the given TCO_2 concentration for CRM 61 (1998 µmol/kg), and three of the four linear regression intercepts are 2 to 3 µmol/kg higher. The concentrations also exhibit a slightly positive slope. The uncertainty in the values, based on replicate analyses, is ~2 µmol/kg. The average of the regression intercepts is 2 (±2) µmol/kg; the regression slopes average .005 (±.004) $\mu molkg^{-1}day^{-1}$. Thus, at worst, repackaging may introduce a change of ~ 2 µmol/kg that can be assessed by analysis after repackaging. The slope values, if real, translate to a drift of ~1 µmol/kg over the six months that RATS is designed to operate.

While the above documents long term stability of the standards, in a few instances we have observed larger changes in TCO_2 concentration as a result of the transfer of CRMs to the aluminized bags (up to 6 µmol/kg). It is thus important that concentration be determined after transfer.

The modified CRMs, described above, that extend the standard concentration range behave similarly to the untreated CRMs. In the preparation of these solutions the opportunity for contamination is greater. Two solutions were followed for ~250 days (Figure 9b). Neither solution evinces a significant trend for at least six months. However, the last sample (~250 days) of each departs somewhat from the preceding values. The

slope of MCRM.2 is not statistically significant (1σ); that of MCRM.1 is > 1σ but < 2σ greater than the slope uncertainty. Based on the regressions of Figure 9b, the drift in MCRM.1 and MCRM.2 could be ~3 µmol/kg and ~2 µmol/kg, respectively, over 6 months.

6.2 NaOH Stability

Because we employ standards to determine TCO_2 calibration, small changes in the NaOH solution (a few cts or ≤0.1%) over periods of months are not critical. It is critical that the NaOH not drift significantly over the period in which a calibration is used for TCO_2 measurement. For mooring deployment we anticipate that each standard would be run once every ~10 days, requiring a period 1 to 2 months to complete a calibration with 4 analyses of each standard. The stability of stored 7 mM NaOH has been discussed above in regard instrument stability in both the laboratory time series experiment (5.2.1) and the *in situ* deployment of RATS at the WHOI dock (5.2.2). In neither instance was a significant change in conductivity detected over the period of the experiment (5 and 10 weeks, respectively). It thus appears that drift of the 7 mM NaOH in the Cali-5-Bond™ storage bags does not introduce significant error in TCO_2 concentration calibration, as determined both in the laboratory and *in situ*.

7. Summary

We have built and tested a robotic analyzer for TCO_2 (RATS) suited for unattended operation on oceanographic moorings. TCO_2 determination is based on the change in conductivity of a NaOH solution resulting from the addition of CO_2 diffusing across a gas permeable membrane from an acidified sample. Concentration calibration is based on *in situ* standardization using four onboard TCO_2 standards. The analyzer carries sufficient power and reagents for ~850 analyses over a period of at least 6 months. Based on component pressure tests RATS can operate to depths of at least 1000m.

A Laboratory experiment was carried out for a period of 38 days at temperatures ranging from 8° to 25°C to assess instrument performance. Calibration over the 38-day period gave a TCO_2 concentration for a Certified Reference Material (CRM), treated as an unknown, of 2043 ±2.7 µmol/kg vs. a given value of 2044 µmol/kg. Calibration over 5 day intervals at each of the temperatures studied yields similar results.

In situ tests of RATS were carried out at the Woods Hole Oceanographic Institution dock at 10 m depth over a period of 56 days. Based on *in situ* standardization, the TCO_2 concentration of a CRM standard, treated as a sample, was determined to be 2034 ±3.6 μmol/kg vs. laboratory determined concentration before and after deployment of 2036± 4 μmol/kg.

The long term stability of standards stored in the metallized plastic sample bags used for *in situ* operation were carried out in the laboratory for periods up to ~2 years. CRM samples evince drift in TCO_2 concentration corresponding to ≤ +1 μmol/kg over 6 months. Modified CRM standards exhibited drift equivalent to ≤2 to 3 μmol/kg over 6 months. The base indicator (7mM NaOH) was followed for periods of 38 (laboratory) to 68(*in situ*) days. No significant change in conductivity was detected.

8. Acknowledgments

Many discussions with Mike DeGrandpre have been essential in moving the development of RATS forward.

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TABLES

Table 1- Summary of Li-cor analyses of standards used on the 8-week *in situ* test (concentrations in µmol/kg): Analyses were done before and after the deployment on the dates indicated. Standards A and D are CRM Batch 71 solutions; standards B and C are CRM Batch 71 solutions modified with the addition of $NaHCO_3$ and HCl respectively. The 1σ values given are based on replicate analyses the solution.

TABLE 1

Date Run	Std A	±	Std B	±	Std C	±	Std D	±
20-Mar-02	2031.4	2.8	na		na		2035.0	1.4
23-Mar-02	na		2272.0		1652.2		na	
23-May-02	2039.2	2.6	2274.0	2.4	1656.7	0.9	2040.9	0.7
14-Jun-02	2031.9	0.9	2266.3	1.2	1648.5	1.4	2032.9	2.6
Averages:	2034.2	3.6	2270.8	4.0	1652.5	4.1	2036.3	4.1
Given Concentration for CRM Batch 71= 2032.8 µmol/kg								

Table 2- Comparison of the TCO_2 concentration of filtered (0.2 μm) and unfiltered dock water samples collected in a Niskin bottle; concentrations in μmol/kg: Samples were collected simultaneously with an *in situ* RATS analysis and at the same depth. Also shown are the RATS concentration measurements made at the time of Niskin sample collection.

				TABLE 2				
Collection Date	Elapsed Time (days)	Sample Type	Licor TCO2 (μmol/kg)	StDev	Unfilt-Filt	RATS ID	RATS TCO2 (μmol/kg)	[2] Conc. RATS-Niskin (unfiltered)
24-Apr-06	26.50	Unfiltered	1989	4.1	0	04241230.cos	1977	-12
		Filtered	1989	0.1				
3-May-06	35.39	Unfiltered	1981	1.6	0	05030940.cos	1978	-3
		Filtered	1981	1.8				
10-May-06	42.41	Unfiltered	1994	1.8	1	05101009.cos	1992	-2
		Filtered	1993	0.8				
22-May-06	54.43	Unfiltered	1976	2.3	-2	05221040.cos	1986	10
		Filtered	1978	0.2				

FIGURE CAPTIONS

Figure 1- Theoretical speciation and conductivity of a 7 mM NaOH solution with varying TCO_2 content: The concentrations of HCO_3^-, $CO_3^=$ and OH^- have been calculated with the freshwater option of the carbonate equilibrium program CO2SYS (Lewis and Wallace, 1998). The conductivity has been calculated with a program provided by F. J. Millero (Millero, 2000), modified to include OH^- conductivity (Robinson and Stokes, 1959). Relative Specific Conductivity is the specific conductivity of 7mM NaOH + CO_2 normalized to the specific conductivity of 7 mM NaOH (i.e. TCO_2= 0). The shaded area is the optimum range of TCO_2 content for 7mM NaOH.

Figure 2- A schematic of the CO_2 analysis instrument used for *in situ* studies: The components labeled RV1 and RV3 are rotary distribution valves. The "Home" position is the indexed port to which all valve rotations are referenced. The "balloons" are representations of the metallized plastic bags that are used to store the reagents and standards. The exchange cell (see inset Figure 2) is described in the text. The sample loop fixes sample volume at 500 μl. The knotted mixer is used to enhance mixing of the acid and sample (or standard) prior to entering the exchange cell. The loop between the exchange cell and the conductivity cell stores the most recently added base (the 3rd base flush) that is used to determine the pre-peak baseline. The color-coding denotes the various flow paths.

Figure 3- The correction factor, CorF, used to adjust peak area to a common reference temperature as a function of measured temperature (see section 5.1.2): The reference temperature used was 16.5 °C (Correction Factor=1). All of the standard runs over the 6-week period are plotted along with linear regression fits for each of the three solutions.

Figure 4- A summary of the 35 TCO_2 analyses of the reference seawater (CRM) over the course of the laboratory experiment: The temperature for each set of runs is indicated. The individual and the average (± 1σ) concentrations are plotted. The dashed

line is the average of our measurements; the solid line is the independently determined TCO_2 concentration of this batch of reference seawater.

Figure 5- The *in situ* version of the TCO_2 analyzer following a 3-week deployment during the month of Dec. at the WHOI dock at a depth of 10 m: The cylinders in the picture, left to right, are the spectrometer housing (silver color) with Teflon AF housing (on top of cylinder) and the dye pump, both used in pH analysis (not discussed here), the sample pump, and the controller and battery housing. The uppermost cylinder houses the conductivity cell. The large stainless steel box at the bottom contains the reagent and standard bags.

Figure 6- TCO_2 concentration calibration from *in situ* peak area measurements over the course of the 8-week deployment at the WHOI dock: The peak areas have been corrected to 8.5°C as discussed in the text (section 5.2.2). The TCO_2 concentrations are averages of Li-cor IR analyses made in the laboratory before and after the deployment. The highest and lowest standards are modified CRM reference seawater; the middle standard is an unmodified CRM. Also shown on the figure is the polynomial fit used to calculate the TCO_2 concentrations of a fourth CRM, standard “D”, and samples.

Figure 7- The concentration of TCO_2 determined *in situ* on Standard “D” over the 8-week deployment at the WHOI dock: Two points (open symbols) have been omitted from the *in situ* average. Standard D is a CRM with pre- post-deployment Li-cor analyzed TCO_2 of 2036 (±4) µmol/kg. The *in situ* measurements average 2034 (±3.6) µmol/kg.

Figure 8- Characteristics of the dock water sample analyses:

a. The TCO_2 concentration of samples measured over the duration of the deployment at the WHOI dock: Sample analysis intervals were 5 hours throughout the 8-week deployment. Superimposed on the TCO_2 data is the *in situ* temperature record as measured on the samples at the time of TCO_2 analysis.

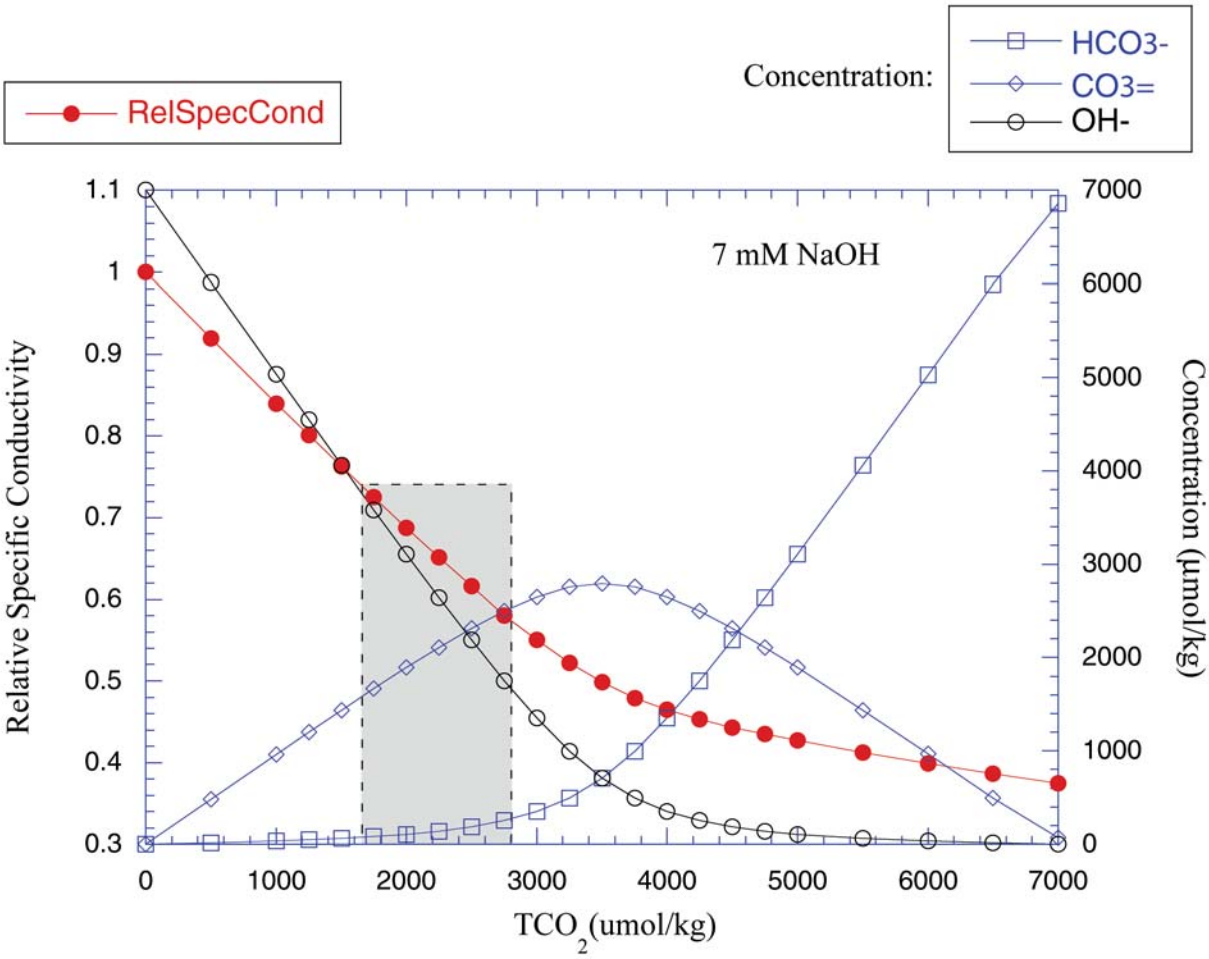
b. The power spectrum density of the sample data (bottom of panel): The frequency cutoff at 0.1/hr is the Nyquist frequency dictated by the 5 hr sample interval. Also presented is the null probability (top of panel), i.e. the probability that the peaks are due to random noise. The two frequencies that are significant, .042/hr and .080/hr, correspond to periods of 23.8 hr and 12.5 hr, respectively, diurnal and tidal signatures.

Figure 9- Stability tests of TCO_2 standards:

a. Long term tests of four CRM reference seawater samples stored in the Cali-5-Bond™ sample bags used for *in situ* deployments: The symbol legend gives the date on which each bag was filled. The linear regression equation for each bag is given in the order that the bags are listed in the legend. The significance of the intercepts and slopes is discussed in the text (section 6.). The error of ±2 µmol/kg shown is the typical uncertainty in our replicate Li-cor analyses rather that the uncertainty in the individual data points.

b. Stability tests of modified CRM reference seawater samples (MCRM) stored in the Cali-5-Bond™ sample bags used for *in situ* deployments: MCRM.1 and MCRM.2 have been modified by the addition of a small amount of HCl and $NaHCO_3$, respectively, and subsequent equilibration with laboratory air for ~18 hours. The significance of the intercepts and slopes is discussed in the text (section 6.1). The error of ±3 µmol/kg shown is the typical uncertainty in our replicate Li-cor analyses rather than the uncertainty in the individual data points.

Fig 1


RelSpecCond
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HCO3-
CO3=
OH-
7 mM NaOH
Relative Specific Conductivity
Concentration (µmol/kg)
TCO2 (umol/kg)
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1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
7000
6000
5000
4000
3000
2000
1000
0
0
1000
2000
3000
4000
5000
6000
7000

Fig 2

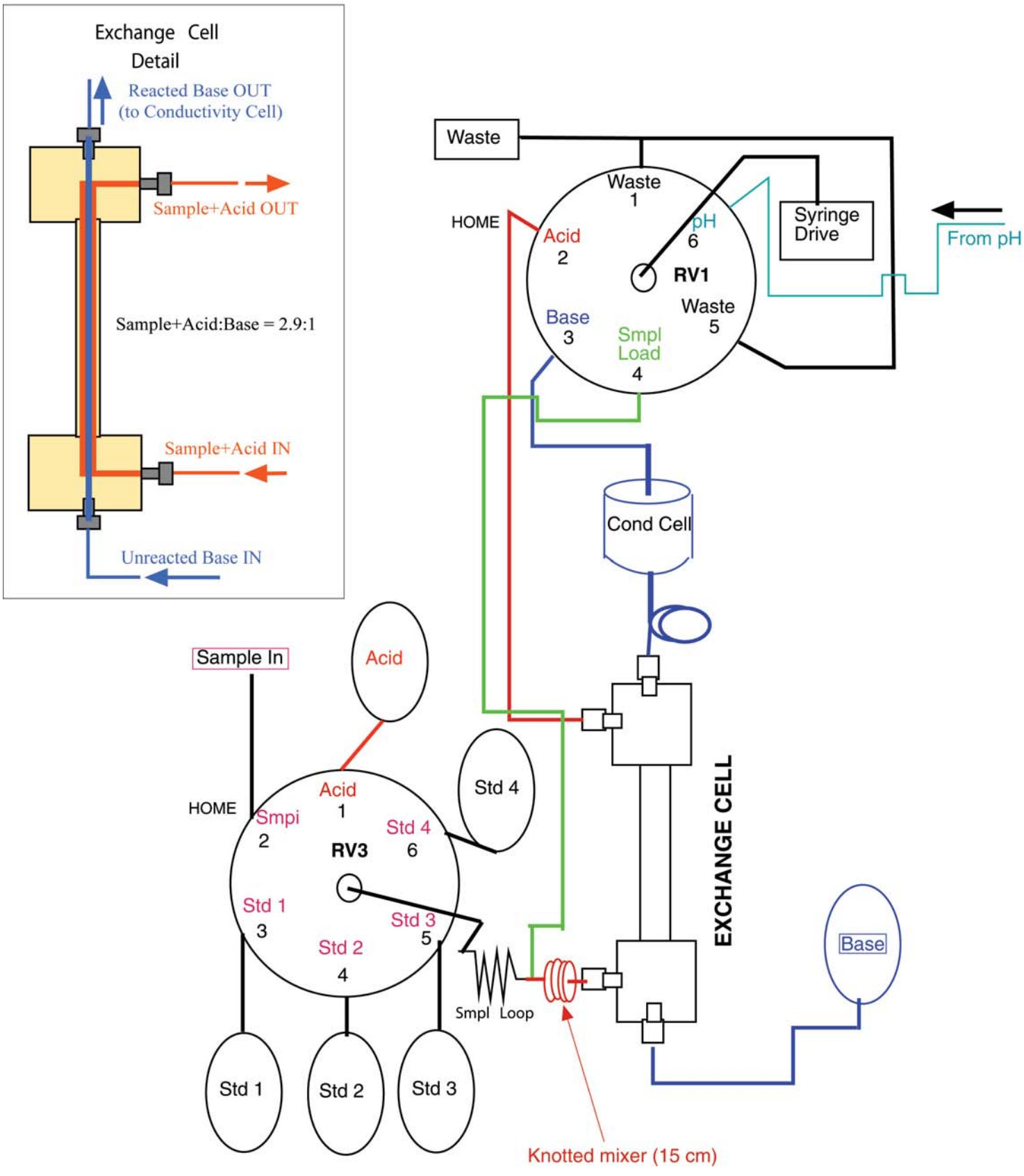
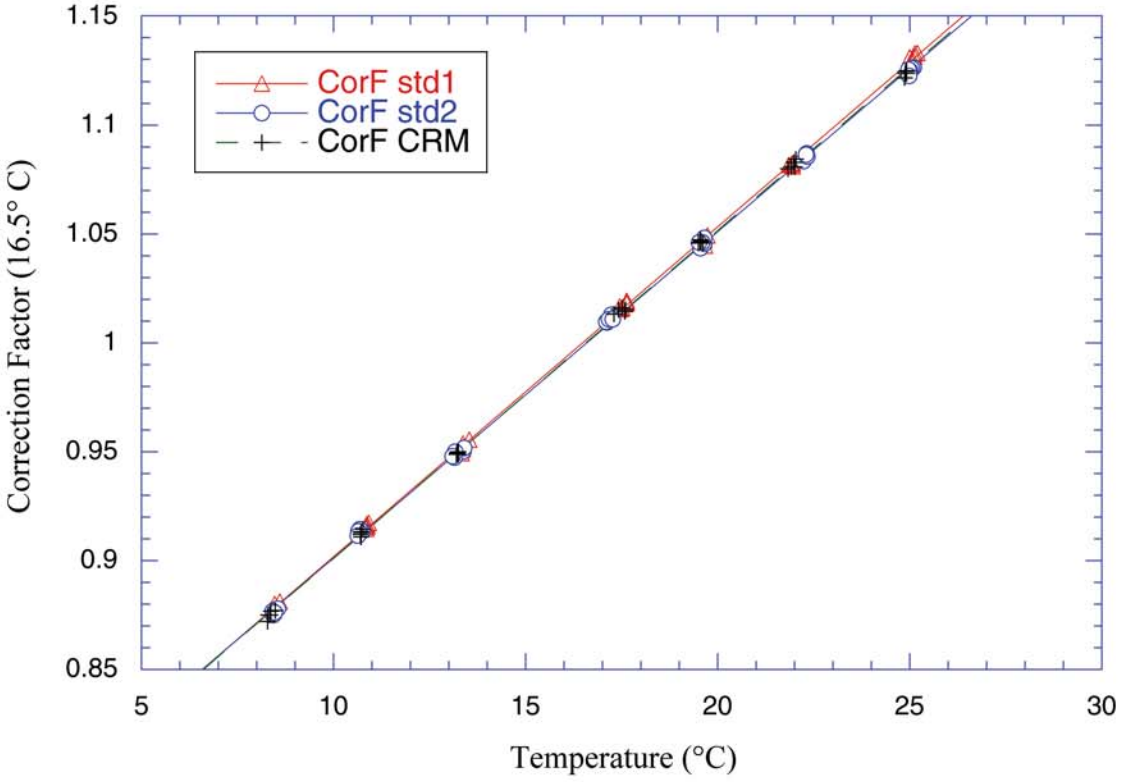

Exchange Cell Detail
Reacted Base OUT (to Conductivity Cell)
Sample+Acid OUT
Sample+Acid:Base = 2.9:1
Sample+Acid IN
Unreacted Base IN
Waste
Waste 1
HOME
Acid 2
pH 6
RV1
Waste 5
Base 3
Smpl Load 4
Syringe Drive
From pH
Cond Cell
Sample In
Acid
HOME
Acid 1
Smpi 2
Std 4 6
RV3
Std 1 3
Std 3 5
Std 2 4
Std 4
Smpl Loop
EXCHANGE CELL
Base
Std 1
Std 2
Std 3
Knotted mixer (15 cm)

Fig. 3


CorF std1
CorF std2
CorF CRM
Correction Factor (16.5° C)
Temperature (°C)
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1.1
1.05
1
0.95
0.9
0.85
5
10
15
20
25
30

Fig. 4

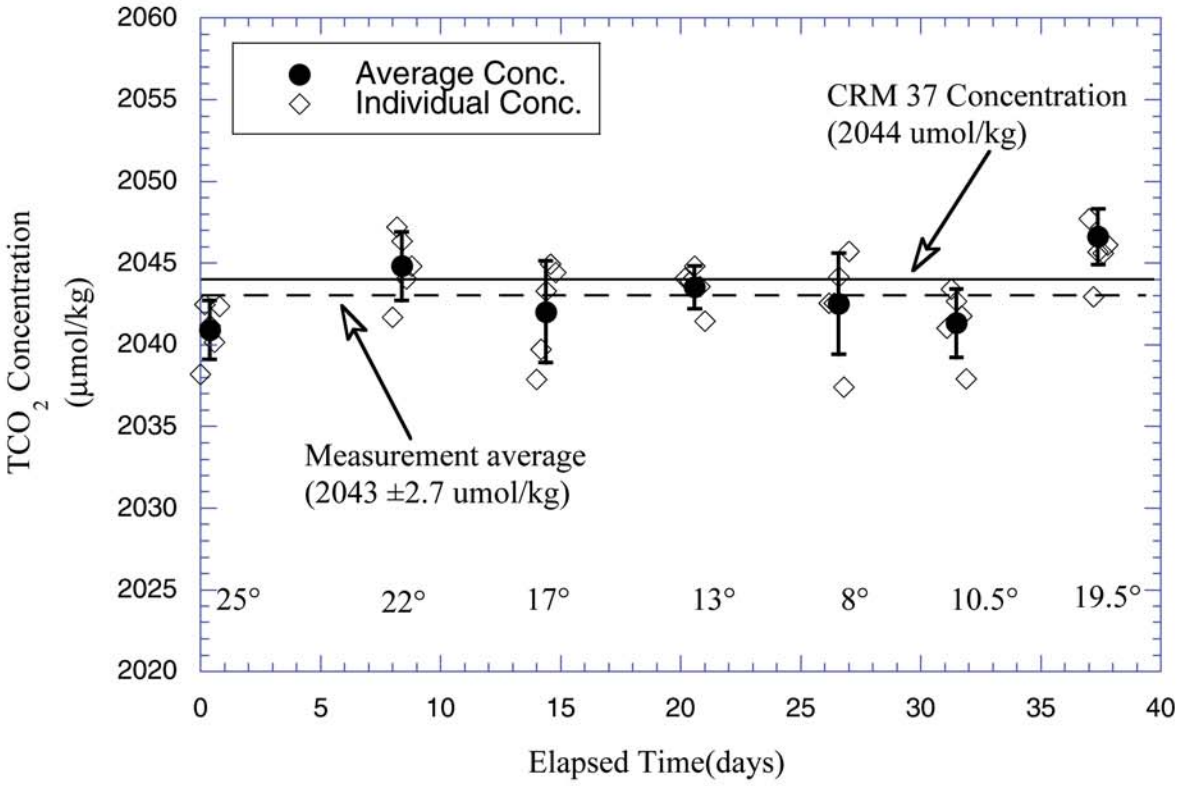

Average Conc.
Individual Conc.
CRM 37 Concentration
(2044 umol/kg)
Measurement average
(2043 ±2.7 umol/kg)
25°
22°
17°
13°
8°
10.5°
19.5°
TCO2 Concentration
(µmol/kg)
Elapsed Time(days)
2060
2055
2050
2045
2040
2035
2030
2025
2020
0
5
10
15
20
25
30
35
40

Fig. 6

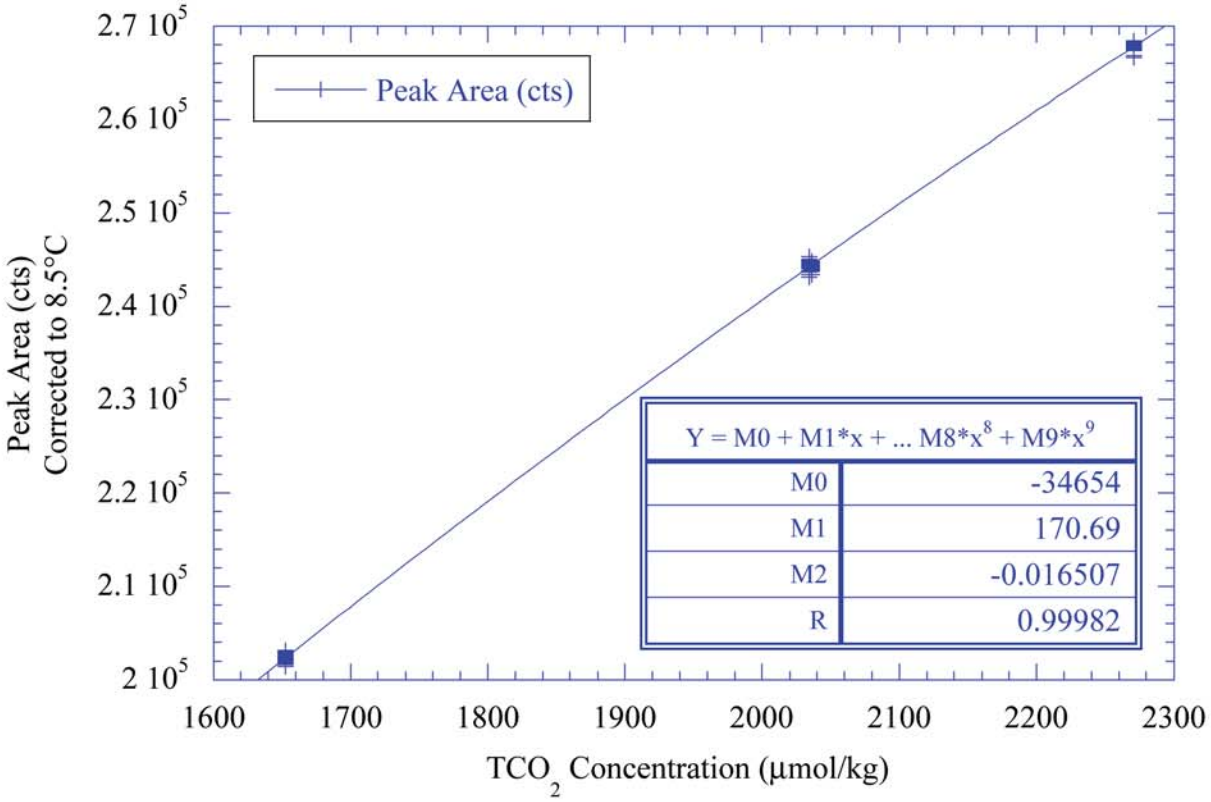

Peak Area (cts)
Peak Area (cts)
Corrected to 8.5°C
2.7 10^5
2.6 10^5
2.5 10^5
2.4 10^5
2.3 10^5
2.2 10^5
2.1 10^5
2 10^5
1600
1700
1800
1900
2000
2100
2200
2300
TCO2 Concentration (µmol/kg)
Y = M0 + M1*x + ... M8*x^8 + M9*x^9
M0 -34654
M1 170.69
M2 -0.016507
R 0.99982

Fig. 7

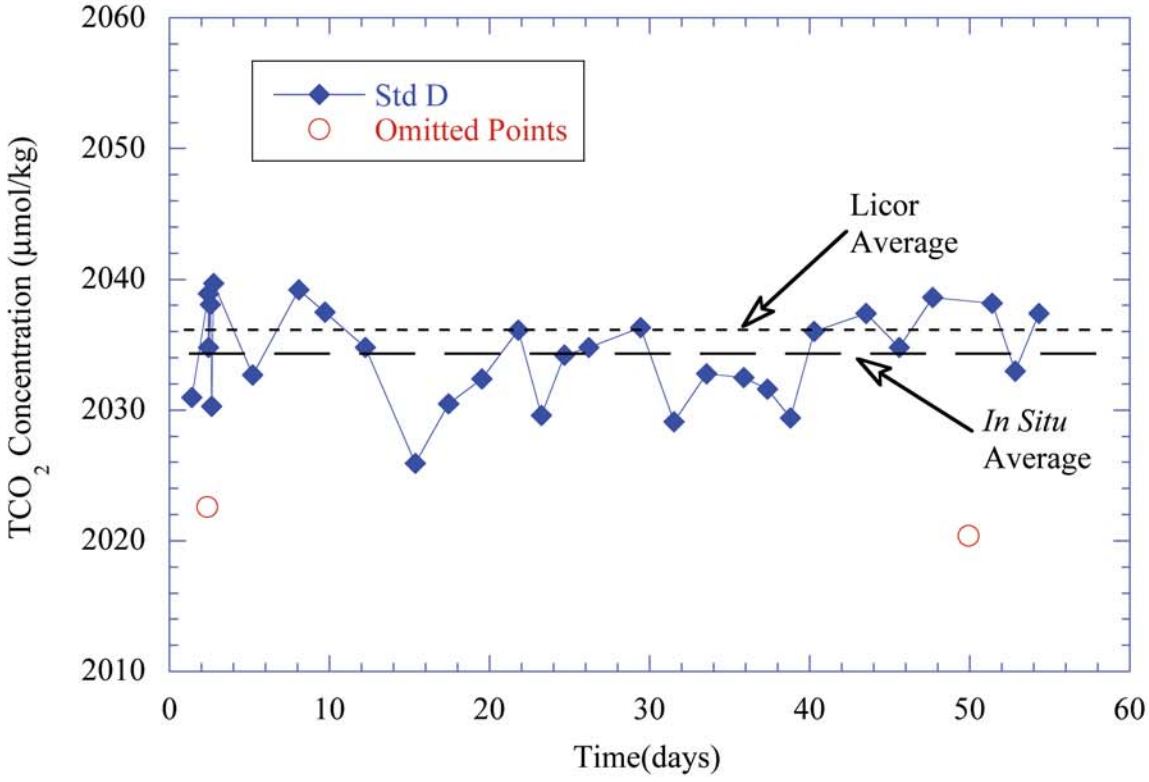

Std D
Omitted Points
Licor
Average
In Situ
Average
TCO2 Concentration (µmol/kg)
Time(days)
2060
2050
2040
2030
2020
2010
0
10
20
30
40
50
60

Fig. 8a

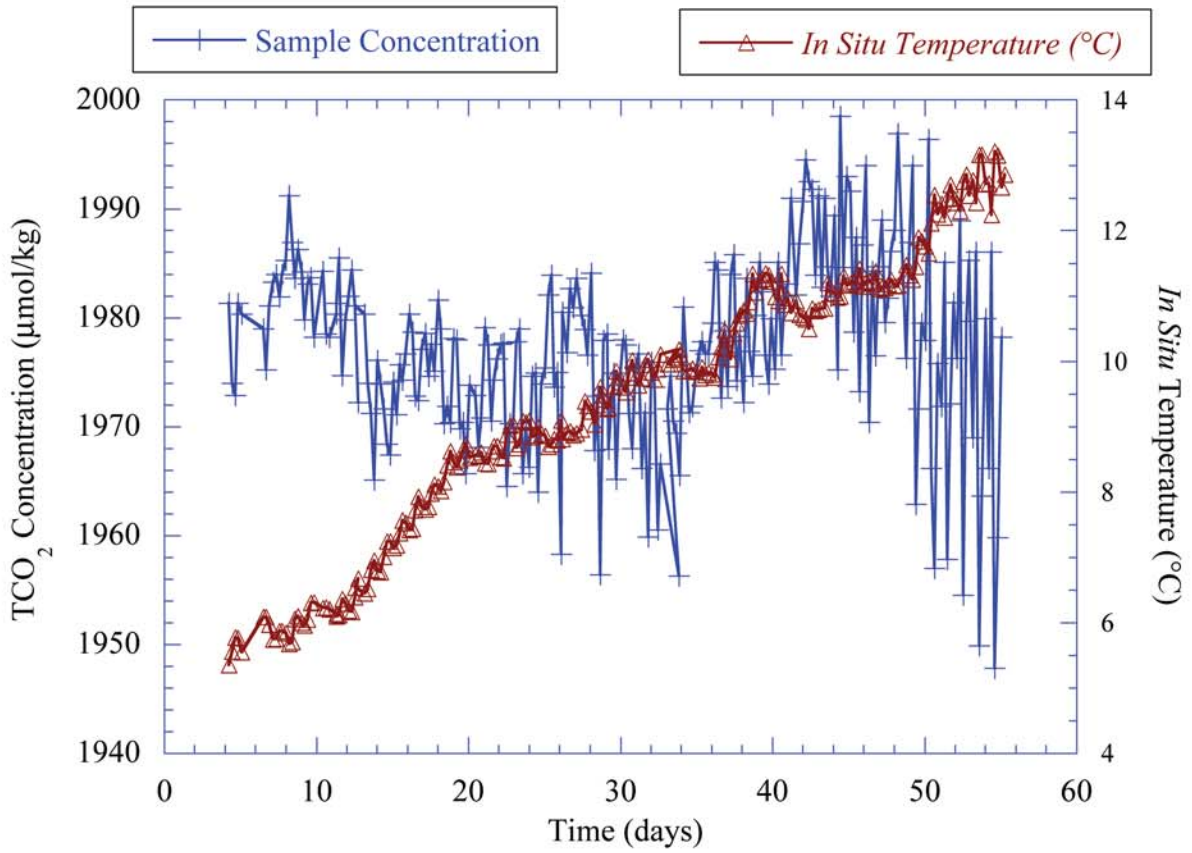

Sample Concentration
In Situ Temperature (°C)
TCO2 Concentration (µmol/kg)
In Situ Temperature (°C)
Time (days)
2000
1990
1980
1970
1960
1950
1940
14
12
10
8
6
4
0
10
20
30
40
50
60

Fig. 8b

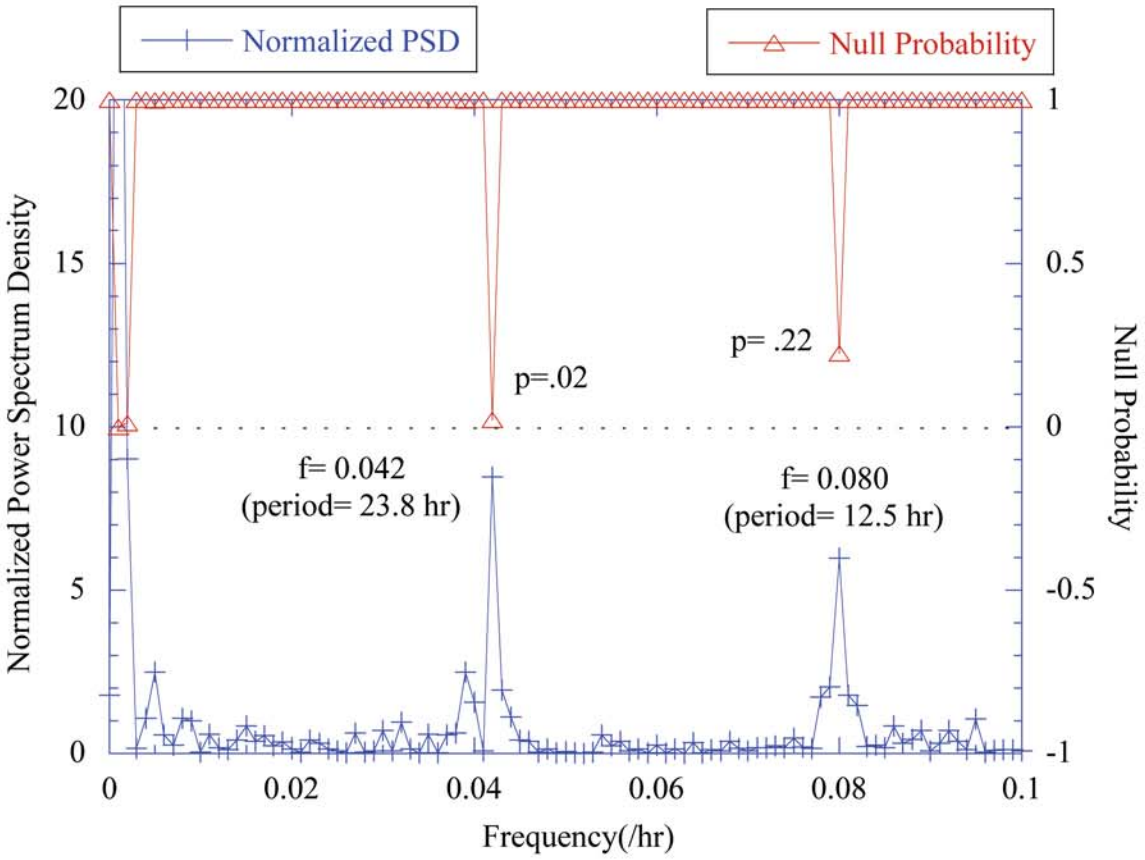

Normalized PSD
Null Probability
Normalized Power Spectrum Density
Null Probability
Frequency(/hr)
20
15
10
5
0
1
0.5
0
-0.5
-1
0
0.02
0.04
0.06
0.08
0.1
p=.02
p= .22
f= 0.042
(period= 23.8 hr)
f= 0.080
(period= 12.5 hr)

Fig. 9a

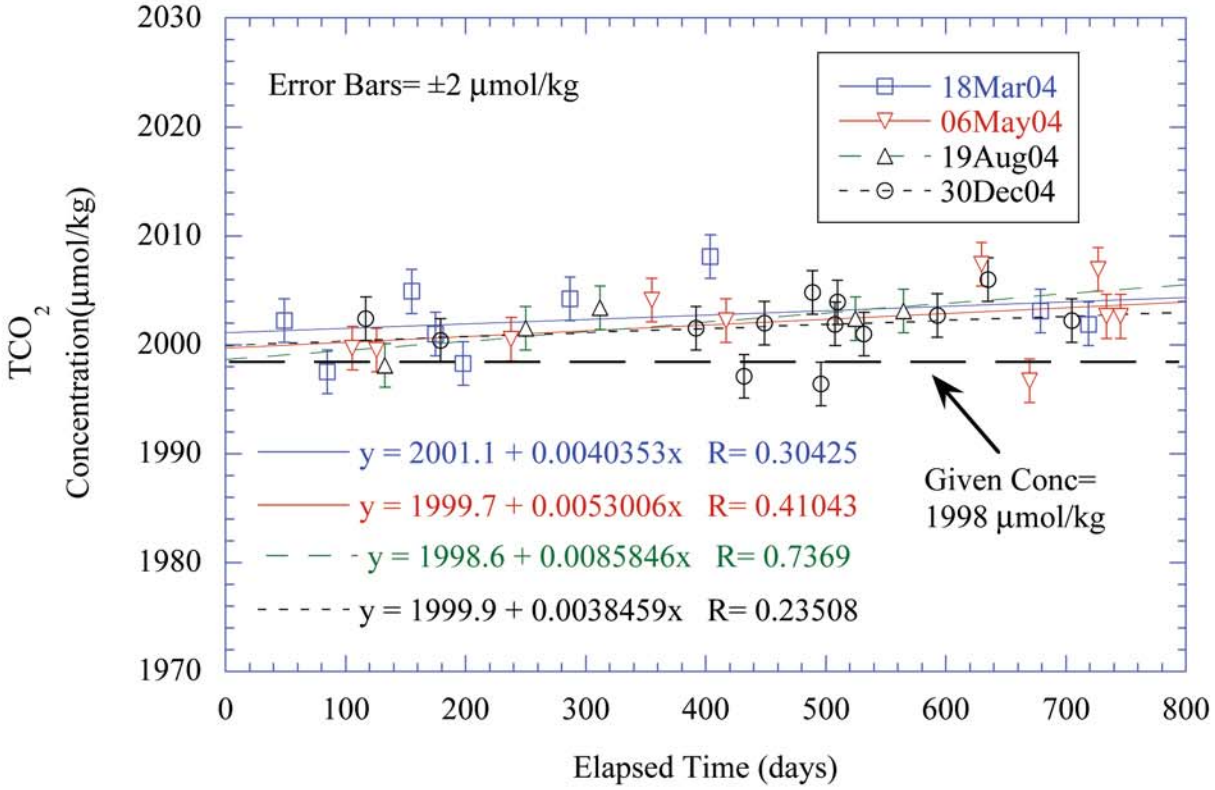
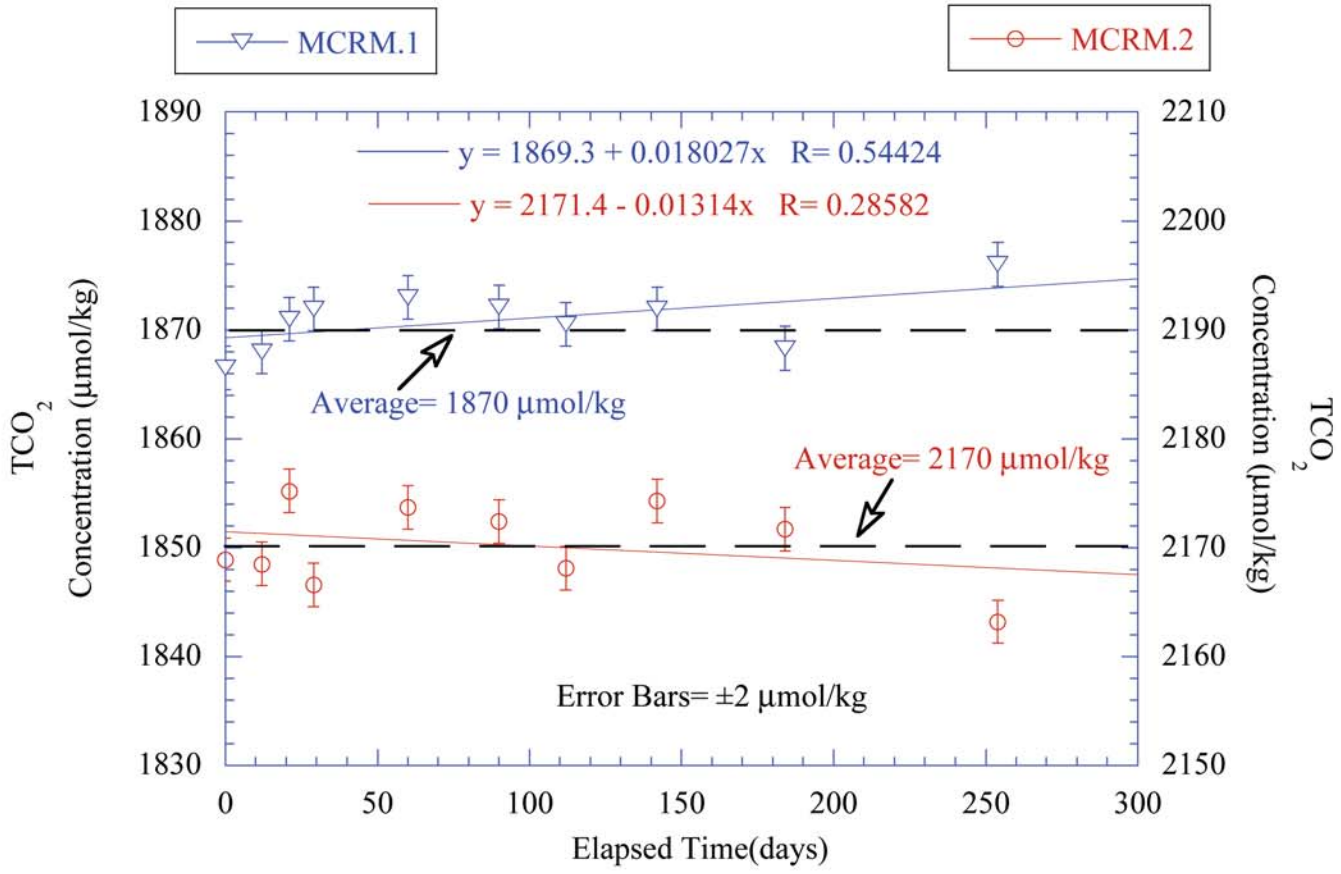

Error Bars= ±2 µmol/kg
18Mar04
06May04
19Aug04
30Dec04
y = 2001.1 + 0.0040353x R= 0.30425
y = 1999.7 + 0.0053006x R= 0.41043
y = 1998.6 + 0.0085846x R= 0.7369
y = 1999.9 + 0.0038459x R= 0.23508
Given Conc= 1998 µmol/kg
TCO2 Concentration(µmol/kg)
Elapsed Time (days)
2030
2020
2010
2000
1990
1980
1970
0
100
200
300
400
500
600
700
800

Fig. 9b


MCRM.1
MCRM.2
y = 1869.3 + 0.018027x R= 0.54424
y = 2171.4 - 0.01314x R= 0.28582
Average= 1870 µmol/kg
Average= 2170 µmol/kg
Error Bars= ±2 µmol/kg
TCO2 Concentration (µmol/kg)
TCO2 Concentration (µmol/kg)
Elapsed Time(days)
1890
1880
1870
1860
1850
1840
1830
2210
2200
2190
2180
2170
2160
2150
0
50
100
150
200
250
300