A TRANSATLANTIC SECTION OF SHIPBOARD DIC CONCENTRATION AND δ^{13}C-DIC MEASUREMENTS AND RESULTS FROM A WORLDWIDE INTER COMPARISON STUDY

by

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Abstract

I developed and optimized a Cavity Ring-Down Spectroscopy based method for simultaneous determination of dissolved inorganic carbon concentration ([DIC]) and its stable carbon isotope composition (δ^{13}C-DIC) in seawater. The new method was tested on the research vessel Celtic Explorer during an occupation of the Go-Ship A02 trans-Atlantic hydrographic section in 2017. The precision and accuracy for [DIC] determinations achieved ±3 µmol/kg. Precision of δ^{13}C-DIC determination was better than ±0.12‰. Using deep seawater samples (DSW) collected during the cruise and a DIC Reference Material (RM), I organized a worldwide inter comparison experiment for the measurement of δ^{13}C-DIC in seawater samples. Results from 16 participating groups showed excellent agreement (1σ of 0.11‰ and 0.10‰ for RM and DSW respectively). A normalization procedure increased the inter-laboratory precision of the DSW' δ^{13}C-DIC even further (1σ of 0.05‰). I also give a very brief overview of the δ^{13}C-DIC results from the cruise.

List of Abbreviations and Symbols Used

Abbreviations	Description
AOU	apparent oxygen utilization
CRDS	Cavity Ring-Down Spectroscopy
CRM	Certified Reference material for oceanic CO_2 measurements
CTDO	conductivity, temperature, depth and O_2
DIC	dissolved inorganic carbon
DO	dissolved oxygen
DSW	deep seawater samples
EOVs	Essential Ocean Variables
GOOS	Global Ocean Observing System
IQR	inter-quartile range
IRIS	Isotope Ratio Infrared Spectrometer
IRMS	Isotope Ratio Mass Spectrometry
JGOFS	Joint Global Ocean Flux Study
MS	Mass Spectrometry
NDIR	non-dispersive infrared detector
pCO_2	partial pressure of CO_2
PTFE	Polytetrafluoroethylene
QC	quality control
Q-Q	quantile-quantile
Q_1	first quartile
Q_3	third quartile
RM	reference material
R.V.	research vessel
SOP	standard operating procedures
TA	total alkalinity
VPDB	Vienna PeeDee Belemnite
WOCE	World Ocean Circulation Experiment
2nd QC	secondary quality control

Symbols	**Description**
C_{ant}	anthropogenic carbon
$[CO_2]$	carbon dioxide concentration
δ	isotope ratio
δ^{13}C-DIC	stable carbon isotope composition of dissolved inorganic carbon
$\delta^{13}C\text{-}CO_2$	stable carbon isotope composition of carbon dioxide
$\delta^{13}C^0$	preformed δ^{13}C-DIC value
$\delta^{13}C_{org}$	δ^{13}C of particulate organic matter
σ	standard deviation
R^2	square of correlation coefficient
R_A	measured isotope ratio of a specific sample
R_{std}	defined isotope ratio of a standard reference material

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Chapter 1

Introduction

1.1 Importance of Ocean Carbon Cycling Studies

Since the industrial revolution, a huge amount of anthropogenic CO_2 has been released into the atmosphere due to fossil fuel combustion, and this has created global climatological and ecological problems (e.g. Keeling 1979). For instance, as a greenhouse gas, increased CO_2 in the atmosphere has significantly reduced the infrared radiation from earth to space, and thus caused global warming.

As the third largest reservoir of carbon after precipitated carbonates and organic carbon in the sediments, the ocean plays an important role for buffering the increasing concentration of CO_2 in the atmosphere (Pilson 2012). However, in the mean time, oceanic uptake of excess CO_2 from the atmosphere has also caused ocean acidification (Feely et al., 2008), because the CO_2 system is responsible for approximately 95% of the acid–base buffering in seawater (Pilson 2012). Decrease in seawater pH has put some marine organisms in danger, especially the species that form calcium carbonate shells and/or skeletons, because decrease in pH would increase the dissolution rate of calcium carbonate, and makes it harder to precipitate (Caldeira & Wickett 2003).

Further, carbon serves as one of the most important elements for marine life forms. By monitoring the short term variations of the ocean CO_2 system, we can learn a great deal about processes related to biological activities of marine organisms such as photosynthesis, respiration, and remineralization.

Therefore, it is of great importance for us to study the origin, movements and distribution of carbon in the ocean and the related ocean carbon cycling processes, in order to clarify human impacts on the ocean environment.

1.2 Chemistry of CO_2 in Seawater

When CO_2 in the atmosphere dissolves into seawater, the following chemical reactions take place: CO_2 (g) ⇌ CO_2 (aq) + H_2O (l) ⇌ H_2CO_3 (aq) ⇌ H^+ (aq) + HCO_3^- (aq) ⇌ CO_3^{2-} (aq) + H^+ (aq), where (g), (aq) and (l) denotes the state of each carbon species which are gas, solution and liquid, respectively. Due to analytical difficulties, the concentration of each individual carbon species in seawater cannot be quantified. Generally, researchers measure the following four parameters of seawater: dissolved inorganic carbon (DIC), total alkalinity (TA), partial pressure of CO_2 (pCO_2) and pH, and use them, and other ancillary information such as the equilibrium constants for the reactions above, to describe the seawater CO_2 system.

Specifically, DIC is the sum of CO_2 (aq), H_2CO_3 (aq), HCO_3^- (aq) and CO_3^{2-} (aq) concentration in seawater. This parameter can be determined by acidification of a seawater sample and measurement of the extracted CO_2 concentration by, for example, coulometric titration system (Dickson et al., 2007) or with a Non-dispersive Infrared Detector (NDIR) (Call et al., 2017). Generally, DIC measurement results are standardized by "Certified Reference material for oceanic CO_2 measurements" (CRM) provided by University of California, San Diego, Scripps Institution of Oceanography (Dickson et al., 2003). To observe the ocean carbon system, both precision and accuracy of ±2 μmol/kg in DIC measurements are required and have been achieved by various analytical and standardization methods (Dickson et al., 2007).

TA is defined as "the number of moles of hydrogen ion equivalent to the excess of proton acceptors in 1 kilogram of seawater sample" (Dickson et al., 2007). It reflects alkaline substance amounts in seawater. Similar to DIC measurements, TA concentration of seawater can be determined by potentiometric titration system and is generally standardized by CRM.

pH reflects the total hydrogen ion concentration in seawater, and is defined as the following: $\mathrm{pH} = -\log[H^+]$. The pH of seawater can be determined by various analytical instruments such as glass electrode cell, spectrophotometer (Dickson 1993) or, more recently, in-situ pH sensors (Martz et al 2010).

$p\mathrm{CO_2}$ in the air that is in equilibrium with a seawater sample is usually measured by coupling a gas-liquid equilibration system with a detector such as gas chromatography, non-dispersive infrared sensor (NDIR) or Cavity Ring Down Spectroscopy (CRDS) for $\mathrm{CO_2}$ mole fraction determination.

1.3 Stable Carbon Isotope Composition of Dissolved Inorganic Carbon (δ^{13}C-DIC)

Isotopes are atoms of specific elements with the same number of protons but different numbers of neutrons in the nucleus. They can be divided into two categories which are stable isotopes and radioactive isotopes. For instance, carbon has two stable isotopes (^{12}C and ^{13}C) and one radioactive isotope (^{14}C), with approximately 98.9% of the carbon in the form of ^{12}C (https://www.esrl.noaa.gov/gmd/ccgg/isotopes/chemistry.html). Similar to carbon, for most elements, the stable isotope ratio is relatively small (in the order of 0.001), so it is easier to express the stable isotope composition of an element using the delta (δ) notation: $\delta_A=(R_A/R_{std}-1)*1000‰$, where R_A denotes the measured isotope ratio of a specific sample, and R_{std} refers to a defined isotope ratio of a standard reference material. In the case of carbon, PeeDee Belemnite (carbonate) with ^{13}C/^{12}C of $1.123*10^{-2}$ is generally used as a standard reference material. In this study, all stable isotope composition of carbon are reported in ‰VPDB.

As the chemical properties of an element are mainly determined by the number and arrangement of electrons, isotopes share similar chemical properties. However, different masses of isotopes lead to subtle difference in their physical or chemical properties. These differences can cause separation/partition of isotopes during various physical, chemical and biological processes. "This partitioning of isotopes between two substances or two phases of the same substance with different isotope ratios is called isotope fractionation." (Hoefs 1997). There are two types of isotopic fractionation processes, which are "equilibrium isotope exchange" and "kinetic fractionation processes". Equilibrium isotope exchange fractionation is the case that in chemical equilibrium reactions, there is no net reaction but a change in isotope distribution between different chemical substances or phases. (Tiwari et al., 2015) This type of fractionation is usually caused by the slight difference in zero point energies of molecules with different isotopic composition. Generally, in an equilibrium reaction, the heavier isotopes with lower zero point energy tend to concentrate in the chemical species which has the strongest bond to its atom, thus causing isotope fractionation. For example, between each inorganic carbon species in seawater, that is CO_2 (aq), H_2CO_3 (aq), HCO_3^- (aq) and CO_3^{2-} (aq), ^{13}C tends to be more concentrated within HCO_3^- (aq), because it has the stronger bonds compared to other species. Kinetic fractionation processes mainly depend on the different reaction rates and/or different movement speed of molecules with different isotope composition. This type of fractionation is associated with incomplete and unidirectional processes where no isotope equilibrium is attained. For instance, in most diffusion process, lighter isotopes tend to move faster than the heavier isotopes, thus, causing isotope fractionation. Another example of this type of fractionation is photosynthesis. Plants preferably assimilate lighter ^{12}C rather than heavier ^{13}C, therefore their organic matter tends to have a lighter $\delta^{13}C$ value compared to atmospheric CO_2. The magnitude of isotope fractionation can be expressed using difference fractionation factors (ε), which represents the difference between the δ value of a product and its correlated reactant (Emerson & Hedges 2008). In the case of carbon, $\varepsilon^{13}C = \delta^{13}C_{product} - \delta^{13}C_{reactant}$.

The distribution of carbon stable isotope composition of DIC ($\delta^{13}C$-DIC) in seawater is affected by various physical and biogeochemical processes such as photosynthesis and respiration, degradation of organic carbon, carbonate dissolution and precipitation, air-sea exchange of CO_2 including oceanic uptake of anthropogenic CO_2. Isotope fractionation of carbon is involved in some of these processes. Table 1.1 shows $\delta^{13}C$-DIC variation of seawater during various processes together with approximate difference fractionation factors (ε) of carbon isotope fractionations involved in those processes. Specifically, during photosynthesis, $\delta^{13}C$-DIC of seawater increases due to significant kinetic fractionation of carbon. In contrast, during respiration, carbon isotopes fractionate very little, although the degradation of organic matter with lighter $\delta^{13}C$ would result in a decrease of $\delta^{13}C$-DIC. In addition, $CaCO_3$ precipitation in seawater causes slightly decrease in $\delta^{13}C$-DIC, because heavier ^{13}C tends to be concentrated within solid-phase

$CaCO_3$. On the other hand, dissolution of carbonate with slightly heavier $\delta^{13}C$ value in seawater would increase the $\delta^{13}C$-DIC. Further, when gas phase CO_2 dissolves in seawater, equilibrium isotope exchange fractionation of carbon between the gas phase and dissolved CO_2 lead to a slight decrease in seawater $\delta^{13}C$-DIC. Particularly, due to fossil fuel combustion, anthropogenic CO_2 with very light $\delta^{13}C$ values was emitted into the atmosphere. Uptake of this anthropogenic CO_2 lead to reduction in the seawater $\delta^{13}C$-DIC value.

Table 1.1. $\delta^{13}C$-DIC variation of seawater during various processes.

Process	**$\delta^{13}C$-DIC variation**	**Approximate difference fractionation factor (‰)**	**Reference**
equilibrium isotope exchange when CO_2 (g) dissolves in seawater	decrease	-1.1	Knox et al., 1992; Emerson & Hedges. 2008
equilibrium isotope exchange during $CaCO_3$ precipitation	decrease	+1	Romanek et al., 1992; Emerson & Hedges. 2008
kinetic fractionation during photosynthesis	increase	-14 to -19	O'Leary et al., 1981; Emerson & Hedges. 2008
kinetic fractionation during respiration	little variation	0	O'Leary et al., 1981; Emerson & Hedges. 2008
oceanic uptake of anthropogenic CO_2	decrease		
degradation of organic matter	decrease		
dissolution of carbonate	increase		

Fig. 1.1 shows 3 $\delta^{13}C$-DIC depth profiles derived from the same location of north Atlantic over the past 20 years. We could notice that significant isotope fractionation during photosynthesis and little fractionation during respiration of marine plankton in the

euphotic zone leaves the surface seawater enriched in ^{13}C. The produced organic matter with lower δ^{13}C value sinks and degrades, which depletes the ^{13}C in subsurface water (e.g. within the ocean thermocline). Also, we can notice a decrease in δ^{13}C-DIC in surface water over a 20 years' interval, and that the magnitude of this decrease in δ^{13}C-DIC weakens with increasing depth. Oceanic uptake of anthropogenic CO_2 with lower δ^{13}C value may contribute to this temporal decrease in δ^{13}C-DIC of seawater.

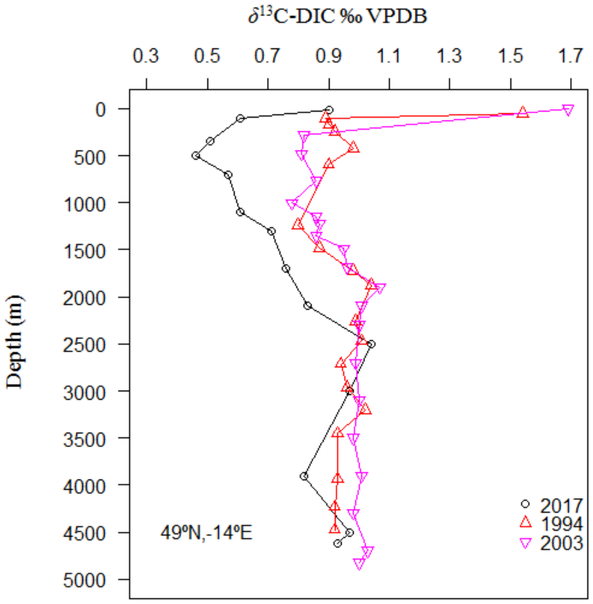

Fig. 1.1. δ^{13}C-DIC depth profiles derived from the same location of north Atlantic.

This brief discussion shows that measurements of seawater δ^{13}C-DIC can provide information about sources of carbon, help specify processes that affect the carbon distribution in the ocean, quantify phtotosynthetically driven export of organic matter to the deep ocean, and help to evaluate the human impact on ocean carbon cycling.

1.4 Aims of Study

In this study, I developed and optimized a new simultaneous DIC and δ^{13}C-DIC measurement system and tested it on the R.V. Celtic Explorer during the 2017 A02 Go-Ship trans-Atlantic cruise. Also, I evaluate the δ^{13}C-DIC data derived from the new system, and the level of consistency of measurements made by multiple groups, by conducting a world-wide δ^{13}C-DIC inter-comparison experiment and through comparison with the historic δ^{13}C-DIC data from the A02 section. Finally, I provide a brief description and initial interpretation of the DIC and δ^{13}C-DIC data derived from the 2017 Go-Ship A02 cruise, including consideration of anthropogenic CO_2 uptake on temporal changes over the past 2 decades.

1.5 Structure of Thesis

This thesis consists of the following parts: 1) this chapter of introduction and background. 2) an article entitled "Shipboard measurement of DIC and δ^{13}C-DIC on discrete seawater samples using Cavity Ring-Down Spectroscopy: system testing and performance during a trans-Atlantic research cruise" which will be submitted to" *Limnology and Oceanography: Methods*" for publication. As the corresponding author, I conducted the measurements, processed the experimental data, interpreted the results, and wrote the manuscript. 3) an article entitled "An international inter-comparison of stable carbon isotope composition measurements of dissolved inorganic carbon in seawater", which has been submitted to "*Limnology and Oceanography: Methods*" As the corresponding author, I conducted the measurements, processed the experimental data, interpreted the results, and wrote the manuscript. 4) a chapter of preliminary interpretations of A02 cruise δ^{13}C-DIC data. 5) a short conclusions chapter.

Chapter 2

Shipboard Measurement of DIC and δ^{13}C-DIC on Discrete Seawater Samples Using Cavity Ring-Down Spectroscopy: System Testing and Performance During a Trans-Atlantic Research Cruise

This Chapter is an article that will be submitted to" *Limnology and Oceanography: Methods*" for publication.

2.1 Abstract

We coupled a Cavity Ring-Down Spectroscopy (CRDS) analyzer with a seawater acidification interface to allow high-frequency, simultaneous measurement of dissolved inorganic carbon concentration ([DIC]) and its stable carbon isotope composition (δ^{13}C-DIC).The approach uses small volume water samples (1 to 4 mL) and its measurement frequency is >4 samples per hour. The paper presents system design, operation and results of laboratory and field-testing. The precision and accuracy of this method for [DIC] determinations are both ±3 μmol/kg, and there is potential for further improvement. Precision of δ^{13}C-DIC determination was ±0.12‰, and the δ^{13}C-DIC results for deep-ocean seawater samples derived from the method have high consistency (better than 0.12‰) with data measured using traditional Isotope Ratio Mass Spectrometry (IRMS) of the same water mass. This method has been tested for its performance during a trans-Atlantic research cruise, and results show that it is suitable for research vessel onboard [DIC] and δ^{13}C-DIC measurements.

2.2 Introduction

The oceanic concentration of Dissolved Inorganic Carbon ([DIC]) and its carbon isotope composition (δ^{13}C-DIC) are both designated as Essential Ocean Variables (EOVs) by the Global Ocean Observing System (GOOS) (http://www.goosocean.org/components/com_oe/oe.php?task=download&id=35906&version=2.0&lang=1&format=1). Measurement of these parameters provides information on inorganic carbon sources, sinks and transformations within the ocean and supports enhancement of understanding of processes such as anthropogenic CO_2 uptake, biological production/respiration, microbial biogeochemistry, deep ocean circulation etc., (e.g. Kroopnick 1985; Sweeney et al., 2000; Gruber et al., 2002; Körtzinger et al., 2003;Olsen et al., 2006; Quay et al., 2007). In particular, the lighter isotopic composition of CO_2 released through combustion of fossil fuels and the corresponding reduction of the $^{13}C/^{12}C$ of atmospheric CO_2 (i.e. the ^{13}C Suess effect; Keeling 1979) has caused a reduction of oceanic $\delta^{13}C$-DIC which has proven a useful tracer of anthropogenic CO_2 uptake (Quay et al., 2003).

However in spite of the considerable importance of oceanic [DIC] and $\delta^{13}C$-DIC measurements, currently, the spatial and temporal coverage of paired [DIC] and $\delta^{13}C$-DIC measurements remains limited (Becker et al., 2016; Call et al., 2017). This is mainly because conventionally, $\delta^{13}C$-DIC measurements are made using Isotope Ratio Mass Spectrometers (IRMS), which are large, lab-based instruments, that preclude at-sea measurement of $\delta^{13}C$-DIC. Further, the preservation, transportation and pre-processing of the water samples, as well as, calibration of the Mass Spectrometry are often time and labor consuming and may introduce unexpected analytical difficulties (e.g. Salata et al., 2000; Assayag et al., 2006; Kim et al., 2014). In contrast, DIC measurements are made at sea routinely using considerably less complex and more compact instruments. Measurement at-sea allows for 24-hour sample measurement on board vessels, and avoids the high cost and considerable risk of shipping large quantities of bottles of seawater samples from remote ports to land-based laboratories. A further advantage of ship- and field-based measurement is that sampling can be adapted or repeated depending on the results obtained on the vessel or in the field.

In this study, we describe a newly-developed method for simultaneous measurement of [DIC] and $\delta^{13}C$-DIC on small-volume water samples using Cavity Ring-Down Spectroscopy (CRDS), that is appropriate for making measurements on-board research vessels and during other field deployments. We describe testing and quality assurance procedures which demonstrate that the method is capable of approaching the demanding accuracy and precision requirements (precision and accuracy of ±2 μmol/kg and ±0.05‰

in [DIC] and δ^{13}C-DIC measurements respectively) established by GOOS. Results are presented from an at-sea deployment on R.V. Celtic Explorer during the 2017 Go-Ship A02 trans-Atlantic cruise (McGovern et al., 2018).Our results and methodology are compared with other recently published studies of δ^{13}C-DIC analysis of seawater using CRDS, and recommendations are made concerning future developments.

2.3 Methods

2.3.1 Instrumentation and Analysis Procedure

A schematic of the measurement system is presented in Fig. 2.1. It consists of: a) an Apollo SciTech AS-D1 acidification interface (Apollo SciTech, LLC., Newark, DE. USA) for water sample acidification, CO_2 conversion & extraction; b) a Picarro G2201*i* CRDS detector (Picarro Inc., Santa Clara, CA. USA) for determination of both the CO_2 concentration ([CO_2]) and its carbon stable isotope composition (δ^{13}C-CO_2); and c) a laptop computer which runs Apollo SciTech's ADIC software and which is used to control the acidification interface as well as collection of data from the CRDS detector. The system uses CO_2 free compressed air as the carrier gas.

The basic principle and operation of Picarro’s G2201*i* CRDS detector has been described by Law 2000, Crosson 2008, and Peeters et al., 2001. The detector measures the individual stable carbon isotopes (^{12}C &^{13}C) of CO_2 simultaneously, and reports the δ^{13}C-CO_2 in ‰VPDB scale and the [CO_2] in ppm, at a measurement interval of ~3 seconds. Under the detector’s "CO_2 Isotope-only mode", the manufacturer’s stated precision of [CO_2] and δ^{13}C-CO_2 are 200 ppb (1-σ, 30 sec. average) and 0.12‰ (1-σ, 5 min. average) respectively when the gas stream to the detector contains CO_2 in air mixing ratios that are within the instrument’s specification range (i.e. 380 to 2000 ppm; https://www.picarro.com/products_solutions/isotope_analyzers/13c_for_ch4_co2).

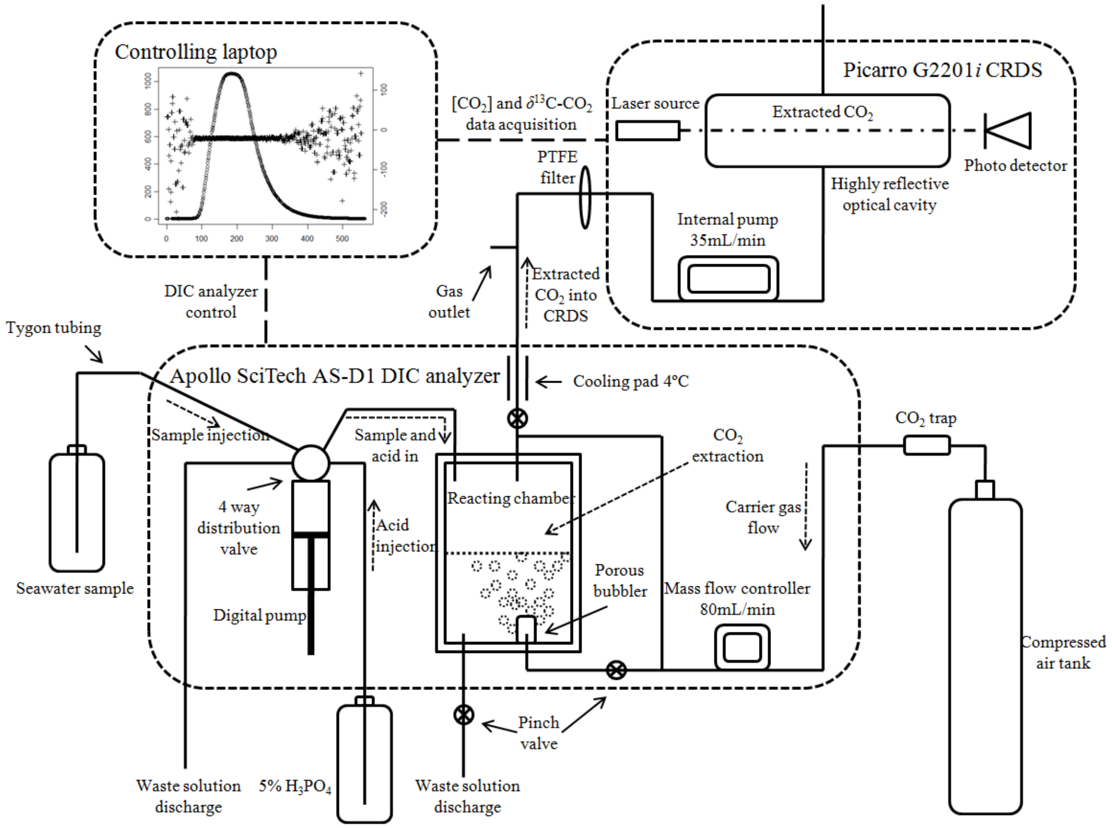

Fig. 2.1. Schematic of our simultaneous [DIC] and δ^{13}C-DIC measurement system.

Apollo SciTech's AS-D1 acidification interface consists of: 1) a mass-flow-controller (CMOSens® Technology Mass Flow Controller SFC 5400) to control the carrier/ stripping gas flow rate precisely; 2) a digital syringe pump (Kloehn Inc. Versa Pump 655 Series SYRINGE DISPENSER MODULE) for transferring precise volumes (1~4 mL) of 5% H_3PO_4 reagent and sample water into the gas-stripping chamber (approximate total volume 30 mL) where DIC species are converted to CO_2 and extracted by the carrier/ stripping gas; 3) a Peltier-based electronic cooling system to keep the stripping chamber temperature at 4ºC and to reduce the water vapor concentration before the extracted CO_2 is transferred into the Picarro G2201*i* CRDS detector for CO_2 concentration and δ^{13}C-CO_2 determination.

The measurement procedure for a water sample is as follows: firstly, 0.2 mL of 5% H_3PO_4 is introduced into the syringe pump (via Tygon tubing; Saint-Gobain™ Tygon S3™ E-3603 Flexible Tubings) and then injected into the stripping chamber, thereby flushing the entire sample pathway with acid. After discharge of this acid rinse, 1 mL of 5% H_3PO_4 is injected into the stripping chamber with a further 1 mL of acid remaining in the syringe pump. Next, an aliquot of water sample (1~4 mL) is introduced into the syringe pump on top of the pre-introduced acid. The combined volume of water sample and acid is injected into the stripping chamber (that is already pre-loaded with 1 mL of acid) at a speed of 150 µL/sec. A bubbling frit (pore size about 10 µm) ensures mixing of sample and acid, and that the resulting CO_2 is extracted efficiently from the liquid phase by the carrier/ stripping gas stream (typical flow rate 80 mL/min) and delivered to the Picarro G2201*i* CRDS for concentration and isotopic composition detection. A PTFE membrane filter is added at the inlet of the Picarro G2201*i* CRDS to protect it from the water vapor produced during CO_2 extraction. As the internal pump of Picarro G2201*i* CRDS has a flow rate of 35 mL/min, which is less than the carrier/ stripping flow rate, a split is used to avoid pressure build-up at the CRDS inlet.

The measurement process is controlled with Apollo SciTech's ADIC software, which acquires $[CO_2]$ and $\delta^{13}C\text{-}CO_2$ data from the Picarro G2201*i* CRDS at a frequency of 1Hz. The [DIC] of a water sample is derived from the integral of $[CO_2]$ data at each time point over the integration period (Fig. 2.2; see next section).

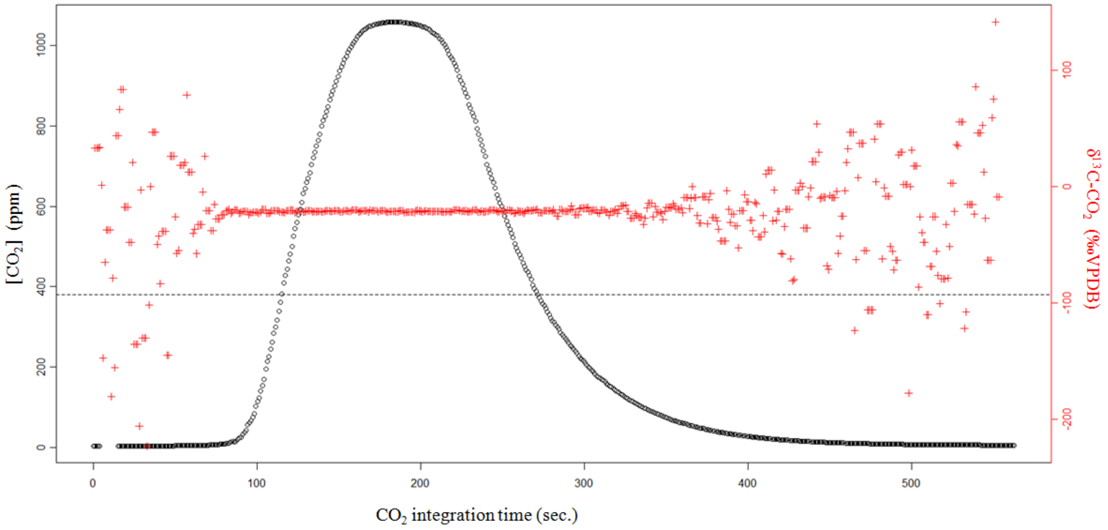

Fig. 2.2. CO_2 integration curve. [DIC] is derived from the integration area of $[CO_2]$ at each time point, and $\delta^{13}C$-DIC is calculated from the weighted average of $\delta^{13}C\text{-}CO_2$ by $[CO_2]$ between 380 ppm (dotted line) and 2000 ppm.

Typical sample analysis time is <12 minutes, and different sample injection volumes (1~4 mL) and different measurement schemes for [DIC] can be set up within the ADIC software interface to fulfill various measurement requirements, such as high-precision or high sample-throughput (discussed below).

2.3.2 Calibration of DIC Concentration

The integrated signal from the Picarro CRDS (in the unit of ppm*sec) is converted to concentration using a calibration based on three sets of lab-made $NaHCO_3$ "in-house" standard solutions with different concentrations. Further, Certified Reference Materials for oceanic CO_2 measurements" (CRM) supplied by the University of California, San Diego, Scripps Institution of Oceanography (Dickson et al., 2003) are used for validation of the relationship between [DIC] and [CO_2] integration area, as well as for final correction of [DIC]. Generally, during measurements, [DIC] and [CO_2] integration area of "in house" standard solutions show a strong linear correlation (R^2>0.9999), and "in house" standard calibrated [DIC] and certified [DIC] of CRM agree within 10 μmol/kg as shown in Fig. 2.4.

2.3.3 Calibration of δ^{13}C-DIC

The δ^{13}C-DIC of a water sample is calculated from the weighted average of the δ^{13}C-CO_2 by [CO_2], when the Picarro CRDS is measuring between 380 ppm and 2000 ppm (discussed below). As there is currently no liquid certified reference material for δ^{13}C-DIC, standardization of δ^{13}C-DIC results are conducted using three sets of "in-house" standards made by dissolving $NaHCO_3$ (ACP S-2150), Na_2CO_3 (BDH 9284) and baking soda (ARM & Hammer) powder with different δ^{13}C values into Helium gas purged MiliQ water. The δ^{13}C of each "in-house" standard is determined by ISOPRIME100 IRMS in CERC.OCEAN laboratory, and standardized by international reference material USGS-40 and USGS-41. Defined δ^{13}C values of the international reference material and standardized δ^{13}C values of each "in-house" standards are shown in Table 2.1.

Table 2.1 Defined $\delta^{13}C$ values of the solid international reference material and standardized $\delta^{13}C$ values of each "in-house" standards reported in VPDB‰.

	USGS-40	USGS-41	$NaHCO_3$	Na_2CO_3	SODA
$\delta^{13}C$ (VPDB‰)	-26.39	+37.63	-20.89	-2.55	-10.51

2.3.4 Field Testing on Research Cruise

This new system was tested onboard the research vessel Celtic Explorer in the North Atlantic during the 2017 A02 Go-Ship cruise from St. John's, Canada to Galway, Ireland in May, 2017 (McGovern et al., 2018; GO-SHIP; http://www.go-ship.org/). Water samples for [DIC] and $\delta^{13}C$-DIC analysis were collected at 30 stations (12 depths per station on average), mainly using 160 mL borosilicate serum bottles with flat butyl septa with PTFE coating and aluminum seals for crimping, in accordance with sampling protocols suggested by Dickson et al., 2007. Borosilicate media bottles with polypropylene caps were also used for sampling. Samples from 19 stations were measured onboard, and the rest were analyzed in the lab after the cruise.

2.4 Laboratory and Field Test Results

A series of in-laboratory tests were conducted to evaluate performance and examine procedures for their potential to introduce noise and/or systematic bias in the measurements. The method was then tested on a trans-Atlantic research cruise which allowed for comparison with historical data measured using IRMS.

2.4.1 Precision

The method's precision for [DIC] and $\delta^{13}C$-DIC measurement was evaluated by measuring the same lab-made 2 mmol/kg "in house" $NaHCO_3$ standard solution 15 times, using a constant sample injection volume of 3.8 mL. The measurement precision for [DIC], based on variability of the CO_2 integration area, was 0.12% (1 standard deviation). For a typical seawater sample, where [DIC] might range between 1850 μmol/kg and 2250 μmol/kg, this precision is equivalent to ±2-3 μmol/kg. The measurement precision for

δ^{13}C-DIC based on the same replicate analyses was 0.06‰ (n=15; 1 standard deviation) which is better than the manufacturer's stated precision (0.12‰) for δ^{13}C-CO_2 measurements made with the Picarro G2201*i* CRDS.

2.4.2 Assessment of Accuracy

The system's accuracy for [DIC] and δ^{13}C-DIC measurement was evaluated by measurement of Certified Reference Materials and an inter-comparison exercise (Cheng et al., 2018 submitted), respectively. Specifically, for [DIC], 3 "in-house" standard solutions and 2 CRM (batch 157 and batch 168) were measured. The concentration of [DIC] is calibrated using 3 "in-house" standard solutions and further corrected by a CRM. In this test, one CRM was used for final [DIC] correction, and the other one was treated as a "sample" and vice versa. In both cases, the measured [DIC] of CRM agreed to the certified [DIC] value within 3 μmol/kg. In the case of "accuracy" in δ^{13}C-DIC, it was evaluated in an international inter-comparison exercise of δ^{13}C-DIC in seawater (Cheng et al., 2018 submitted). In this study, 16 participating laboratories measured CRM (batch 157), which is not certified but shows excellent bottle to bottle reproducibility for its δ^{13}C-DIC value (A.G. Dickson, personal communication, 9 August 2016; Humphreys et al., 2016), as well as samples derived from a stable water mass in north eastern Atlantic deep water (DSW) with identical δ^{13}C-DIC value. Various IRMS or Isotope Ratio Infrared Spectrometer (IRIS) based methods were used for δ^{13}C-DIC determination, and the results from this system agree to the average δ^{13}C-DIC results of CRM and DSW from all groups within 0.05‰ and 0.08‰ respectively.

2.4.3 Concentration Effect for δ^{13}C-DIC

A "concentration effect" occurs when the measured δ^{13}C-DIC value of a water sample is a function of its [DIC]. A small concentration effect was reported in the study of Bass et al., 2014 and Call et al., 2017. We tested for this concentration effect by measuring 8 sets of a $NaHCO_3$ standard solution with concentrations ranging from 1800 to 2500μmol/kg. These standard solutions were made by dissolving $NaHCO_3$ powder into deionized water that had been pre-purged with helium, thus precluding the possibility of air-derived CO_2 affecting the measurements.

Results show that with a sample injection volume of 3.8mL, in which the [DIC] ranges from 1800 to 2500 µmol/kg, corresponding to [CO_2] integration areas of 115000 to 159000 ppm*sec, there is no significant concentration effect in measured δ^{13}C-DIC. The standard deviation of the 8 δ^{13}C-DIC results is 0.07‰ and the correlation coefficient between [DIC] and δ^{13}C-DIC is $R^2=0.013$ (Fig. 2.3).

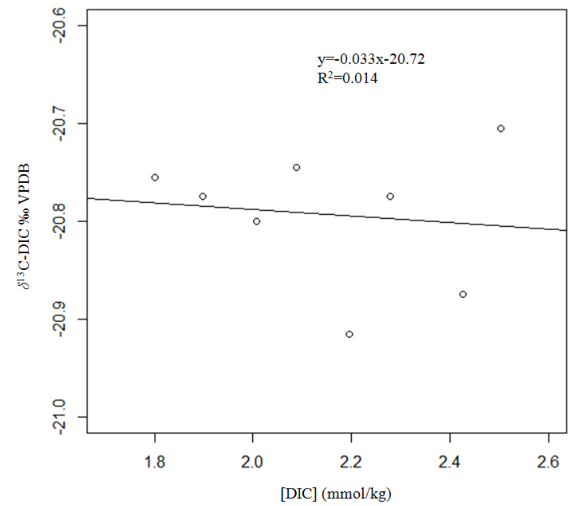

Fig. 2.3. Concentration effect of measured δ^{13}C-DIC. With the same sample injection volume, no significant concentration effect is observed by measuring $NaHCO_3$ solutions with various [DIC].

2.4.4 δ^{13}C-DIC Data Acquisition and Averaging

Use of CO_2-free air as the carrier/stripping gas implies that some of the CO_2 extracted from a water sample, and therefore some of the sample's $\delta^{13}C$-CO_2 signal, is measured when CO_2 mixing ratios lie below the CRDS-detector's specification range for reliable measurement (380 ppm to 2000 ppm; see Fig. 2.2). The [CO_2] and $\delta^{13}C$-CO_2 values collected during measurement of a typical water sample were used to test whether exclusion of $\delta^{13}C$-CO_2 values for mixing ratios <380 ppm affected the calculation of the sample's δ^{13}C-DIC. Specifically, weighted averages of $\delta^{13}C$-CO_2 by [CO_2] were calculated from the same integration curve for the single typical sample using 9 different cut off CO_2 concentrations (15, 20, 30, 100, 200, 300, 380, 400, 500 ppm). The largest discrepancy between calculations was 0.07‰, which is less than the manufacturer's reported precision of the Picarro G2201*i* CRDS for δ^{13}C-CO_2 (0.12‰). Hence, exclusion of δ^{13}C-CO_2 values below 380ppm, at the beginning and end of the analysis, does not affect the calculation of δ^{13}C-DIC significantly.

2.4.5 Risk of Isotope Fractionation Due to Flow-splitting

The split installed between the Apollo SciTech acidification interface and the Picarro CRDS was tested to ensure that separation of carrier/stripping gas flow to the detector did not result in fractionation of carbon isotopes. Both a syringe and a T-shaped-tube were tested as a split. In order to test whether there is any isotope fractionation linked to these connections and flow-splitting (T shape tube or syringes with different headspace volume), we connected a CO_2 reference gas cylinder (PRAXAIR 655ppm CO_2 gas with uncertified δ^{13}C-CO_2 value) to the Apollo DIC analyzer and used it as "carrier/stripping gas". And then, while changing the connecter's headspace volume (0, 2, 4, 6, 8, 10 mL), we observed the δ^{13}C-CO_2 value of the "baseline" (~650ppm CO_2 gas) and the results showed that the δ^{13}C-CO_2 values of the "baseline" did not change significantly with changes of the connector's headspace volume (maximum discrepancy is 0.13‰). These results show that there is no isotope fractionation within the connector's headspace.

2.4.6 Effect of Carrier/Stripping Gas Flow Rate

As changes of carrier gas flow rate at the Apollo acidification interface may lead to different amounts of extracted CO_2 gas being drawn into the Picarro CRDS for δ^{13}C-CO_2 measurements, we tested whether variations of the carrier gas flow rate cause deviations in δ^{13}C-DIC determinations. Specifically, 3 "in-house" δ^{13}C-DIC standards together with a seawater sample taken from North Atlantic was measured under different flow rates (70, 80, 90, 100mL/min) set at Apollo SciTech acidification interface. Largest discrepancies of the 3 "in-house" standards δ^{13}C-DIC results under different flow rate settings is 0.16‰. Further, the δ^{13}C-DIC results of the seawater sample, which was calibrated by "in-house" standards show better stability under different flow rates (maximum discrepancy of 0.09‰). Given that the precision of Picarro CRDS in δ^{13}C-CO_2 measurements is 0.12‰, we could conclude that the system is stable for δ^{13}C-DIC measurements under different main flow rates ranging from 70 to 100 mL/min.

2.4.7 At-sea Measurement Scheme and Quality Control (QC)

As stated above, different sample injection volumes and measurement schemes for [DIC] can be applied using this system. During the research cruise, the measurement scheme was set up as the follows: each sample was injected into the Apollo SciTech DIC analyzer for a maximum of 6 times (3.8 mL per injection). If results from two injections met the requirement of standard deviation/average of [DIC]≤0.12%, the measurement would stop automatically (i.e. no more injections if two valid values appeared).This setting was a compromise between measurement precision and frequency. Generally, the measurements stopped at the third injection, so that it took approximately 30 minutes for one sample to be measured. The final [DIC] and δ^{13}C-DIC results were calculated by averaging the two valid values, and in most cases, standard deviation of 2 valid δ^{13}C-DIC values was less than the reported precision of Picarro G2201*i* CRDS in δ^{13}C-CO_2 measurements (0.12‰).

Four sets of DIC and δ^{13}C-DIC standards were measured throughout the cruise to calibrate the water sample [DIC] and δ^{13}C-DIC data. Also, one bottle of CRM (batch 157) was measured every day for QC of [DIC] and δ^{13}C-DIC data as well as for final correction of [DIC]. During 35 days of measurement on board and in lab of the CRM (batch 157), most of our [DIC] results showed good consistency (±10 µmol/kg) to the certified [DIC] of 2049 µmol/kg (Fig. 2.4), which assures that the [DIC] results are of good quality after final correction using the CRM (batch 157). Results from a few days showed a larger deviation in comparison to the Certified DIC concentration of CRM (batch 157) (up to ±20 µmol/kg). This was caused by inappropriate preparation of the "in-house" DIC standard solutions. In the case of δ^{13}C-DIC data quality control, the average and standard deviation from the 35 days of measurements were 0.80‰ and 0.07‰ respectively, which shows that the system was very stable as well as accurate both onboard and in the lab for δ^{13}C-DIC measurements, because the 35 days average of CRM

δ^{13}C-DIC results is highly consistent with the between lab average of CRM δ^{13}C-DIC results (0.77‰) of the international inter-comparison exercise described above. Further, another CRM (batch 163) was measured onboard at a random time as a "sample", and the measured [DIC] agreed with certified value of 2039 µmol/kg within 2µmol/kg, which again proved the accuracy in [DIC] of the system during onboard deployment.

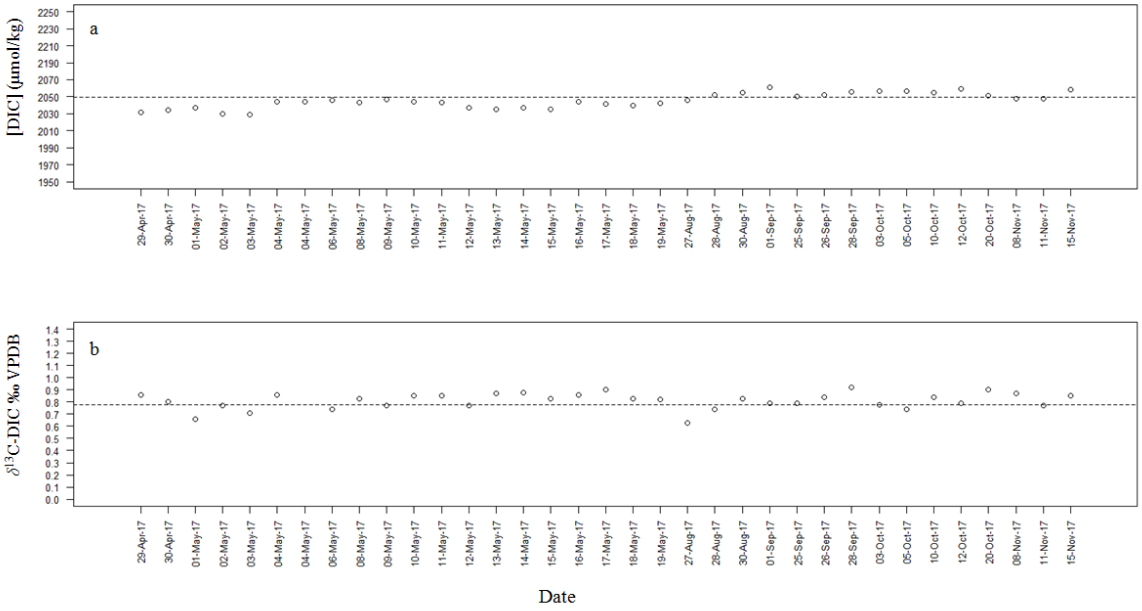

Fig. 2.4. a) Quality control of [DIC] data by on-board and in-lab measurements of the CRM (batch 157) for 35 days. Dotted line shows the Certified [DIC] of CRM (batch 157). b) Quality control of δ^{13}C-DIC data by on-board and in-lab measurements of the CRM (batch 157) for 35 days. Dotted line shows averaged δ^{13}C-DIC results of CRM (batch 157) from 16 participating groups in the study of Cheng et al., 2018 submitted.

2.4.8 In Lab vs Onboard Measurements

During the 2017 A02 Go-Ship cruise, replicate samples were taken at Station 22 and Station 33 at 13 and 14 depths respectively, using borosilicate media bottles with polypropylene caps and stored for further analysis in the lab after the cruise. The average offsets between onboard and in-lab measurements of samples taken from the same Niskin bottle at two stations in δ^{13}C-DIC are 0.09‰ and 0.12‰ respectively and the average offsets between onboard and in-lab measurements of replicate samples in [DIC] are 17 µmol/kg and 5 µmol/kg respectively. For δ^{13}C-DIC measurements, our system show good reproducibility between onboard and in lab analysis. In the case of [DIC], the large offsets of results from Station 22 is because the onboard measurement results were calibrated by inappropriate "in-house" DIC standard solutions. As for the results of Station 33, the average offset is less than the 2-σ precision of our system. As the replicate samples were measured approximately 3 months after the cruise, good reproducibility in both [DIC] and δ^{13}C-DIC suggest that the system is stable for both in lab or research vessel onboard deployments.

2.5 Discussion

The results of precision tests, accuracy tests and comparison experiments show that our system is suitable for simultaneous seawater [DIC] and δ^{13}C-DICdetermination in lab or onboard research vessels. For water samples derived from other environments such as rivers, lakes or some coastal regions, where [DIC] varies in a larger signal, high quality measurements can be achieved by adjusting sample injection volume appropriately to fulfill the guaranteed specification range of Picarro CRDS in δ^{13}C-CO_2 determination.

2.5.1 Comparison with Historical Data from the WOCE A02 Hydrographic Section

Some stations of the Go Ship A02 hydrographic section and nearby stations of other cruises have been sampled and analyzed for [DIC] and δ^{13}C-DIC in 1994 (Koltermann & Meincke 1994), 1997 (Schott et al., 1997), 2001 (Zenk et al., 2002) and 2003 (Rhein 2003). This allows us to compare our results with past surveys, including with historic [DIC] and δ^{13}C-DIC data derived from same locations. Especially, Cheng et al., 2018 (submitted) claimed that the north-eastern Atlantic Ocean deep water mass has likely been unaffected significantly by anthropogenic carbon and is stable in terms of its [DIC] and δ^{13}C-DIC for at least hundreds of years. This allows us to assess the accuracy of the system by comparing our [DIC] and δ^{13}C-DIC results of derived from the 2017 Go-Ship A02 cruise with historic values.

Results of 6 deepwater samples (≥4000 m) collected from three nearby stations (Station 55, 57, 59) during 2017 Go-Ship A02 Cruise are used for comparison with historic [DIC] and δ^{13}C-DIC results of samples with identical salinity, temperature and dissolved oxygen concentration derived from nearby stations of different cruises (Table 2.2). For [DIC], the average of our 6 results (2200 µmol/kg) show excellent consistency with the average of 9 [DIC] results (2200 µmol/kg) from 2001 Go-Ship A02 Cruise, the average of 5 [DIC] results (2202 µmol/kg) from 1997 Go-Ship A02 Cruise, and the average of 5 [DIC] results (2200 µmol/kg) from 1994 Go-Ship A02 Cruise. Also, the maximum discrepancy between individual samples of our [DIC] results and historic data from 2001, 1997 and 1994 are 7 µmol/kg, 7 µmol/kg and 5 µmol/kg respectively. In the case of δ^{13}C-DIC, unlike the [DIC] results which were standardized with CRM, historic δ^{13}C-DIC results were adjusted by secondary quality control procedure (2nd QC) based on crossover analysis (Tanhua et al., 2010; Lauvset& Tanhua 2015; Becker et al., 2016) in order to minimize the between lab discrepancy. The average of our 6 δ^{13}C-DIC results (0.94‰) is highly consistent with the average of 3 δ^{13}C-DIC results (0.94‰) from the 2003 RV METEOR M-59/2 cruise and the average of 5 δ^{13}C-DIC results (0.92‰) from the 1994 Go-Ship A02 Cruise. Also, the maximum between individual sample discrepancy of our δ^{13}C-DIC data and historic results from 2003 and 1994 are 0.12‰ and 0.06‰ respectively.

2.5.2 Comparison with Other CRDS Methods

A number of applications of CRDS and related measurement approaches to δ^{13}C analysis of aquatic samples have been reported in recent years. These have arisen from improvements in optical technologies which allow for smaller, robust, and highly automated stable isotope analysis. using optical spectroscopy techniques such as Cavity Ring-Down Spectroscopy (CRDS) system (Law 2000).

CRDS has been applied to shipboard measurements of partial pressure of dissolved CO_2 and its carbon stable isotope composition through coupling of the detector to an air-water equilibrator systems (e.g. Friedrichs et al., 2010; Becker et al., 2012). In addition, Bass et al., 2014a were amongst the first to couple a water acidification interface with a CRDS detector, allowing for measurement of [DIC] and δ^{13}C-DIC and were likely the first to use such a system on board a research vessel. Their system was used to make high frequency, simultaneous [DIC] and δ^{13}C-DIC determinations on surface waters during a cruise from New Zealand to Antarctica. Precision of their system for [DIC] and δ^{13}C-DIC measurements was ±10 μmol/kg and ±0.1‰ respectively.

This method is suitable for observation of large [DIC] variation signals in various aquatic environments such as mesocosm coral reef system (Bass et al., 2012), floodplains and wetlands (Bass et al., 2013), and tropical catchments (Bass et al., 2014b). However, as stated by GOOS, an accuracy/uncertainty of ±2 μmol/kg in shipboard [DIC] measurements has been achieved, and is generally required for quantifying many processes related to oceanic carbon cycling studies such as long term oceanic anthropogenic CO_2 uptake or daily variation of productivity and respiration in open ocean sea water etc. (e.g. Johnson et al., 1987; Johnson et al., 1998).

Most recently, Call et al., 2017 coupled a non-dispersive infrared detector (NDIR) and a CRDS for high frequency [DIC] and δ^{13}C-DIC measurements, and evaluated the performance of this system through a simulated coastal water algal bloom experiment in laboratory (Call et al., 2017). This method showed relatively high precision in both [DIC] and δ^{13}C-DIC measurements (±2 μmol/kg and ±0.14‰ respectively), and achieved a high sample resolution of 16 minutes per sample. However, it required expensive δ^{13}C-CO_2 reference gas as carrier gas and has not been tested onboard for its performance.

Both Bass et al, 2014 and Call et al., 2017 have reported a small but detectable concentration effect (<0.2‰) on measured δ^{13}C-DIC with increasing [DIC] with a variation range of 1000~3600 μmol/kg and 1000~2100 μmol/kg respectively. However, this concentration effect is not observed in this study. This may because the [DIC] variation range (1800 to 2500μmol/kg) we were testing is relatively small so that the concentration effect is undetectable, or use of Helium-purged MilliQ water for standard solution preparation precludes airborne CO_2 contamination during the concentration effect test, thus, eliminating the concentration effect observed by the earlier studies.

Results from δ^{13}C-DIC data acquisition test and flow rate test indicate that there is no isotope fractionation in terms of ^{12}C and ^{13}C during the water sample acidification and the CO_2 extraction stage, or at the gas flow-splitting between the acidification interface and the CRDS. Therefore, ignoring parts of the extracted CO_2 does not affect the δ^{13}C-DIC measurement result. This suggests that it is not necessary to use expensive δ^{13}C-CO_2 reference gas as carrier gas in order to satisfy Picarro's guaranteed specification range for [CO_2] and δ^{13}C-CO_2 determination. Further, a precision of ±2 μmol/kg is generally required, and has been achieved by culometric titration system or NDIR system in [DIC] analysis. Therefore there is room for further improvement in [DIC] determination by our system. As our system is the first unit commercially provided by the company, Apollo SciTech LLC has suggested that optimizing the gas flow rate in the acidification interface may be a feasible option for improvements in precision of [DIC] measurements. Our test results have proven that adjustments in flow rate does not affect the quality of δ^{13}C-DIC measurements. Also, improving flushing efficiency to avoid contamination by the previous analysis is another option. Further, Apollo SciTech LLC has also automated their newer units by using a multi-port sampler (http://www.apolloscitech.com/).

2.6 Conclusions

Our new simultaneous [DIC] and δ^{13}C-DIC measurement system reached precision and accuracy of ±3 μmol/kg in [DIC] determination, and further improvements can likely be achieved by adjusting the configuration of the Apollo SciTech acidification interface. As for δ^{13}C-DIC determination, the typical precision for seawater δ^{13}C-DIC ranges from ±0.03 to ±0.23‰. Our system reaches a precision of ±0.12‰, which is comparable to that of traditional IRMS based methods. Currently, certified reference material for δ^{13}C-DIC in liquid phase is not available, thus, it is impossible to evaluate fully the accuracy of our system in δ^{13}C-DIC determination. However, δ^{13}C-DIC results derived from our system show high consistency with δ^{13}C-DIC results of IRMS based methods under 2nd QC. Furthermore, our system has proved to be in good performance during its deployment in North Atlantic Ocean. Compared with the traditional approaches for [DIC] and δ^{13}C-DIC measurements, in which separate water samples are collected onboard and then shipped back to lab for analysis using different instruments (Titration systems and IRMS respectively), shipboard high frequency simultaneous determination of [DIC] and δ^{13}C-DIC is more efficient in time and labor. More importantly, onboard measurements allows adjustments of sampling scheme during a cruise base on real time data, and reduces risk of losing samples after a cruise.

Table 2.2. Comparison of [DIC] and δ^{13}C-DIC results with historic results of the same water mass.

Year	Latitude (ºN)	Longitude (ºE)	Pressure (dbar)	Salinity	Temp (ºC)	O$_2$ (µmol/kg)	[DIC] (µmol/kg)	δ^{13}C-DIC (‰VPDB)
2017	48.352	-17.416	4032	34.907	2.506	238.115	2200	0.96
2017	48.352	-17.416	4000	34.907	2.505	237.832	2197	0.91
2017	48.567	-15.944	4525	34.902	2.516	239.795	2203	0.93
2017	48.567	-15.944	4501	34.903	2.516	239.849	2204	0.91
2017	48.781	-14.467	4621	34.902	2.525	238.870	2201	0.95
2017	48.781	-14.467	4500	34.903	2.519	239.393	2197	0.97
1994	48.643	-14.355	4006	34.909	2.516	244.400	2199	0.93
1994	48.643	-14.355	4307	34.904	2.506	244.300	2200	0.92
1994	48.643	-14.355	4558	34.902	2.514	244.700	2199	0.92
1994	48.348	-17.345	4108	34.908	2.515	243.300	2199	0.93
1994	48.348	-17.345	4157	34.908	2.513	244.700	2201	0.91
1997	48.740	-14.733	4211	34.904	2.509	241.600	2201	−
1997	48.740	-14.733	4511	34.903	2.525	241.800	2201	−
1997	48.740	-14.733	4772	34.901	2.535	241.800	2203	−
1997	48.303	-17.688	4030	34.908	2.521	240.800	2202	−
1997	48.303	-17.688	4054	34.908	2.524	240.800	2202	−
2001	48.167	-18.115	4273	34.908	2.549	242.500	2201	−
2001	48.417	-16.397	4068	34.912	2.557	241.700	2197	−
2001	48.417	-16.397	4375	34.907	2.544	242.000	2199	−
2001	48.417	-16.397	4677	34.904	2.550	240.300	2201	−
2001	48.417	-16.397	4997	34.903	2.575	240.900	2202	−
2001	48.550	-15.450	4375	34.906	2.535	241.400	2199	−
2001	48.550	-15.450	4993	34.902	2.571	241.400	2208	−
2001	48.783	-13.785	4068	34.910	2.539	242.000	2197	−
2001	48.783	-13.785	4687	34.901	2.528	240.900	2202	−
2003	48.600	-15.550	4922	−	2.565	241.900	−	1.00
2003	48.600	-15.550	4789	−	2.556	242.700	−	1.03
2003	48.600	-15.550	4379	−	2.537	243.100	−	0.98

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Chapter 3

An International Inter-comparison of Stable Carbon Isotope Composition Measurements of Dissolved Inorganic Carbon in Seawater

This chapter is an article that has been submitted to "*Limnology and Oceanography: Methods*" for publication.

3.1 Abstract

We report results of an inter-comparison of stable carbon isotope ratio measurements in seawater dissolved inorganic carbon (δ^{13}C-DIC) which involved 16 participating laboratories from all over the world. The inter-comparison involved distribution of samples of a Certified Reference Material for seawater DIC concentration and alkalinity and a preserved sample of deep seawater collected at 4000 m in the north-eastern Atlantic Ocean. The between-lab standard deviation of 0.11‰ (1σ) was better than expected despite the participants' use of diverse analytical, detection and calibration methods. The multi-lab average δ^{13}C-DIC value reported for the deep seawater sample was also consistent within 0.07‰ with historical measured values for the same water mass that had been corrected using a secondary quality control procedure. A correction procedure based on a consensus value for the reference material demonstrated that provision of an aqueous phase reference material for δ^{13}C-DIC has the potential to improve between-lab data consistency to 0.05‰, which is the desired accuracy proposed by the Global Ocean Observing System. Our results suggest that the existing Certified Reference Materials used for seawater DIC and alkalinity quality control may be suitable for this purpose, if "Certified" or internally-consistent "Consensus" values for δ^{13}C-DIC can be assigned to various batches. In the meantime, we suggest that these inter-comparison experiments should be extended and repeated periodically.

3.2 Introduction

The concentration and stable carbon isotope composition of dissolved inorganic carbon in ocean waters, referred to henceforth as DIC and δ^{13}C-DIC respectively, is influenced by several important physical and biogeochemical processes including biological uptake and release of inorganic carbon, mixing of water masses and air-sea CO_2 exchange. This makes δ^{13}C-DIC a useful tracer whose geographic and temporal distribution contains information about ocean carbon cycle processes as well as exchanges of oceanic carbon with other carbon reservoirs, such as the atmosphere, sediments, or the terrestrial biosphere (Assayag et al., 2006; Huang et al., 2013).

Over the past 200 years, the oceanic uptake of fossil-fuel-derived CO_2, with depleted values of δ^{13}C, has caused time-dependent depletion of seawater δ^{13}C-DIC. This "^{13}C Suess-effect" signal (Keeling 1979) is a particularly useful tracer for estimation of the anthropogenic carbon (C_{ant}) accumulation rate in ocean waters (e.g. Körtzinger et al., 2003; Olsen et al., 2006; Quay et al., 2007). Because of its utility as a tracer, δ^{13}C-DIC is listed as an Essential Ocean Variable (EOV) by the Global Ocean Observation System (GOOS). Use of the tracer, however, depends on the ability to compare measurements made in different geographical locations at different times by multiple measurement groups. This requires measurements to be very accurate as well as precise, with accuracy being particularly important for resolving small temporal changes associated with the "^{13}C Suess effect". In general, ocean water δ^{13}C-DIC values ranges from –6.56‰ to 3.10‰ (Schmittner et al., 2013), and in order to detect changes in δ^{13}C-DIC values associated with C_{ant} uptake (ca. –0.02‰ year^{-1} in global ocean surface water; Gruber et al., 1999), an accuracy of ±0.05‰ in δ^{13}C-DIC measurements is called for in the GOOS EOV specification.

Unlike the measurement of DIC, for which most measurement groups follow standard operating procedures (SOPs) (Dickson et al., 2007), there is currently no recommendation for an SOP for δ^{13}C-DIC analyses. There are no agreed-upon standardization procedures, or liquid or soluble Certified Reference Materials that can be distributed amongst measurement groups. Further, a variety of analytical methods are in-use, including detection by mass spectrometry and, in recent years, laser-based optical spectroscopy. The result is that the accuracy and inter-comparability of measurements made by different groups worldwide, or by the same group over time, is not well known.

Becker et al. (2016) compiled and examined historical data for δ^{13}C-DIC collected from the North Atlantic Ocean over the years 1981 to 2014 and used "crossover analysis" (Tanhua et al., 2010) of measurements reported from nearby locations at different times to assess offsets between data sets. Offsets between individual data sets ranged from –0.39‰ to 0.17‰, which provides a rough estimate of the inter-comparability of historical data sets.

More direct assessment of measurement inter-comparability can be derived from inter-comparison exercises in which identical, replicate samples are sent to multiple labs for analysis (a so-called "ring test" or "round robin test"). Inter-comparisons of this type have been conducted for oxygen and hydrogen stable isotope compositions of water (δ^2H-H_2O and δ^{18}O-H_2O; e.g. Walker et al., 2015; Wassenaar et al., 2018), seawater nutrient concentrations (Aoyama et al., 2016), and seawater DIC and alkalinity concentrations (Bockmon and Dickson. 2015; Verma et al., 2015). However, to the authors' knowledge, there has been only one published inter-laboratory comparison study of δ^{13}C-DIC measurements on natural waters (van Geldern et al., 2013). In their study, five groups measured a wide variety of natural water samples using IRMS, including replicate samples of seawater. For the latter, results from four groups agreed to within 0.16‰ with one group's result showing a larger discrepancy (1.1‰) from the group mean.

As with other stable isotopic systems (e.g. ^{18}O/^{16}O in water; Walker et al., 2015), the introduction of methods based on optical spectroscopy is likely to lead to a rapid increase in the number of groups measuring δ^{13}C-DIC on seawater samples. Given this, and the status of δ^{13}C-DIC as an EOV, there is an urgent need to evaluate the consistency and traceability of current measurements as a basis for recommendations concerning future data quality control and assurance procedures. In this study, we present results of a worldwide seawater δ^{13}C-DIC inter-comparison exercise involving 16 participating laboratories. The results are used to assess the likely inter-comparability of historical and current data and are used as the basis for proposing steps which would lead to future improvements in seawater δ^{13}C-DIC data quality.

3.3 Materials and Methods

3.3.1 Test Waters and Their Suitability

Two supplies of seawater were used for the inter-comparison study: (1) "Certified Reference Material for oceanic CO_2 measurements (Batch 157)" supplied by the University of California, San Diego, Scripps Institution of Oceanography; (2) samples of deep ocean seawater (DSW) collected in May 2017 from the north-eastern basin of the Atlantic Ocean at depths of >4000 m during the 2017 Go-Ship A02 trans-Atlantic cruise (McGovern et al., 2017; GO-SHIP; http://www.go-ship.org/). One sample of "Certified Reference Material for oceanic CO_2 measurements (Batch 157)" and four samples of DSW (two for some groups) were distributed to 16 laboratories in the USA, Canada, Germany, France, Norway, Australia, Japan and Russia for δ^{13}C-DIC analysis.

The Certified Reference Material is certified, prepared and distributed for the quality control and assessment of accuracy for seawater DIC and alkalinity measurements (Dickson et al., 2003; Humphreys et al., 2016). However it has not been certified for its δ^{13}C-DIC value, so we will refer to it from now on as "RM". The preparation and storage procedures of the RM have been tested extensively for DIC and alkalinity (Dickson et al., 2003) so that it is can be expected that RM's δ^{13}C-DIC value should also show high bottle to bottle reproducibility within each batch (A.G. Dickson, personal communication, 9 August 2016; Humphreys et al., 2016). On this basis, we regard RM from the same batch contained in different bottles as identical, replicate samples suitable for use in a ring test.

The DSW samples were collected and stored in accordance with protocols recommended by Dickson et al. (2007). After rinsing pre-cleaned, 160 mL borosilicate serum bottles 3 times, water samples were introduced into the bottles from the bottom through Tygon tubing, allowing for overflow of the samples prior to closure. Special care was taken to avoid introduction of airborne CO_2 during the filling procedure. The bottles were capped immediately with flat butyl septa with PTFE coating, crimped with aluminum seals, and

1.6 mL of the water sample was removed using a syringe and replaced with CO_2 free air, which had been passed through a sodium hydroxide trap. Finally, 0.1 mL of saturated mercuric chloride solution was injected into each bottle for preservation of the samples, which were stored in the dark at room temperature (20ºC~23ºC) prior to distribution.

In total, 52 DSW samples were collected from 3 x 10-liter Niskin bottles at two nearby stations. Information about these DSW samples is presented in Table 3.1. Saunders (1986) had previously noted "remarkable uniformity" of the temperature-salinity relationship in waters below ca. 3000 m in the north-eastern Atlantic Ocean where "the Mid-Atlantic Ridge, European and African continental rise, Sierra Leone rise and the Rockall Plateau enclose the deep water in the sampling region, permitting significant exchange only south of 15°N". Saunders also noted high uniformity of dissolved oxygen concentrations and used this as the basis for assessment of the accuracy of historical salinity and oxygen measurements. For our purposes it is sufficient that the 52 samples collected from 3 separate Niskin bottles are representatives of one homogeneous water sample. This is supported strongly by the identical values of salinity, temperature, and dissolved oxygen concentrations corresponding to the three Niskin bottles (Table 3.1). This is also supported by *t*-test results of all the δ^{13}C-DIC measurement results derived from different participating groups, which are discussed below.

Table 3.1. Information of DSW samples.

Sample ID	Latitude (°N)	Longitude (°E)	Depth (m)	Niskin bottle number	Temp (℃)	Salinity	O_2 (µmol/kg)
101107-a	48.674	–15.208	4700	2	2.545	34.903	238.4
101107-b	48.674	–15.208	4700	2	2.545	34.903	238.4
101155-a	48.890	–13.723	4300	2	2.500	34.903	239.5
101155-b	48.890	–13.723	4300	2	2.500	34.903	239.5
101156-a	48.890	–13.723	4301	3	2.500	34.903	238.1
101156-b	48.890	–13.723	4301	3	2.500	34.903	238.1

The deepwater below 4000 m contained near-zero concentrations (0.03 pmol/kg) of the anthropogenic compound CFC-12 (CCl_2F_2) (T. Tanhua, personal communication, 31 March 2018) and earlier measurements from further south (ca. ~38 N; Tanhua et al.,

2007) also showed near-zero concentrations of CCl_4. The latter is an anthropogenic compound that was introduced into the environment around 1910. Taken together, these findings imply that this deep water has not been impacted significantly by C_{ant}, has high spatial and temporal uniformity and has likely been stable in terms of its δ^{13}C-DIC composition for at least hundreds of years. This not only allows us to use the DSW samples for the ring-test, but also allows us to compare the δ^{13}C-DIC measurement results from this inter-comparison exercise with historical (and future) δ^{13}C-DIC data from the same region and water mass.

3.3.2 Participating Laboratories and Methods

In most oceanographic and hydro-geological studies, δ^{13}C-DIC of water samples is measured by Isotope Ratio Mass Spectrometry (IRMS) coupled with various front-end peripherals (e.g. Salata et al., 2000; Torres et al., 2005; Assayag et al., 2006; Waldron et al., 2014). In recent years, laser-based optical spectroscopy such as Isotope Ratio Infrared Spectrometer (IRIS) and Cavity Ring-Down Spectroscopy (CRDS) have also been used as a detection method for δ^{13}C-DIC analysis (e.g. Bass et al., 2012; Call et al., 2017). A brief summary of the methods used by the participating groups is presented in the Appendix 1. All the methods for δ^{13}C-DIC measurements applied by different groups are based on the traditional CO_2 conversion technique in which DIC in seawater is converted to CO_2 by adding H_3PO_4, followed by the extracted and equilibrated gaseous CO_2 being introduced into detectors for subsequent δ^{13}C-CO_2 analysis. In this study,14 groups used IRMS systems for δ^{13}C-CO_2 analysis, one group measured δ^{13}C-CO_2 using CRDS, and one group used both IRMS and IRIS for δ^{13}C-CO_2 determination.

Appendix 1 shows that a wide variety of internal reference materials such as $NaHCO_3$, Na_2CO_3 etc. as well as international calibration materials in both solid and gas phase were used by the different groups to standardize their results to the Vienna Pee Dee Belemnite (VPDB) scale and also for internal data Quality Control (QC). The reported measurement precisions of participating laboratories ranged from 0.03‰ to 0.40‰ (1σ). Due to long distance sample transportation and unexpected analysis system breakdowns encountered by some groups, sample analysis time by participating laboratories varied from 4 months to 10 months after the DSW were collected and preserved on board R.V. Celtic Explorer.

3.4 Results and Assessment

The δ^{13}C-DIC results reported by the participating laboratories are shown in Table 3.2. All the δ^{13}C-DIC results reported by participating groups are in the standard delta notation in per mil (‰) versus VPDB (Craig 1957) and have been rounded to 2 decimal places. Lab 11 reported their results to one decimal place. In addition, Lab 1 reported unexpected difficulties during analysis, thus the δ^{13}C-DIC results of RM and DSW derived from Lab 1 are likely not representative of their normal operations. It should be noted that Lab 12 also reported potential errors during their analyses. A plot of the raw δ^{13}C-DIC results from participating laboratories is shown in Fig. 3.1.

Table 3.2. Raw δ^{13}C-DIC results reported by participating laboratories. All δ^{13}C-DIC results are reported in ‰VPDB.

Lab #	**δ^{13}C-DIC RM**	**δ^{13}C-DIC 101107-a**	**δ^{13}C-DIC 101107-b**	**δ^{13}C-DIC 101155-a**	**δ^{13}C-DIC 101155-b**	**δ^{13}C-DIC 101156-a**	**δ^{13}C-DIC 101156-b**
1	0.68	1.22	0.61	0.67	0.82		
2	0.85	0.99	0.99	0.95	0.96		
3	0.83	0.94	0.92	4.55	2.29		
4	0.87	0.98	0.97	0.95	0.94		
5	0.74	0.89	0.88	0.86	0.86		
6	0.75					0.89	0.88
7	0.70	0.91	0.78	0.81	0.80		
8	0.61	0.74	0.75	0.73	0.72		
9	0.82	0.95	0.90	0.82	0.87		
10	0.90					1.09	1.03
11						0.70	0.80
12	0.90					1.01	1.00
13	0.65					0.68	0.78
14	0.58	0.74	0.79	0.80	0.75		
15	0.82					0.94	0.88
16-a	0.88	0.96	0.90	0.96	0.94		
16-b	0.85	0.92	0.93	0.86	0.86		

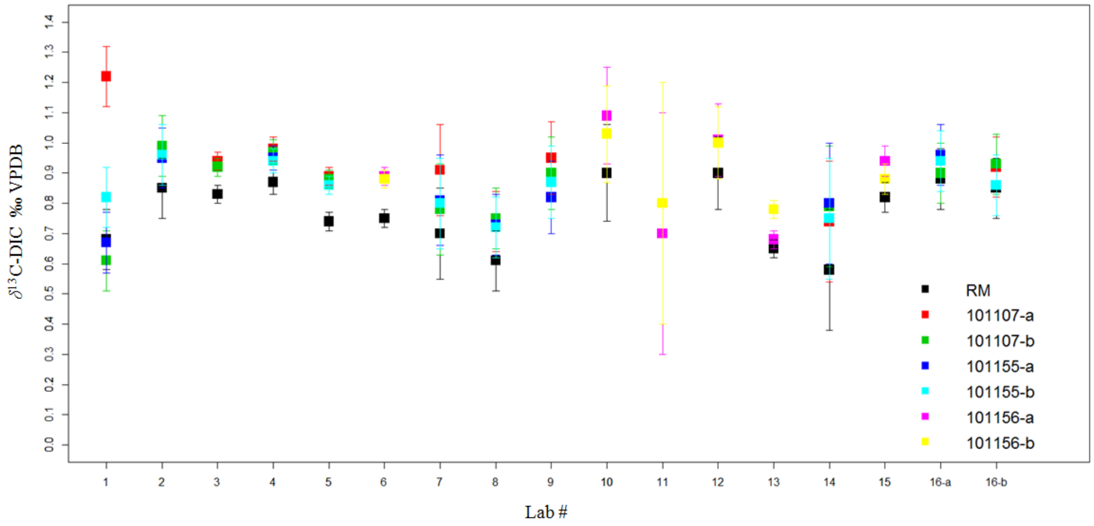

Fig. 3.1. Reported δ^{13}C-DIC results of reference material (RM) and deep sea water samples (DSW) by participating laboratories. δ^{13}C-DIC results of 101156-a and 101156-b from Lab 3 are not shown in the figure (see text).

Many statistical analyses are based on the assumption that data are normally distributed (Ghasemi and Zahediasl 2012). The normality of these two data sets is visualized by quantile-quantile (Q-Q) plot shown on Fig. 3.2, which indicates that the δ^{13}C-DIC results of RM are normally distributed, whereas those of DSW are not. Use of the Shapiro-Wilk tests (Shapiro and Wilk 1965) with the δ^{13}C-DIC results derived from all the groups gave the same result. (W = 0.90889, p-value = 0.1121, n=16 for RM δ^{13}C-DIC results; W = 0.34614, p-value = 2.218 x 10^{-14}, n=56 for DSW δ^{13}C-DIC results).

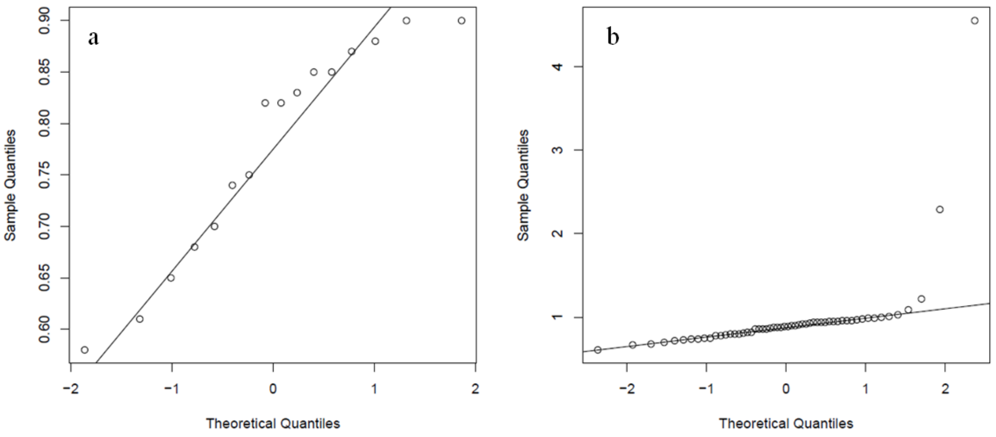

Fig. 3.2. Normal quantile-quantile (Q-Q) plots of RM (a) and DSW (b) δ^{13}C-DIC results.

We subsequently examined the data set further in order to identify outliers. The first quartile (Q_1), third quartile (Q_3) and the inter-quartile range (IQR), defined as the difference between first and third quartiles, were calculated for RM and DSW δ^{13}C-DIC results, respectively. Traditionally, data points outside the interval of [Q_1-1.5IQR, Q_3+1.5IQR], named as the "fence", are treated as outliers (Rousseeuw and Hubert 2011). The fences of δ^{13}C-DIC results of RM and DSW are [0.46,1.55] and [0.58,1.18] respectively, therefore, no outlier was identified in the RM analyses. A single δ^{13}C-DIC value for DSW sample 101107-a determined by Lab 1, was identified as an outlier. As noted above, Lab 1 reported difficulties during their analyses. The δ^{13}C-DIC results for DSW samples 101155-a and 101155-b determined by Lab 3 were also identified as outliers. These two results deviated considerably from the other groups' results by 0.61‰ to 1.10‰, as well as from the historical deep water (>3000 m) δ^{13}C-DIC results from the North Atlantic Ocean, which range between 0.18‰ and 1.30‰ (Becker et al., 2016). As the DSW samples 101155-a and 101155-b were collected from the same Niskin bottle and no damage or leakage of the samples was reported, the cause for the deviations is likely to be misconduct in sample fixation, as the large δ^{13}C-DIC values imply decomposition of dissolved organic matter in samples, which were observed in seawater samples without $HgCl_2$ solution fixation. After eliminating the three outliers from the data set, we conducted another Shapiro-Wilk test with the δ^{13}C-DIC results of DSW, and the result (W = 0.97588, p-value = 0.3564, n=53) shows that the remaining δ^{13}C-DIC data for DSW are also normally distributed.

In order to test whether all DSW samples can be considered representative of the same water, three t-tests were conducted between the δ^{13}C-DIC results of DSW samples taken from the three Niskin bottles. P values of 0.29, 0.79, and 0.32 for 99% confidence interval indicate that there is no significant difference in the means of δ^{13}C-DIC results of DSW samples taken from the three Niskin bottles. Subsequently, all δ^{13}C-DIC results of DSW taken from the 3 Niskin bottles will be considered together.

We used Z-scores (e.g. Wassenaar et al., 2012; Aoyama et al., 2016) to measure how well sample analyses agreed with each other. The Z-score is defined as $Z = \left|\frac{M-A}{\mu}\right|$, where M is the δ^{13}C-DIC result for a particular sample as reported by a participating laboratory

($\delta^{13}\mathrm{C}_{\mathrm{meas}}$), A is the average of all δ^{13}C-DIC results for that sample reported by the participating laboratories ($\delta^{13}\mathrm{C}_{\mathrm{ave}}$), and μ is the standard deviation of the reported values. Generally, a *Z*-score value of <2 is treated as acceptable, a value from 2 to 3 is considered questionable and a value of >3 is unacceptable (Wassenaar et al., 2012). The *Z*-score values of each δ^{13}C-DIC value of RM and DSW samples are shown in Table 3.3. All the RM δ^{13}C-DIC results are classified as acceptable. In the case of DSW, all results are acceptable except for results reported for samples 101107-b and 101156-a by Lab 1 and Lab 10 respectively which are classified as questionable. Again, this appears to be consistent with Lab 1's reporting of unexpected difficulties during their analyses. The questionable result from Lab 10 may have been a consequence of unexpected difficulties during analysis or other unknown errors.

Table 3.3. *Z*-scores of each δ^{13}C-DIC value of RM and DSW samples.

Lab #	***Z*-score RM**	***Z*-score 101107-a**	***Z*-score 101107-b**	***Z*-score 101155-a**	***Z*-score 101155-b**	***Z*-score 101156-a**	***Z*-score 101156-b**
1	0.91		**2.49**	1.91	0.47		
2	0.69	1.17	1.17	0.78	0.88		
3	0.53	0.71	0.46				
4	0.88	1.07	0.97	0.78	0.69		
5	0.35	0.20	0.10	0.08	0.12		
6	0.25					0.21	0.11
7	0.73	0.40	0.85	0.56	0.66		
8	1.57	1.23	1.11	1.38	1.42		
9	0.41	0.78	0.30	0.47	0.01		
10	1.16					**2.13**	1.55
11						1.62	0.66
12	1.16					1.36	1.26
13	1.24					1.81	0.90
14	1.86	1.24	0.76	0.66	1.14		
15	0.41					0.69	0.11
16-a	0.97	0.88	0.30	0.88	0.69		
16-b	0.69	0.49	0.59	0.08	0.08		

Statistical properties for the δ^{13}C-DIC results for RM and DSW are reported in Table 3.4. The $\delta^{13}\mathrm{C}_{\mathrm{ave}}$ and standard deviation ($\delta^{13}\mathrm{C}_{\mathrm{stdev}}$) of all RM δ^{13}C-DIC results are 0.78‰ and

0.11‰ respectively, and the absolute maximum between lab difference is 0.32‰. In the case of DSW, the $\delta^{13}C_{ave}$ and $\delta^{13}C_{stdev}$ of DSW δ^{13}C-DIC results derived from all laboratories are 0.87‰ and 0.10‰ respectively, and the absolute maximum between-lab difference is 0.48‰. Note that if the two questionable DSW results from Lab 1 and Lab 10 are eliminated, the corresponding $\delta^{13}C_{stdev}$ and absolute maximum between-lab difference are reduced to 0.09‰ and 0.36‰ respectively.

Table 3.4. Statistical properties for RM and DSW δ^{13}C-DIC results reported in ‰VPDB.

All uncorrected RM δ^{13}C-DIC results		**All uncorrected DSW δ^{13}C-DIC results**		**Uncorrected DSW δ^{13}C-DIC results exclude two questionable results**	
average	0.78	**average**	0.87	**average**	0.87
std dev	0.11	**std dev**	0.10	**std dev**	0.09
median	0.82	**median**	0.88	**median**	0.88
max	0.90	**max**	1.09	**max**	1.03
min	0.58	**min**	0.61	**min**	0.67
max–min	0.32	**max–min**	0.48	**max–min**	0.36
n	16	***n***	53	***n***	51

All corrected DSW δ^{13}C-DIC results		**Corrected DSW δ^{13}C-DIC results exclude all questionable results**	
average	0.88	**average**	0.88
std dev	0.06	**std dev**	0.05
median	0.89	**median**	0.89
max	1.00	**max**	1.00
min	0.71	**min**	0.78
max–min	0.29	**max–min**	0.22
n	51	***n***	45

Fig. 3.1, shows that there are systematic between-lab differences of $\delta^{13}C_{meas}$ values that are reflected in the results of both RM and DSW analyses. This is reflected in the significant correlation (R^2=0.72) between the residuals (i.e. $\delta^{13}C_{meas}$–$\delta^{13}C_{ave}$) of RM with those for DSW, as shown in Fig. 3.3. This correlation is also stronger when the two questionable results from Lab 1 and Lab 10 are removed (R^2=0.77; Fig. 3.3).

Using the empirical relationship between the residuals for RM and DSW, we corrected the reported DSW δ^{13}C-DIC values based on each lab's average deviation from the $\delta^{13}C_{ave}$ of RM using the following equation:

$$\delta^{13}C_{DSW\text{-}corr} = \delta^{13}C_{DSW\text{-}meas} - (\delta^{13}C_{RM\text{-}meas} - \delta^{13}C_{RM\text{-}ave}), \qquad (1)$$

where $\delta^{13}C_{DSW\text{-}corr}$ denotes the corrected DSW δ^{13}C-DIC values, $\delta^{13}C_{DSW\text{-}meas}$ is the reported DSW δ^{13}C-DIC result from a participating laboratory, $\delta^{13}C_{RM\text{-}meas}$ is the reported RM δ^{13}C-DIC result from the same participating laboratory and $\delta^{13}C_{RM\text{-}ave}$ is the average of all RM δ^{13}C-DIC results from all participating laboratories.

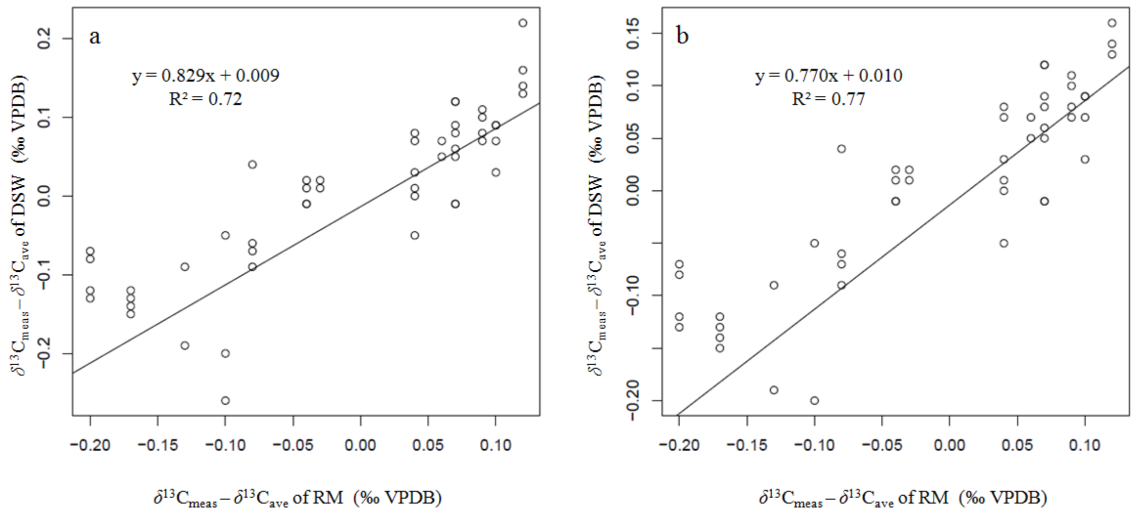

Fig. 3.3. Correlation between the residuals of all RM results ($\delta^{13}C_{meas}$–$\delta^{13}C_{ave}$) with residuals of all DSW results (a), and correlation between the residuals of RM results with residuals of DSW results excluding two questionable results (b) (see text).

Here, we must emphasize that the $\delta^{13}C_{RM\text{-}ave}$ does not represent a "Certified" value, and hence the correction procedure does not necessarily make the results more accurate. The resulting $\delta^{13}C_{DSW\text{-}corr}$ are shown in Table 3.5, and visualized in Fig. 3.4. The correction based on RM results reduces the inter-laboratory standard deviation for the DSW δ^{13}C-DIC results from 0.10‰ to 0.06‰. Further, if we remove the statistically-evaluated questionable results reported by Lab 1 and Lab 10, and potentially questionable results reported by Lab 1 and Lab 12, the average value and standard deviation of all the corrected DSW results are 0.88‰ and 0.05‰ respectively (Table 3.4)

Table 3.5. Corrected DSW δ^{13}C-DIC results reported in ‰VPDB.

Lab #	**$\delta^{13}C_{DSW\text{-}corr}$ 101107-a**	**$\delta^{13}C_{DSW\text{-}corr}$ 101107-b**	**$\delta^{13}C_{DSW\text{-}corr}$ 101155-a**	**$\delta^{13}C_{DSW\text{-}corr}$ 101155-b**	**$\delta^{13}C_{DSW\text{-}corr}$ 101156-a**	**$\delta^{13}C_{DSW\text{-}corr}$ 101156-b**
1		0.71	0.77	0.92		
2	0.92	0.92	0.88	0.89		
3	0.89	0.86				
4	0.89	0.88	0.86	0.85		
5	0.93	0.92	0.90	0.89		
6					0.92	0.91
7	0.99	0.86	0.89	0.88		
8	0.91	0.92	0.89	0.89		
9	0.91	0.86	0.78	0.83		
10					0.97	0.91
11						
12					0.89	0.88
13					0.81	0.91
14	0.94	0.99	1.00	0.95		
15					0.90	0.84
16-a	0.86	0.80	0.86	0.84		
16-b	0.85	0.86	0.79	0.79		

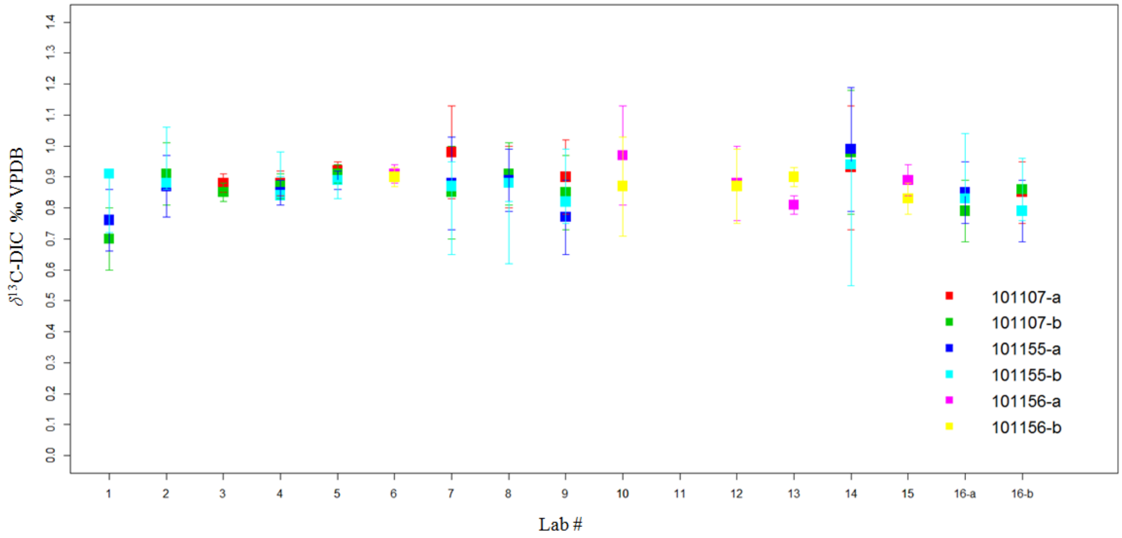

Fig. 3.4. Corrected DSW δ^{13}C-DIC results from participating laboratories. The correction procedure is based on each lab's deviation from the averaged RM δ^{13}C-DIC results reported by all labs.

3.5 Discussion

3.5.1 Comparison of Within-lab and Between-lab Precision with Prior Estimates of Precision

The 16 participating laboratories reported within-lab analytical precision (±1σ) ranging from 0.03‰ to 0.40‰ with a median value of 0.10‰. These levels of precision are comparable with those reported previously by individual laboratories in the literature that range from 0.03‰ to 0.23‰. (e.g. Olsen et al., 2006; Quay et al., 2007; McNichol et al., 2010; Humphreys et al., 2016). In this study, however we were encouraged, that the between-lab precision ($\delta^{13}C_{stdev}$) for both RM and DSW, before any correction based on RMs but after outlier removal, was better than 0.11‰. As noted earlier, van Geldern et al. (2013) reported between-lab precision from a study with significantly fewer groups (*n*=5), with a $\delta^{13}C_{stdev}$ of 0.07‰ for four laboratories and 0.47‰ when results from all 5 participating laboratories were included.

During this ring-test, the absolute maximum between-lab differences for uncorrected RM and DSW values (0.32‰ and 0.48‰, respectively) were close to the typical 2σ precision for seawater $\delta^{13}C$-DIC measurements in most oceanographic studies (Humphreys et al., 2016). Taken together, the level of agreement achieved without any RM-based correction, is encouraging. However, between-lab precisions remained a factor of two worse than the uncertainty of ±0.05‰ proposed by GOOS. This 0.11‰ between-lab precision suggests that current analytical approaches, which are based on individual laboratory calibration of aqueous phase sample analyses using solid and/or gas-phase standards, are not sufficient to meet analytical needs for scientific research (see Appendix 1 for methods).

3.5.2 Comparison of Deep Seawater Sample Analyses with Historical Data

The GO-SHIP A02 hydrographic section across the North Atlantic Ocean and other cruises have been occupied several times since the 1990's and samples from the same location have been collected and analyzed for $\delta^{13}C$-DIC in 1994 (Koltermann and Meincke 1994), 1997 (Schott et al., 1998), 1999 (Friis et al., 2003), 2003 (Rhein, 2003) as well as this data set which was collected in 2017. The mean and between-lab standard deviation of these raw (i.e. uncorrected), previously reported data (including the average value reported from this study) for deep seawater collected at approximately the same location and depth as our samples are 1.00‰ and 0.10‰ respectively. These values refer to the data reported by individual laboratories without any additional corrections and/or normalization. Following secondary quality control (2nd QC) procedure and data adjustment based on "crossover analysis" (Tanhua et al., 2010; Becker et al., 2016), the mean value for deep seawater from this location was reported to be 0.95‰. It is important to note that the empirical adjustment procedure is based on the assumption that the ocean's deepwater values are not changing, rather than on any standard- or calibration-based adjustment. Nevertheless, the close agreement of our mean value (0.88±0.10‰) for DSW with the mean of the adjusted historical data of 0.95‰ from the same location, suggests that overall inter-comparability of δ^{13}C-DIC data using the approaches currently employed by experienced laboratories on the order of 0.07‰ is close to the GOOS accuracy requirement.

3.5.3 Sampling, Sample Preservation and Analysis Methods

As noted, the DSW samples were analyzed 4 to 10 months after they were collected on board. The good inter-laboratory agreement in DSW δ^{13}C-DIC results and the consistency of our average value for DSW with historical δ^{13}C-DIC results from the same water mass suggest that the sampling and preservation methods employed in this study are appropriate for seawater δ^{13}C-DIC analysis and for long-term preservation of the samples (at least 10 months).

The inter-laboratory agreement also demonstrates that, for a typical seawater sample with DIC concentration of 2050 to 2200µM, the use of a wide range of δ^{13}C-DIC determinations and standardization methods does not necessarily lead to large inter-laboratory differences. It appears that the operating procedures used by the laboratories involved in this study, including calibrations and corrections (e.g. corrections for fractionation effects during CO_2 equilibration; corrections for linearity effect, and system drift) are implemented effectively.

Further, there is no significant difference in the δ^{13}C-DIC results measured with IRMS-based methods and from the two laboratories that used CRDS and IRIS for detection. For the IRMS based methods, various methods of pre-processing of water samples (e.g. different front-end peripherals; various equilibration times after CO_2 extraction; different sample volumes) do not result in large scatter in the δ^{13}C-DIC results. In addition, the use of a variety of calibration procedures based on both internal reference materials and international calibration materials in solid and gas phase does not appear to cause major discrepancies between participating laboratories.

3.5.4 Value and Importance of a Reference Material

Nevertheless, Fig. 3.1 shows that there were systematic, between-lab differences of the order of 0.10‰ in the results for both RM and DSW analyses. Until now, systematic analysis-derived differences in δ^{13}C-DIC data collected on different cruises have been identified and corrected using 2nd QC based on crossover analysis of data collected on multiple expeditions in nearby locations, and on the assumptions that deep ocean δ^{13}C-DIC is invariant (Tanhua et al., 2010; Lauvset and Tanhua 2015; Becker et al., 2016) or can be corrected for using some form of correction based on a simultaneously measured parameters such as dissolved oxygen, DIC or temperature. This 2nd QC procedure can achieve an internal consistency of carbon stable isotope data collected on multiple cruises in the order of 0.02‰ (Becker et al., 2016). However, the approach is effectively a "consensus" approach based on forcing agreement between groups that happen to have sampled in the same geographic region, rather than on an absolute reference or widely-used reference material. Further it cannot be used effectively in locations where there are strong spatial gradients of δ^{13}C-DIC data exist or where temporal changes are likely. Hence Lauvset and Tanhua (2015) highly recommends to completed all CRM-based calibration and/or corrections prior to crossover analysis. This step is presently not possible for δ^{13}C-DIC analyses.

In order to examine the potential for improvement in data quality with use of a CRM, we applied a data correction procedure to the results from the various laboratories, in which we assigned the $\delta^{13}C_{ave}$ for RM analyses reported by all participating groups as a consensus δ^{13}C-DIC value. We then used each laboratory's average reported deviation from this consensus value as a means to correct for inter-laboratory bias. This procedure lowered the between-lab standard deviation to 0.05‰ which matches the target uncertainty level proposed by the Global Ocean Observing System. This is particularly encouraging, as this target uncertainty was achieved not only with different analytical and standardization approaches, but also included nearly all variables potentially influencing sample integrity such as sample collection, transportation, and handling prior to analysis.

Although our consensus value is not necessarily accurate, application of this correction did bring the DSW δ^{13}C-DIC results from all groups into very close agreement (0.07‰ absolute difference) with the previously proposed δ^{13}C-DIC values of the same water mass following 2nd QC approach (Becker et al., 2016). We therefore conclude that provision and use of CRMs in the form of aqueous-phase samples, including certification of the existing "Certified Reference Material for oceanic CO_2 measurements" from Scripps Institution of Oceanography" for δ^{13}C-DIC value, would make a significant contribution to data quality and allow the GOOS accuracy specification to be met. It is of note that at least 2 CRMs with the upper and lower range of typical oceanic δ^{13}C-DIC values should be employed, in order to ensure that GOOS specifications are achieved in different oceanic regions.

3.6 Conclusions, comments and recommendations

In conclusion, a seawater δ^{13}C-DIC inter-comparison exercise involving 16 globally-distributed participating laboratories showed rather than expected and surprisingly good agreement between laboratories. Without further correction, the 1σ inter-laboratory agreement was better than ±0.11‰. This is comparable to or better than the only previous such estimate of inter-laboratory agreement for seawater analyses (van Geldern et al., 2013). Also, between-lab average δ^{13}C-DIC value reported from this study is highly consistent (0.07‰ absolute difference) with mean historic values for deep seawater from this location which were adjusted by 2nd QC.

Our uncorrected results suggest that use of different analytical and detection methods and/or standardization procedures does not necessarily lead to major systematic differences between laboratories. For example, the consistency between CRDS and IRMS based measurements is promising and may open the door to novel methods allowing high frequency and/or autonomous δ^{13}C-DIC measurement without sacrificing data accuracy.

A correction procedure based on use of a RM demonstrates that availability of an aqueous phase reference material for δ^{13}C-DICcould result in a significant (e.g. factor of 2) improvement in between-lab data consistency. Our results suggest that the demanding specifications proposed by the Global Ocean Observing System (±0.05‰) are within reach, but only if such a reference material is available and used. Further, our results support the suggestion that the existing Certified Reference Materials used for seawater DIC and alkalinity quality control may be suitable for this purpose, but only if the various batches can be assigned a "Certified" δ^{13}C-DIC value. A possible alternative could be the centralized distribution of a readily-soluble carbon-containing compound, coupled with a standard operating procedure for its introduction into the aqueous phase. However, this option remains to be tested.

The authors of this study suggest that this stable isotope analysis ring-test be extended and repeated periodically, at least until a suitable Certified Reference Material for δ^{13}C-DIC becomes available. Overall, the results are encouraging and confirm that the gradients and temporal variability of δ^{13}C-DIC in the ocean can be measured reliably and consistently on a global scale.

3.7 Acknowledgements

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Chapter 4

Preliminary Interpretation of A02 Cruise δ^{13}C-DIC Data

4.1 Introduction and Background

As stated in Chapter 1, oceanic uptake plays an important role for buffering the excess anthropogenic CO_2 released into the atmosphere. The storage of anthropogenic CO_2 in the North Atlantic has been proved to be particularly large (Wallace 2001; Friis et al., 2005). According to Sabine et al., 2004, the North Atlantic stores 23% of the global oceanic anthropogenic CO_2, and highest vertically integrated carbon concentrations has been detected in this region. Especially, in the high latitude area of the North Atlantic Ocean such as the Nordic Sea and the Labrador Sea, it is considered that there is a strong anthropogenic CO_2 sink (Racapé et al., 2014). Therefore, knowledge of the oceanic CO_2 uptake in the North Atlantic Ocean is of particular importance.

In order to clarify the distribution and movement of anthropogenic carbon in the ocean and its impact to the global climate, international scientific research programs such as World Ocean Circulation Experiment (WOCE), Joint Global Ocean Flux Study (JGOFS) and Global Ocean Observation System (GOOS) etc. have been conducted. As part of the GOOS program, the Go-Ship hydrographic investigation cruises aims at specifying Essential Ocean Variables such as salinity, temperature, dissolved oxygen (DO) concentration, nutrients and inorganic carbon parameters (DIC, TA, pCO_2, pH) etc., and use these parameters to investigate the ocean ecological system.

The 2017 Go-Ship A02 cruise (McGovern et al., 2018) took place in April to May 2017, and covered the Newfoundland Basin, Mid Atlantic Ridge and Western European Basin across the 40ºN-50ºN section of North Atlantic Ocean. During this cruise, discrete samples were taken for δ^{13}C-DIC analysis at 30 stations. In this chapter, I give a very brief, preliminary overview of the δ^{13}C-DIC results from the cruise. Also, as the A02 section has been sampled for δ^{13}C-DIC in 1994, and some stations of the A02 sections were also sampled for δ^{13}C-DIC during other past research cruises. I attempt some preliminary interpretation of the δ^{13}C-DIC results in comparison with the historical δ^{13}C-DIC data from nearby stations. It is important to note that hydrographic data from the A02 cruise are still undergoing quality-control and that some post-cruise adjustments (e.g. to oxygen concentration data and/or DIC data) are likely to be applied that might impact the conclusions presented here.

4.2 Results and Discussion

First, the δ^{13}C-DIC results derived from the 2017 Go-Ship A02 cruise and historic δ^{13}C-DIC results of A02 section sampled in1994 are plotted using Ocean Data View, as shown in Fig. 4.1. The X-Axis shows the distance along the A02 section from the Newfoundland Basin to the Western Europe Basin, and the Y-Axis shows pressure with the color bar showing the δ^{13}C-DIC results reported in ‰VPDB. The sampling stations of the A02 section in 2017 and 1994 are shown in Fig. 4.2. All the δ^{13}C-DIC data derived from both onboard and in lab measurements of the samples taken at different depths during the 2017 A02 Go-Ship cruise were in the range of 0.4‰ to 1.5‰, which is consistent with earlier δ^{13}C-DIC related studies conducted in the same region (e.g. Racapé et al., 2014; Becker et al., 2016). Also, as shown in Fig. 4.2, due to insufficient sampling stations and depths in 1994, there are some gaps in δ^{13}C-DIC results from that earlier occupation of the section, which makes it hard to describe the δ^{13}C-DIC distribution across the A02 section in 1994. Compared with 1994, better sampling coverage was achieved during the 2017 A02 Go-Ship cruise using our on-board simultaneous DIC concentration and δ^{13}C-DIC measurement system.

Quantification of anthropogenic changes in oceanic δ^{13}C-DIC has proven to be very difficult, not only because of a general lack of historical δ^{13}C-DIC data, but also because the measured δ^{13}C-DIC results are subjected to short-term, natural variability at seasonal and inter-annual timescales (e.g. Bates et al., 1996; Gruber et al., 1999; Körtzinger et al., 2003). In order to detect the anthropogenic changes in oceanic δ^{13}C-DIC at a time interval of approximately 20 years and attempt to remove the effects of natural variability, I applied the back-calculation approach proposed by Körtzinger et al., 2003, to calculate "preformed" δ^{13}C-DIC.This preformed δ^{13}C-DIC approach attempts to reconstruct the " δ^{13}C-DIC values which formed during the last contact with atmosphere" (Körtzingeret al., 2003). Specifically, the preformed δ^{13}C-DIC values ($\delta^{13}C^0$) are calculated using the following equation:

$$\delta^{13}C^0 = \frac{(\delta^{13}C*DIC-\delta^{13}C_{org}*\left(\frac{C_{org}}{-O_2}\right)*AOU)}{(DIC-\left(\frac{C_{org}}{-O_2}\right)*AOU)}$$

Where $\delta^{13}C^0$ denotes the preformed δ^{13}C-DIC value; $\delta^{13}C$ is the observed δ^{13}C-DIC value; DIC denotes the measured DIC concentration value derived from our simultaneous DIC concentration and δ^{13}C-DIC measurement system; AOU refers to apparent oxygen utilization, for the preliminary A02 data set used here, is calculated from dissolved O_2 concentration (DO) data derived from the CTDO sensor; $\delta^{13}C_{org}$ is an approximation of δ^{13}C of particulate organic matter produced during primary production; $\frac{C_{org}}{-O_2}$ is the stoichiometric ratio of organic matter and oxygen consumption during respiration, also denoted as the "Redfield ratio" (Körtzinger et al., 2003). This calculation attempts to remove the biologically-mediated changes in δ^{13}C-DIC of a parcel of seawater within the ocean interior and after it lost contact with the atmosphere.

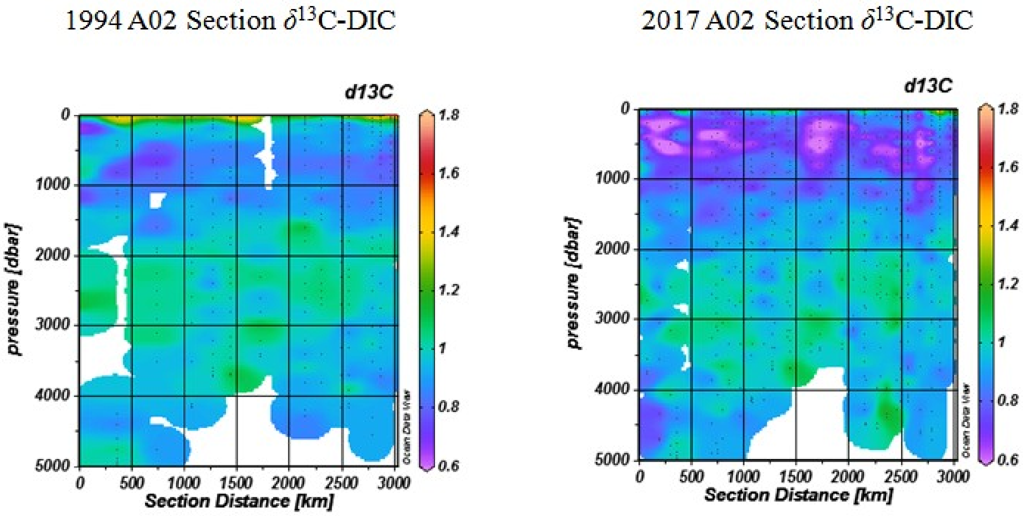
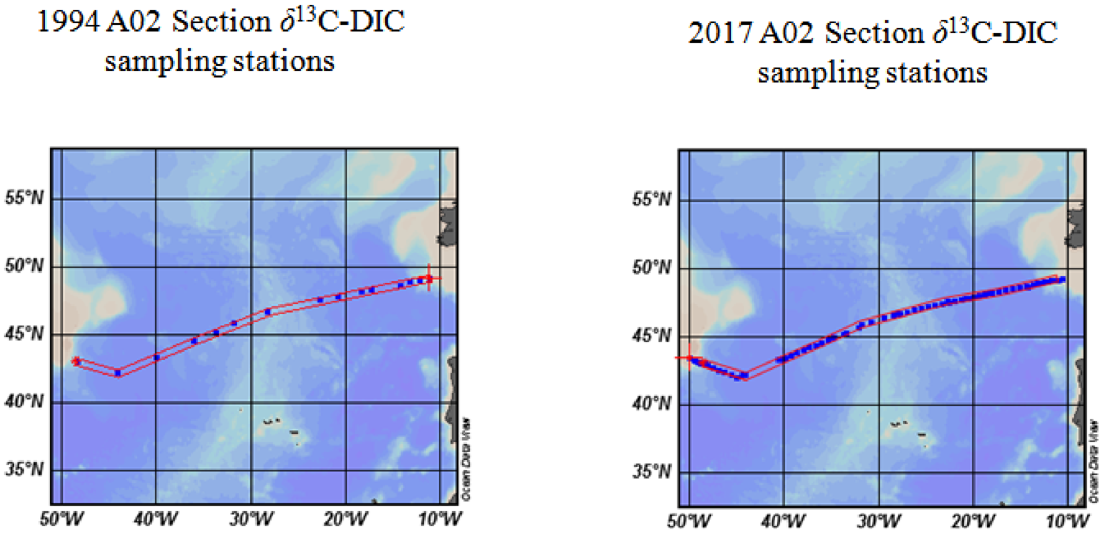


Fig. 4.1. δ^{13}C-DIC results derived from the 2017 Go-Ship A02 cruise and historic δ^{13}C-DIC results of A02 section sampled in1994.


1994 A02 Section δ^{13}C-DIC sampling stations

2017 A02 Section δ^{13}C-DIC sampling stations

Fig. 4.2. The sampling stations of the A02 section in 2017 and 1994 for δ^{13}C-DIC.

In an initial attempt to look for temporal changes along the A02 section, four sets of δ^{13}C-DIC data and preformed δ^{13}C-DIC data profiles derived from the 2017 A02 cruise were compared with historical δ^{13}C-DIC data and preformed δ^{13}C-DIC data profiles collected in 1994, 1999, 2001, and 2003 at nearby locations (±1° in latitude and longitude). These comparisons are shown in Figures 4.3 and 4.4, respectively.

Fig. 4.3, where corrections to “preformed” values have not been applied, shows a decrease in observed δ^{13}C-DIC over approximately 20 years, with the decrease extending to approximately 2600 m depth in our investigation area, and its magnitude weakened with increasing depth. This may be contributed by the oceanic "^{13}C Suess-effect". In addition, no significant decrease in δ^{13}C-DIC was observed in deep water (>3000 m) during this twenty year period, in either the Newfoundland Basin, or Western European Basin.

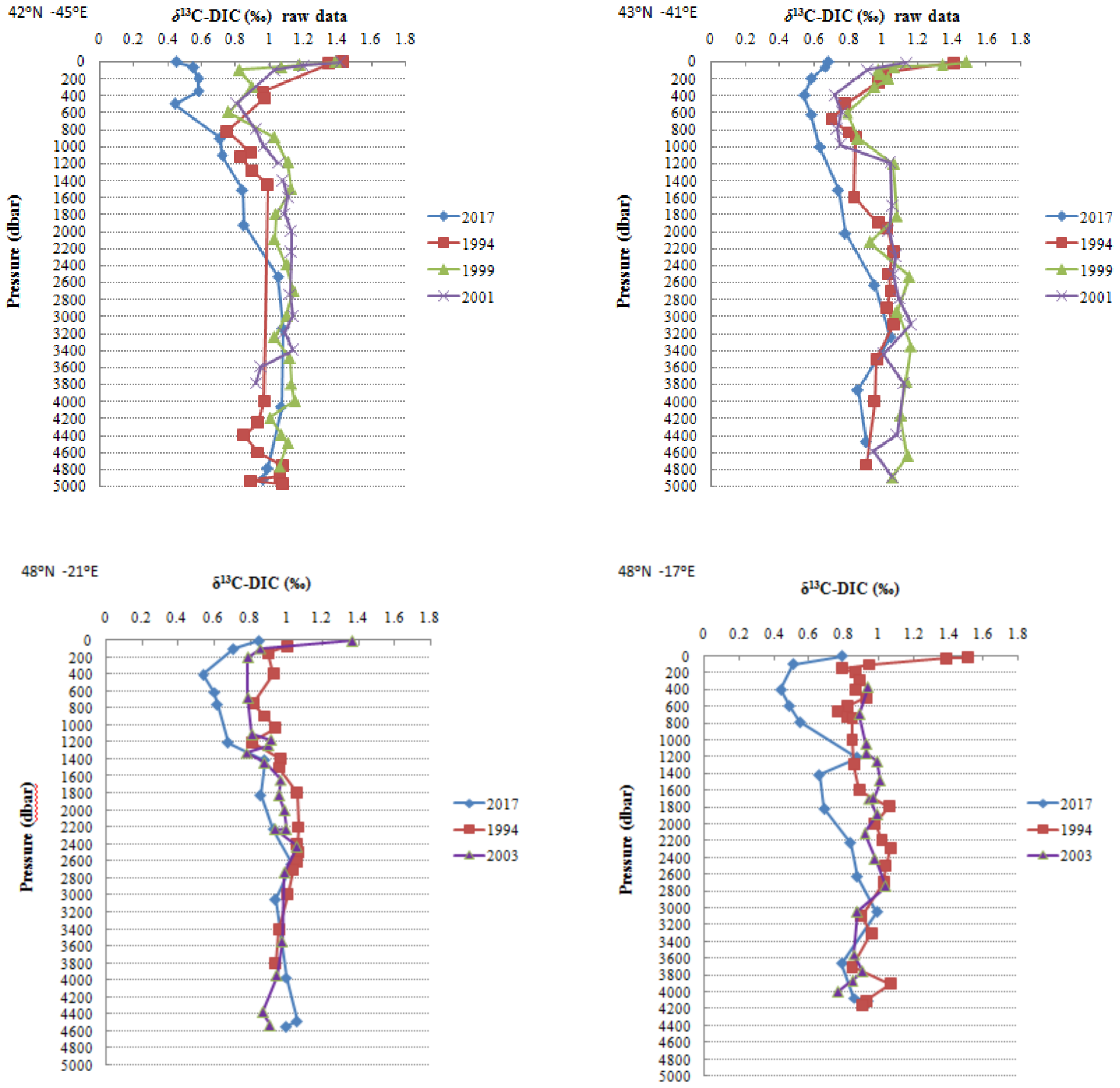


Fig. 4.3. Observed δ^{13}C-DIC data profiles derived from the 2017 A02 cruise in comparison with the historic δ^{13}C-DIC data in 1994, 1999, 2001, and 2003 at the nearby locations (±1° in latitude and longitude).

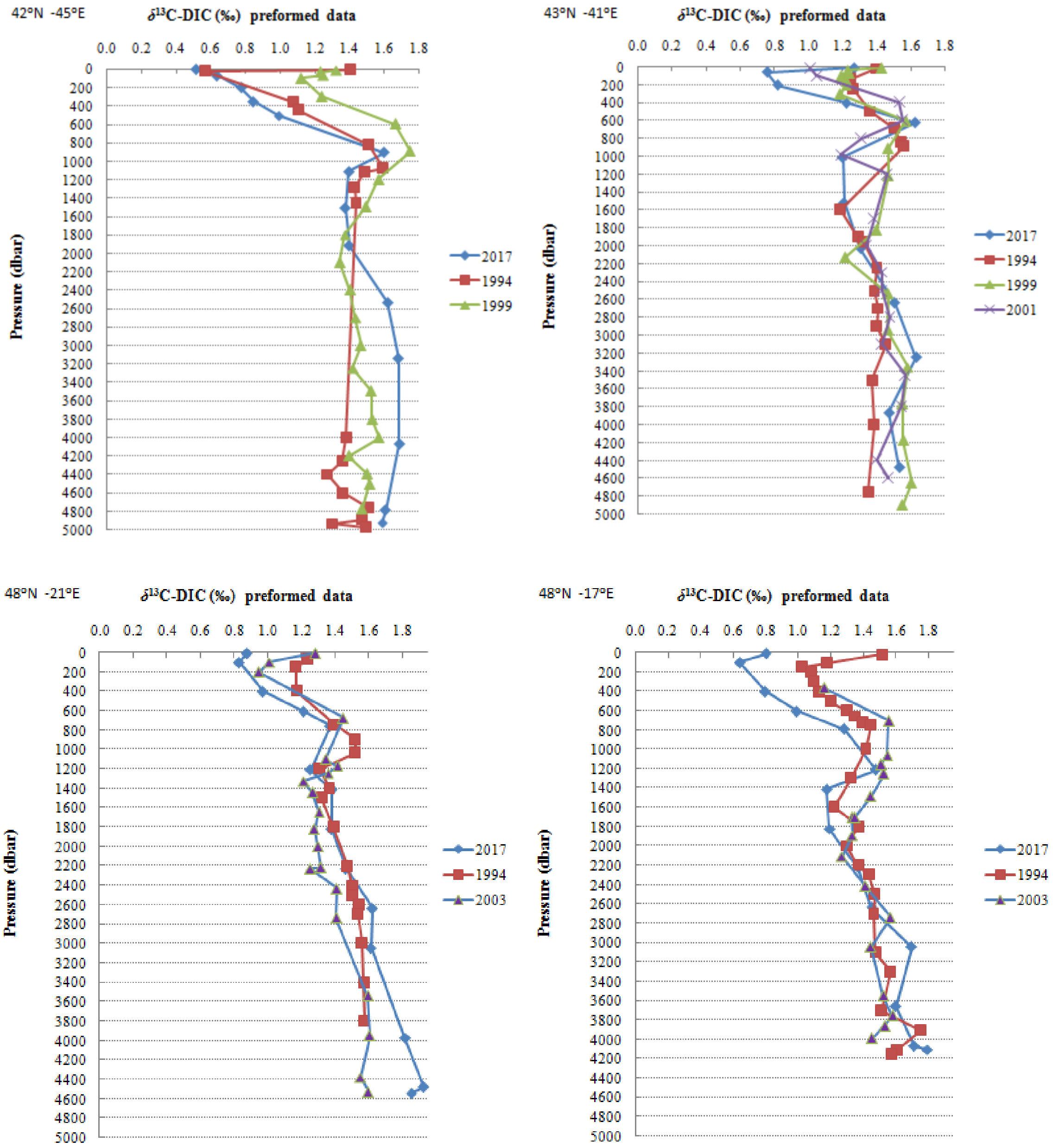


Fig. 4.4. Preformed δ^{13}C-DIC data profiles derived from the 2017 A02 cruise in comparison with the historic δ^{13}C-DIC data in 1994, 1999, 2001, and 2003 at the nearby locations (±1° in latitude and longitude).

However, Fig. 4.4, in which measured values have been corrected to “preformed” values using preliminary AOU data from the A02 cruise according to equation above, shows an unexpected and difficult-to-explain increase in preformed δ^{13}C-DIC values of deep seawater (>3000 m) during the 20 year interval. I suspect that this may not reflect the real world change but is a result of calculation or calibration error, possibly as a result of using preliminary oxygen data for the preformed correction. During my calculations of preformed δ^{13}C-DIC results of year 2017, DO results derived from CTDO sensor set were used which may be subject to relatively large errors. More importantly, unlike the historical oxygen data, the DO data collected from the 2017 Go-Ship A02 cruise have not been adjusted by secondary quality control (2nd QC). Therefore, the preliminary nature of the DO data may be a possible source of error in calculation of preformed δ^{13}C-DIC results and the reason for the apparent temporal increase of ^{13}C in the deep water masses.

This discussion of the difficulties of comparisons with historical data, emphasizes the importance of high accuracy or at least inter-comparability of data sets collected by different investigators at different times. As stated in Chapter 3, the levels of 1-sigma precision reported previously by individual laboratories ranges from ±0.03‰ to ±0.23‰. (e.g. Olsen et al., 2006; Quay et al., 2007; McNichol et al., 2010; Humphreys et al., 2016). Therefore, comparison of δ^{13}C-DIC profiles from nearby locations faces some fundamental limitations for identification of the oceanic "^{13}C Suess-effect", especially detection of the possible very small anthropogenic δ^{13}C-DIC change in deep water (>3000 m) over a timescale of 2 decades.

4.3 Conclusions

My attempt at initial interpretation of the δ^{13}C-DIC results in comparison with historic data proves that, in order to quantify the anthropogenic δ^{13}C-DIC variation in the ocean, a better coverage of high quality δ^{13}C-DIC results is essential. And our newly developed simultaneous DIC concentration and δ^{13}C-DIC measurement system could improve the sample frequency remarkably. Also, the inter-comparison exercise described in Chapter 3 proves that use of liquid phase CRM for δ^{13}C-DIC could significantly improve the δ^{13}C-DIC data quality and hence inter-comparability of data sets.

Chapter 5

Conclusion

In conclusion, a newly developed simultaneous DIC concentration and δ^{13}C-DIC measurement system was tested for its performance both in-lab and onboard a research vessel during a trans-Atlantic research cruise. Results show that this CRDS based method is suitable for high frequency shipboard determination of DIC concentration and δ^{13}C-DIC. Also, results from a worldwide inter-comparison exercise for δ^{13}C-DIC measurement show that all 16 participating laboratories are in good agreement in determination of δ^{13}C-DIC in seawater. Use of a liquid phase certified reference material for δ^{13}C-DIC may further improve the inter-comparability of δ^{13}C-DIC data derived from multiple measurement groups. Finally, my attempt on preliminary interpretation of δ^{13}C-DIC data derived from the 2017 Go-Ship A02 cruise shows the importance of high quality δ^{13}C-DIC data in ocean carbon cycling studies.

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Appendix 1

δ^{13}C-DIC analysis methods used by participating laboratories

Lab #	1
Sample arrival date	September-01-17
Storage after arrival	dark and cool (4 ºC)
Sample analysis date	December-09-17
Front end peripherals	Modified OI Analytical model 1030 wet TOC analyser with 1088 auto sampler interfaced to trapping device
Detector	Finnigan Mat DeltaPlusXP IRMS
Detector mode	Continuous flow
Method description	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for δ^{13}C-CO_2 analysis via trapping of CO_2 on molecular sieve.
Equilibration time after CO_2 extraction (if applicable)	N/A
Sample volume	1 mL
Calibration procedure	Data is normalised using two different internal organic standards prepared in solution three days before beginning analysis. These were calibrated with USGS40 and USGS41 for ^{13}C on elemental analyser.
Reference material (internal standard)	2 internal organic standards (sucrose and Kph)
International calibration material	USGS 40 and USGS41 (DOC curve is applied to DIC)
Method precision (±1σ)	±0.10‰

2	3
September-26-17	October-23-17
dark and cool	dark and room temp
October-05-17	October-26-17
Thermo GasBench II	A vacuum line designed for CO_2 gas stripping
Thermo Delta plus XP IRMS	Finnigan MAT 253 IRMS
Continuous flow	Discrete measurement
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis	CO_2 conversion by H_3PO_4, stripping in a cooling jar (-196 ºC) in N_2 atmosphere for 7 min, removal of H_2O in another cooling jar (-20 ºC), pressure measurement, and sealing in a glass tube. Stripped CO_2 was introduced into IRMS for $\delta^{13}C$-CO_2 analysis manually
> 20 h	about 10 min between extraction (stripping) and sealing in a glass tube
0.85 mL	RM: 240 mL, seawaters: 160 mL
2 points calibration by international calibration material; internal standard for quality control	1 point calibration by gas standard
QC standard ($CaCO_3$)	Oztech CO_2 standard gas (for reference)
NBS19, LSVEC	Oztech CO_2 standard gas
±0.10‰	±0.03‰

4	5
N/A	N/A
N/A	light and room temp
October-10-17	October-12-17
Finnigan GasBench II	N/A
Finnigan DELTA plusXL IRMS	Finnigan MAT 253 IRMS
Continuous flow	Dual inlet
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis
8 h	N/A
0.7 mL	100 mL
three points calibration by 1 internal and 2 international calibration material, gas standard for QC	1 point calibration using international CO_2 gas standard and internal lab $DI^{13}C$ standard analysis
$NaHCO_3$, QC gas standard	internal CO_2 gas standard
Wiley $CaCO_3$, NBS 19	NBS 19
±0.04‰	±0.03‰

6	7
N/A	September-25-17
light and room temp	dark in refrigerator (4 ºC)
October-31-17	October-30-17
Finnigan Gasbench II	Finnigan GasBench II
Thermo DeltaV plus IRMS	Thermo Electron Delta V Plus IRMS
Continuous flow	Continuous flow
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for δ^{13}C-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for δ^{13}C-CO_2 analysis
>15 min	24 h
0.5 mL	2 mL
1 point calibration by international calibration material. QC by two biogenic calcium carbonate internal reference material.	2 points calibration by 1 international calibration material and 1 internal standard; QC by 1 international calibration material and 1 internal standard
$CaCO_3$ (Coral JCp-1, Giant Clam JCt1)	$NaHCO_3$
NBS 19	LSVEC, Acros Li2CO3
±0.03‰	±0.15‰

8	9
September-20-17	August-01-17
dark in refrigerator (~2 ºC)	dark and room temperature (~23 ºC)
October-11-17	November-08-17
Thermo Gas Bench	Apollo SciTech AS-D3 DIC analyzer
Thermo Advantage IRMS	Picarro G2201*i* CRDS analyzer for isotopic carbon in CO_2 & CH_4
Continuous flow	CO_2 isotope only mode
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for δ^{13}C-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into CRDS for δ^{13}C-CO_2 analysis
N/A	N/A
0.33 mL	3.8 mL
3 points calibration by international calibration material; corrections for CO_2 partitioning between the liquid and headspace	dissolve internal solid standard (δ^{13}C value predetermined by IRMS) material into MiliQ water; 3 points calibration
N/A	$NaHCO_3$, Na_2CO_3, Baking soda
NBS 18, NBS 19, LSVEC	NBS-18, IAEA-CO-9
± 0.10‰	±0.12‰

10	11
September-27-17	October-31-17
dark and room temperature (~21 ºC)	dark in refrigerator (~2 ºC)
November-11-17	November-08-17
Thermo GasBench II	Thermo GasBench II
Thermo Delta V Plus IRMS	Delta V Advantage IRMS
Continuous flow	Continuous flow
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis
12-18 h	18 h with shaking
1 mL	0.5 mL
3 points calibration by 2 international calibration material and 1 internal standard; corrections for CO_2 partitioning between the liquid and headspace	2 point calibration by internal calibration material (standardized by 2 international standard material); 1 internal calibration material for low DIC concentration sample correction
Yule marble	$NaHCO_3$, $CaCO_3$, Baking soda
NBS-19, Harding Iceland spar	NBS 18, NBS 19, LSVEC
±0.16‰	±0.40‰

12	13
N/A	November-27-17
dark in refrigerator (5 ºC)	dark and cool (4 °C)
November-08-17	November-28-17
Finnigan GasBench II	manual vacuum system
Delta V Advantage IRMS	SIRA9 VG
Continuous flow	CO_2 isotope only mode
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis
24 h at 30 ºC	N/A
100 mL	160 mL
3 points calibration by comparison to 1 international standard and 2 internal standards (standardized by comparison to 2 international standards); corrections for CO_2 partitioning between the liquid and headspace	monthly calibration by "in house" standard ($NaHCO_3$ solution dissolved in water)
$CaCO_3$: 1 marble, 1 belemnite	Carrare marble from Lycee Marceau (2.13‰ VPDB)
NBS-19, NBS-18	NBS18, NS19
±0.12‰	±0.03‰

14	15
N/A	October-20-18
dark in refrigerator	dark in refrigerator
December-19-17	February-06-18
Finnigan GasBench II	Finnigan GasBench II
Conflo IV, Delta V Plus IRMS	Conflo IV, Delta V Plus IRMS
Continuous flow	Continuous flow
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis
N/A	48 h at 32 ºC
0.8 mL	1 mL
4 points calibration by 3 international calibration material (1 gas phase, 2 solid phase) and 1 internal standard	2 points calibration by international calibration material; 1 internal standard for drift check; 2 internal standards for correction of CO_2 partitioning between the liquid and headspace
SIL bicarbonate	Dry carbonate KH-2, Dry $NaHCO_3$, Solution $NaHCO_3$ (100-400 mg/L)
Oztech 2130, NBS 20, USGS 44	NBS 18, NBS 19
±0.2‰	±0.05‰

16-a	16-b
N/A	N/A
dark and dry in refrigerator	dark and dry in refrigerator
March-05-18	March-05-18
Thermo Gas Bench II	Universal Reference Interface (URI) Connect, and CTAC auto sampler (ASX-7100)
Delta V plus IRMS	Delta Ray CO_2 isotope ratio IR spectrometer
Continuous flow	N/A
CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRMS for $\delta^{13}C$-CO_2 analysis	CO_2 conversion by H_3PO_4, extracted CO_2 introduced into IRIS for $\delta^{13}C$-CO_2 analysis
N/A	N/A
1 mL	0.8 mL
3 points calibration by international calibration material; 1 internal standard for QC	3 points calibration by international calibration material; 1 internal standard for QC
$NaHCO_3$, Carrera marble	Carrera marble
NBS19, NBS18, CO-8	NBS19, NBS18, CO-8
± 0.10‰	± 0.10 ‰

Appendix 2

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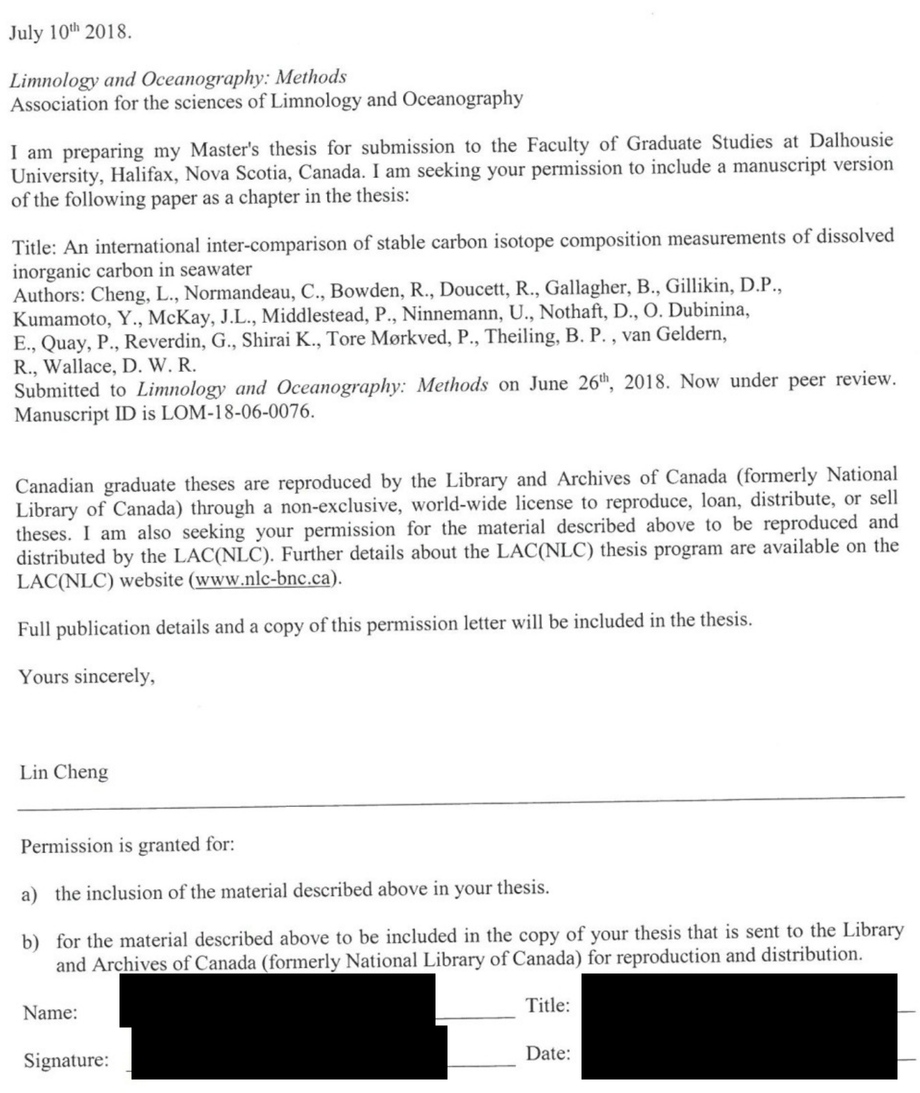

July 10th 2018.

Limnology and Oceanography: Methods
Association for the sciences of Limnology and Oceanography

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Title: An international inter-comparison of stable carbon isotope composition measurements of dissolved inorganic carbon in seawater
Authors: Cheng, L., Normandeau, C., Bowden, R., Doucett, R., Gallagher, B., Gillikin, D.P., Kumamoto, Y., McKay, J.L., Middlestead, P., Ninnemann, U., Nothaft, D., O. Dubinina, E., Quay, P., Reverdin, G., Shirai K., Tore Mørkved, P., Theiling, B. P. , van Geldern, R., Wallace, D. W. R.
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