GO-SHIP Repeat Hydrography: Determination of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) in seawater using High Temperature Combustion Analysis.

Elisa Halewood[1], Keri Opalk[1], Lillian Custals[2], Maverick Carey[1], Dennis A. Hansell[2], and Craig A. Carlson[1]

1. Marine Science Institute/Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, CA, USA
2. Department of Ocean Sciences, Rosenstiel School of Marine and Atmospheric Science, University of Miami, FL, USA

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1. Introduction

Dissolved organic matter (DOM), operationally defined as organic matter that passes through a submicron filter, is a complex mixture of organic molecules comprised of carbon, hydrogen and oxygen as well as nitrogen, phosphorous and sulfur. Resolving the dynamics of each DOM fraction helps to elucidate the greater questions of DOM biogeochemical cycling. At ~662 ±32 Pg (10^{15} g) C, oceanic dissolved organic carbon (DOC) is one of the largest bioreactive pools of carbon in the ocean (Williams and Druffel, 1987; Hansell and Carlson, 1998a; Hansell *et al.*, 2009), and is comparable to the mass of inorganic C in the atmosphere (MacKenzie, 1981; Eppley *et al.*, 1987; Fasham *et al.*, 2001). Perturbations in the sources or sinks of the oceanic DOC pool impact the balance between oceanic and atmospheric CO_2, perhaps making it climatically significant (Ridgwell and Arnt, 2014). In addition, most of the standing stock of fixed nitrogen in the surface ocean (<200m) is in the form of dissolved organic nitrogen (DON) (Bronk, 2002; Aluwihare and Meador, 2008; Letscher *et al*., 2013). As such, it is important to understand the processes that control DOC and DON distribution, inventories and fluxes in the global ocean.

Prior to the 2000's, there was a lack of high-quality data to adequately describe and quantify DOM in the ocean. In the 1980's, controversy over methods of DOC and total dissolved nitrogen (TDN) analyses in seawater (Williams and Druffel, 1988) resulted in efforts by the marine geochemistry community to improve accuracy of the measurement and establish inter-comparability of data sets (Sharp et al. 1995; Sharp et al., 2002a, Sharp et al. 2002b), proper blank procedures (Benner and Strom, 1993) and methods using reference materials (Hansell, 2005). The High Temperature Combustion (HTC) method using commercial instruments such as the Shimadzu Total Organic Carbon (TOC) Analyzer are now common for measuring DOC and TDN in seawater. Advances in analytical skill and increased frequency of global ocean sampling (through time-series sites and in conjunction with basin scale programs such as the U.S. Global Ocean Ship-Based Hydrographic Investigation Program (U.S. GO-SHIP)) have greatly improved temporal and spatial resolution of DOC variability (Hansell et al., 2009; Carlson et al. 2010). Further, DOM's contributions to the ecology and biogeochemistry of the ocean's water column have been illuminated.

This paper describes procedures for collection and measurement of DOC and TDN (the latter used to derive dissolved organic nitrogen concentration) in discrete seawater samples and is suitable for the assay of oceanic levels of DOC (typically <80 µmol C kg^{-1}) and total dissolved nitrogen (<40 µmol N kg^{-1}). It presents best practices for achieving improved determination using the HTC method following the approach of Carlson et al. (2010), which has been used on U.S. GO-SHIP cruises since 2003. The basic approach remains the same but the analyzers have been optimized over the years. The instruments discussed and procedures described are those specific to the methods employed in the Hansell Lab at the University of Miami's Rosenstiel School of Marine and Atmospheric Science and the Carlson Lab at the University of California Santa Barbara. This document builds upon existing guidelines for analysis of DOC in seawater (Tappin and Nimmo, 2019), and seeks to provide detailed updates and step by step protocols on sample collection & storage, optimizing Shimadzu TOC systems for high throughput of seawater samples, and quality assessment/quality control (QA/QC) practices using calibration and reference materials. In addition, we present methodological procedures for coupled TDN

analysis using Shimadzu TOC systems. We have chosen to highlight Shimadzu Scientific Instruments because of ease of use of their off-the-shelf TOC instruments and excellent limit of detection, but other manufacturers with equivalent detection capabilities or custom-built machines may also be appropriate.

2. Sample Collection and Storage

Proper sampling techniques and handling are essential to provide high quality data. Open ocean waters contain relatively low concentrations of DOC (~35-80 µmol C kg^{-1}, Hansell et al. 2009) compared to Dissolved Inorganic Carbon (DIC) (~1900-2200 µmol kg^{-1}) and are easily contaminated via poor handling, inadequately cleaned apparatus, inadvertent atmospheric exposure to volatile contaminants, or improper storage conditions. The methods described here aim to minimize these sources of error.

2.1 Sample bottles

It is recommended that samples be filtered directly from the collection bottle (i.e., Niskin bottle) through an in-line filter (see below) and into a pre-cleaned sample bottle. To minimize handling, we recommend pre-combusted 40 mL glass vials that fit the Shimadzu TOC auto-sampler. These vials are made of chemically inert Type I borosilicate glass. While these can be purchased certified clean (meeting the requirements of the US Environmental Protection Agency (EPA) for the testing of potentially harmful environmental contaminants in water or soil samples and TOC analysis) we have found these not sufficiently clean for low concentration oceanic DOC measurements. We prepare vials in house as below to be clean and free of substances that might influence analysis. If glass is logistically challenging, samples can also be collected into acid washed high-density polyethylene (HDPE) or polycarbonate (PC) bottles. Tests have shown that DOC concentration measured from glass, PC and HDPE bottles are comparable at the µmol L^{-1} resolution (Appendix A). Both glass and plastic sample containers are re-usable after proper cleaning. Prior to first use, or between uses, HDPE or PC bottles should be soaked in 1M hydrochloric acid (HCl Certified ACS Plus grade, see Appendix B), rinsed with low total organic carbon (TOC) water (UV -Nanopure™ or UV- MilliQ® generated and hereafter referred to as "ultrapure water/UW"), and air dried completely before capping. Glass vials are easiest to prepare and ensure that they are clean. These are emptied, rinsed 3x with UW, dried and heated at 450°C for ≥4 hours to remove organics (manufacturer's maximum recommended working temperature for this type of borosilicate glass is 500°C.) The use of Polytetrafluoroethylene (PTFE) lined silicone septa or cap is recommended for the glass vials, and it is recommended that those be soaked in 1M HCl, rinsed with ultrapure water and dried between uses. See step by step standard operation procedure (SOP1) for detailed cleaning procedures and Appendix B for suggested equipment.

2.2 Filters

DOM is operationally defined as the fraction of total organic matter passing through a submicron filter (i.e., 0.22 - 0.7 µm). In practice, oceanographers commonly use Whatman® GF/F filters (nominal pore size 0.7 µm) as the cutoff between particulate and dissolved organic matter fractions (Knap et al. 1996). These borosilicate glass fiber filters are most commonly used for

bulk measures of DOC and TDN (Carlson et al. 1998) as they can be easily prepared by pre-combustion and the flow rate through the filters is ideal for rapid in-line sampling. GF75 filters (0.3 µm nominal pore size) are also appropriate as they can be combusted, and may be preferred when concurrently measuring subfractions of DOM (such as amino acids) where maximum particle exclusion from the dissolved pool is desired. For bulk dissolved organic matter concentrations, we do not resolve differences between GF75 and GF/F use. The GF75 and/or GF/F filters are prepared by combusting at 450°C for 4 hours in foil packets. We do not exceed 450°C because the filter matrix may become altered at higher temperatures. After the filter packets are cooled, the foil pack containing filters is sealed into secondary plastic bags until use. It is advised to pack only enough filters needed for a single cast in each foil packet to avoid long exposure of combusted filters to airborne volatile organic contaminants

In preparation for sampling, a filter is placed into a pre-cleaned 47 mm polycarbonate filter cartridge (see SOP2). Gravity filtration is always recommended to avoid cell rupture and tearing of filters. Refer to SOP1 for details on filter preparation and in-line cartridge cleaning, and Appendix B for relevant product information.

2.3 Niskin sampling procedure

It is important to select a DOM-clean workspace in the shipboard laboratory (i.e., well ventilated and free of volatile organics, organic fixatives, fresh paint, permanent markers, smoke, etc.) and to maintain this area in a clean fashion for storing, cleaning and preparing sampling gear on a daily basis. Cover the benchtop with absorbent liner and replace frequently. The sampling equipment (e.g., filter holders, silicone tubing) should be cleaned in a dilute acid solution (1M HCl) prior to each use (SOP1). It is recommended that pre-printed labels be used; alternatively, labeling with markers should only be done when vials are tightly sealed as permanent markers contain solvent that may contaminate samples.

Gloves should be worn during DOM collection and handling to minimize contamination. Powder-free nitrile, polyethylene and latex-free vinyl gloves are safe options as they have low organic leaching when exposed to seawater. Because DOM samples can be easily contaminated, it is recommended that collection from the CTD rosette occur as soon as possible after gas sampling. It is also recommended that anyone sampling from the rosette prior to collection of DOM samples wear gloves. If that is not possible, every effort must be made not to touch the Niskin bottle's spigot (i.e., the path of the water stream, from Niskin to sample bottle, must be kept very clean). Most importantly, any sampling preceding DOM must avoid use of grease or Tygon® tubing as these are known to contaminate DOM at the µmolar level. If Tygon® is unavoidable for other samplers, supplying a small silicone tubing section as an adapter between the Niskin and Tygon® is advised. Mechanical grease from ship operations (e.g., CTD wire lubricant) should never come in contact with the Niskin bottle's sampling valve or spigot.

Whether or not a sample is filtered prior to analysis depends on the goal of the measurement. If DOC and TDN are the variables of interest, then all samples should be filtered. However, the handling of filters and apparatus can increase potential for contamination, so in some cases filtration can be bypassed. In most oligotrophic waters or depths >250m away from ocean margins, DOC is the dominant component of TOC, exceeding the carbon inventory of organic

particles by several orders of magnitude (Cauwet 1979; Hansell et al. 2012). In high productivity areas, a substantial portion of organic carbon in the euphotic zone may be present in particulate form, and many of those particles may be large and heterogeneously distributed in a sample, such that these sample types should be filtered. Figure A2 presents vertical profiles of TOC and DOC in contrasting regions as an example. As important components of global carbon cycles, accurate measurement of each fraction is critical for constraining mass balance of carbon in ocean models. For consistency when sampling in both oligotrophic and eutrophic environments, filtering is recommended, at a minimum, for all ≤250-m samples. In oligotrophic environments, one filter may be re-used for several consecutive samples around the rosette to conserve resources. It is recommended to filter samples from the greatest depth to the shallowest; particulate concentrations will typically increase nearer the surface ocean, which could cause the filter to clog or the particles to disrupt, requiring more filters to be used for one station. Studies have shown that DOC can sorb to active sites on GF/F filters, which raises the question whether filtration through GF/F strips organic matter from the DOC filtrate. Approximately 60 mL of sample are passed through a new filter during the flushing and vial rinsing procedure. Tests after the filter and bottle rinsing step show no further stripping of organic carbon from DOC filtrate can be resolved at the μmol kg^{-1} level (Figure A3). These results suggest that sorption of dissolved organic matter to combusted GF/F filters saturates the active sites on a combusted filter rapidly (within ~ 60 mL) and is not a DOC stripping concern when filtering samples for bulk DOM analysis.

Samples should be gravity filtered at the rosette via an in-line filter cartridge housing a combusted GF/F filter and attached directly to the Niskin spigot via acid clean platinized silicone tubing (Cole-Parmer, Appendix B). This type of platinum cured silicone tubing offers durability and minimizes organic leaching compared to Tygon®. Rinse the sample container and cap three times with sample water prior to filling it three quarters full (refer to SOP2 for step-by-step instructions). It is important to collect sufficient volume for analysis and to minimize surface area to volume ratio of the container (a minimum of 15 mL in a glass vial or 30 mL in an HDPE bottle for each analyte desired, DOC or TDN) while also taking care to not overfill the sample container. It bears repeating that care should be taken during sampling to avoid any obvious contaminants such as cigarette smoke, paint fumes, excessive engine fumes in the sampling bay, or organic solvents in the laboratories, etc. Sampling equipment (combusted filters and glassware in particular) should be kept carefully sealed right up to the time of sampling to avoid sorption of airborne contaminants onto cleaned surfaces. Always log unusual events regarding the samples; add notes that may be useful for explaining results.

Example Collection Plan- For U.S. GO-SHIP sections, 24-36 Niskin bottles (24-36 depths over the entire water column) are sampled at alternating stations (i.e., station sampling for DOM occurs at ~60 nautical mile intervals). For other campaigns the sampling decisions with regard to horizontal or vertical resolution will depend on the scientific aims of the project. To assess sample handling error, it is recommended that replicate samples be collected randomly from a subset of depths over a hydrographic profile. For current U.S. GO-SHIP sections, the standard practice is to replicate 2 Niskin bottles per 36 bottle cast (~6% replication in sample set).

2.4 Sample preservation and storage

Many DOM analytical instruments are not stable enough to conduct at-sea analyses; thus, safe storage of samples is essential. After collection at the rosette, samples can be preserved and stored for later analysis in a shore-based laboratory using several methods.

Frozen storage- Seawater samples collected into glass should not be stored at temperatures below -20°C as colder temperatures (e.g., < -40°C) can result in breakage of the glass upon thawing. If storing at temperatures < -20°C is the only option available then the use of plastic sampling containers (HDPE or PC) is a safe alternative for bulk DOC/TDN analyses. Frozen samples that have been acidified should only be stored in glass as plastic will leach upon long term exposure to acid. For samples collected in plastic and not acidified it is important to freeze as promptly as possible after collection to avoid changes in organic matter due to biological activity. Upon storing frozen samples, it is imperative that these samples not be overfilled as water will expand with freezing. Tests have shown that a salinity gradient is set up during freezing with high brine / high DOC water potentially being displaced through the cap threads if the bottle is overfilled (Figure A4). This extrusion results in a diluted DOM concentration, rendering the sample compromised. Care should be taken to freeze samples in an upright position, and check that caps are tightly sealed prior to freezing and storage and again before shipping. Segregate frozen samples from any other volatile organic material in storage to prevent airborne volatile organic contamination. Frozen samples can be safely stored for periods of years (Appendix A). Prior to analysis, frozen samples must be completely thawed at room temperature and homogenized. Use of a mechanical device such as a vortex mixer is ideal. The mixer should be set to a high enough speed that a vortex is visible and extends from the surface of the sample through to the bottom of the container.

Acidified and liquid storage- Shipping of frozen samples is costly and often unreliable; thus, an alternative to frozen storage is collection in glass vials, acidification and storage in liquid form. Samples should be acidified soon after collection by adding 2µl of 4M hydrochloric acid (ACS or trace metal grade) per 1 mL of sample. This ratio of acid/sample should bring the sample to pH 2-3. Periodically check samples to ensure this low pH is reached. This can be done by drawing out a few mL of sample (using a non-sterile tip and DOC clean pipette) and using this volume to wet a pH strip. Never immerse a pH strip directly into a sample as this will result in contamination. At pH 2-3 biological activity is halted, ensuring safe storage, and inorganic carbon species are converted to CO_2 and later degassed from the sample solution with sparging on the TOC system at the time of analysis (sparging at the time of sample collection is not recommended as less handling is best for preventing contamination). A repeater pipette with an acid-cleaned tip is recommended for acid addition (refer to SOP2). It is recommended to prepare a 100 – 500 mL batch of 4M HCl using high purity (Certified ACS Plus, Appendix B) acid diluted with ultrapure water and then aliquot and seal the 4M HCl into 1-2 mL pre-combusted glass ampoules. It is advised that a new ampoule be employed for each new station sampling and unused remnants discarded to avoid contamination.

The timing of acidification will be dependent on the biological activity of the environmental system but open ocean samples remain stable if acidified within an hour of collection. It is advised that samples be stored in a dark, volatile organic-free lab space at room temperature, or a refrigerator (4°C) or environmental chamber (<20°C). Never use caps with pierced septa when

collecting samples as contamination of sample can occur during shipping and storage; if septa are used, always ensure that the PTFE lining faces the sample (Figure A5). With these precautions, tests show that acidified samples can also be stored on the order of years (Figures A6 and A7).

Shipping of acidified samples in glass vials is a viable option as long as the shipping container is well cushioned to prevent breakage during shipping. Foam inserts in corrugated plastic field boxes, or cardboard flats with sample dividers placed into a rigid container or cooler work well (see SOP2 and Appendix B for parts). Most importantly, samples in plastic or glass should be tightly capped and remain upright to minimize contamination during transport.

3. Instrumentation

There are several custom and commercial HTC systems that have been described previously (Peltzer and Brewer, 1993; Benner et al., 1993; Hansell, 1993; Carlson et al, 1994; Sharp et al., 2002; Hansell and Carlson, 1998); however, we find the Shimadzu TOC-V_{CSH} and the newer TOC-L_{CSH} series are high throughput HTC instruments that provide appropriate ranges, reliability and sensitivity for seawater measurements. Thus, while other instruments may also be appropriate for HTC analyses of seawater, we limit our discussion to the Shimadzu TOC-V and TOC-L systems in this best practices guide. These models are coupled with Shimadzu ASI-V/ASI-L auto-samplers, which accommodate 40 mL glass vials for added processing efficiency. A Shimadzu TNM-1/TNM-L Analyzer unit can be coupled to the instrument to provide TDN analysis. The TNM units share the combustion tube and catalyst with the TOC unit so that maintenance is minimized for the added operation. In this system configuration it is possible to run DOC or TDN analysis stand-alone, or to run coupled analyses (DOC & TDN) as each detector functions independently.

DOC Analysis- The DOC content of seawater is defined as the concentration of carbon remaining in a seawater sample after particulate and inorganic carbon have been removed. DOC concentrations are determined by an HTC method performed on a modified Shimadzu TOC as previously described by Carlson et al. (2010). A pre-acidified sample (filtered at time of collection to remove POC) is drawn into a 5 mL injection syringe and sparged (100 mL/min) for a minimum of 1.5 minutes with CO_2-free gas, producing a sample containing only non-purgeable organic carbon. Replicates (100 µL) of the resulting sparged sample are injected into a quartz combustion tube heated to 680 - 720°C, where the organic carbon is combusted / oxidized to CO_2. The resulting CO_2 and carrier gas (flow rate of 168 mL/min) are passed through the Shimadzu internal electronic dehumidifier, a magnesium perchlorate water trap (when nitrogen analysis is not being conducted), a copper mesh halide trap, a 0.45 µm particulate filter, and then into the Shimadzu non-dispersive infrared gas analyzer (NDIR). The CO_2 signal results in a sample peak in which the peak area is integrated with Shimadzu chromatographic software.

TDN Analysis- The TDN content of seawater is similarly defined as the concentration of combined nitrogen remaining in a seawater sample after particulate nitrogen has been removed. TDN is determined independently via the high temperature combustion method (Walsh, 1989) on a modified Shimadzu TOC with attached Shimadzu TNM analyzer. Carrier gas is supplied at 168 mL/min flow rate, and ozone (O_3) is generated by the TNM unit at 0.5 L/min flow rate.

Replicates (100µL) of filtered sample are injected into the combustion tube heated to 720°C, where the TN in the sample is converted to nitric oxide (NO). The resulting gas stream is then passed through the Shimadzu internal electronic dehumidifier, a copper mesh halide trap, a 0.45 µm filter, and into the chemiluminescence analyzer, where the dried NO gas reacts with O_3 to produce an excited nitrous oxide. The resulting fluorescence signal is detected by the Shimadzu TNM chemiluminescence detector. The resulting peak area is integrated with Shimadzu chromatographic software. Note the absence of a magnesium perchlorate water trap in this configuration as this trap removes NO (see below, Section 3.1).

Coupled DOC/TDN Analysis- A dual method is possible using Shimadzu software to provide both DOC & TDN analysis on one sample simultaneously. A filtered sample is analyzed for each analyte as detailed above, with the TOC furnace set to 720°C, the omission of the in-line magnesium perchlorate water trap, and each detector reporting separately. Coupled analyses can lead to backpressure in the analytical system that can affect the NDIR peak quality; thus, it is recommended that analysts closely monitor the quality of peak shape of NDIR (DOC) output under dual analyses mode of operation.

3.1 Modified Shimadzu HTC system for signal optimization

Users should first refer to the manufacturer's instrument manuals for the specifics on start-up, operation, and maintenance. To optimize for seawater samples, the operating conditions of the Shimadzu TOC analyzers are slightly modified from the manufacturer's model system.

The condensation coil is removed and the headspace of the purewater trap is reduced to minimize the system's dead space. The purewater trap is a glass reservoir that accumulates water vapor that condenses upon exiting the combustion tube. This reservoir can be used to determine the instrument blank- if properly maintained this can result in blanks equivalent to analysis of laboratory ultra-pure water injections. However, we do not recommend this option as we have found accumulation of sediment in this trap can damage the syringe. In addition, frequent sampling of the condensate from this trap can alter the "dead" space within the system that can affect peak shape and consistency of results throughout an analytical run. We found that keeping a reduced headspace in the purewater trap and removal of the condensing coil results in better peak shape. See Appendix C for details.

Seawater contains on average ~ 2.3 mmol kg^{-1} of dissolved inorganic carbon (DIC) in the form of CO_2, bicarbonate and carbonate. DIC is removed from the sample prior to injecting the water into the combustion column by acidifying to a pH of 2-3 (4M HCl, ACS grade, Appendix B) and sparging with CO_2-free carrier gas for several minutes (i.e., 3 mL of sample sparged for 1.5 minutes at a flow rate of 100 mL min^{-1}). After sparging, an aliquot of sample (50 -200 µL depending on DOC concentration) is injected into the combustion column. The organic carbon is combusted to CO_2 and the carrier gas moves the resulting water vapor, halides and CO_2 out of the column through a series of traps and filters in order to purify the CO_2 signal.

Water vapor interferes with the NDIR detection and must be removed. After passing the combustion column the carrier gas is passed to the Shimadzu electronic dehumidifier, a chilled Peltier cooler set to 1ºC, where a significant fraction of the water vapor condenses and is

removed from the gas stream. We have found that the addition of an in-line water trap containing magnesium perchlorate $Mg(ClO_4)_2$ (Appendix B) helps to further remove water vapor, sharpens the peak shape and minimizes tailing peaks of the NDIR trace; thus, improving the reproducibility of injections. For DOC analyses, the $Mg(ClO_4)_2$ trap should be replaced at a minimum of every two days or as soon the desiccant appears saturated (see Appendix D for instructions). Note that the $Mg(ClO_4)_2$ trap should not be included if TDN is being measured simultaneously as moist $Mg(ClO_4)_2$ removes NO and thus interferes with TDN analysis.

Halogens released with the combustion of seawater can also interfere with the NDIR detection of CO_2; thus, it is imperative to remove halogens from the post combustion gas stream. The proprietary Shimadzu halogen trap (Part No. 630-00992) or bubbling the gas through AgCl solution are effective means of removing halogens. A cost-effective alternative is to pack a halide trap with Cu wool (Appendix B) and connect in line just after the $Mg(ClO_4)_2$ trap. The Cu wool will show signs of discoloration after exposure to halogens; it should be changed when the discoloration reaches within 2 cm of the trap outlet (Appendix D). It is recommended that the $Mg(ClO_4)_2$ and halide traps be placed vertically so gas flow is up through the bottom of the traps.

Prior to entering the NDIR the gas passes through a membrane filter (0.45 µm, Appendix B) to remove particles from the carrier gas. Using a digital flow meter, care should be taken to monitor the carrier gas flow rate before and after the particle trap to ensure that there is no reduction in flow rate. If there is a drop in the flow rate by more than 3 mL min^{-1} from that entering the column the filter should be replaced.

It is recommended that every time the column is replaced the flow rate be checked at points going into the injection port, at the base of the column, before and after the $Mg(ClO_4)_2$ and halogen traps and before and after the particle filter (Appendix C).

3.2 Carrier gas

There are several options regarding the CO_2-free carrier gas needed to operate the HTC system, but high quality is required to obtain low background levels in the detector. Compressed gases such as Ultra High Purity (UHP 99.995%) oxygen or nitrogen can be used. If compressed air is available, a cost-effective option is to integrate a Parker Balston® TOC gas generator into the gas plumbing of the HTC system. This system utilizes catalytic oxidation and pressure swing absorption technologies to remove hydrocarbons and generate CO_2-free gas. Over the long term the gas generator option is a stable and low-cost alternative to compressed gas cylinders. The CO_2-free gas is used both as a carrier and a sparging gas and should be supplied at a pressure of 200-300 kPa.

3.3 Combustion column

Shimadzu offers two sizes of columns, a small diameter column (18 mm ID x 20 mm OD, fits TOC-V and TOC-L) and a large diameter column (27 mm ID x 30 mm OD, TOC-L only with special adaptor kit) that can accommodate more salt loading before changing or reconditioning the column. In our experience, a properly conditioned analytical system can process approximately 30 – 36 seawater samples per analytical run (not including blanks, standards and seawater references) with the small diameter, and 42-48 seawater samples per analytical run with

the large diameter column. Direct comparisons show that either configuration is acceptable for seawater samples (Figure C1). After 4-5 runs (~400-900 saltwater injections), with either column type, we typically observe salt buildup in the column resulting in system back pressure that manifests as poor peak shape of the non-dispersive infrared (NDIR) trace and subsequent poor injection and reference replication. Thus, it is recommended that under high-throughput processing of seawater samples the combustion tube, packing material and various traps be exchanged or cleaned on a weekly basis as described below (refer to Appendix D for an example preventative maintenance schedule).

The combustion tubes are comprised of quartz glass that can be purchased from Shimadzu directly or alternatively, if the researcher has access to a glass blowing shop or a preferred vendor, the quartz can be fabricated using the dimensions in Figure C2. See Appendix B for associated part numbers.

Packing of the combustion column has also been slightly modified from vendor guidance:

Small column configuration - The small column is packed with supplies listed in Appendix B as follows (Figure C2): A 13x13mm single layer of Platinum (Pt) mesh is placed at the base of the column to support the bed of Pt-alumina catalyst beads. 2 mm diameter Pt-alumina beads are added to within 120 mm of the top of the column. An additional layer of Pt gauze, loosely rolled into 5 mm spheres, is placed in a single layer on top of the platinized alumina beads. These Pt spheres serve three purposes: 1) they provide a solid thermal mass that allows for rapid combustion of the sample; 2) the solid surface protects the integrity of the underlying alumina beads; thus, preserving the matrix geometry and preventing the pulverization and "worm holes" that develop if sample is injected directly onto the Pt alumina beads; and, 3) the larger Pt spheres allow salt to penetrate deeper into the column matrix material, thus slowing the development of salt plugs while maintaining good gas flow for a longer period of time. Our experience is that adding Pt pillows improves the peak shape of the NDIR trace and replication of injections, and extends the duration of the column's life when analyzing seawater. Note: we do not recommend using quartz wool to separate the layers of packing material as it devitrifies as salt is loaded onto the column, creating void spaces; thus, changing the geometry of the column's packing material throughout its lifetime.

Large column configuration- The large column is packed with components listed in Appendix B as follows (Figure C2): A ceramic mesh disk is placed at the base of the large column to support the bed of Pt-alumina catalyst beads. The 5 mm diameter Pt- alumina beads are added to within 200 mm of the top of the column, and 2 mm diameter platinized alumina beads are added on top of the larger catalyst beads to a level 120 mm from the top of the column. The smaller catalyst is then topped with 6-10 Pt spheres as described above.

Columns should be removed and reconditioned weekly or at any time poor data quality arises. The column will devitrify as salt infuses into the quartz matrix, becoming "chalky" and fragile after a number of heating and cooling cycles; thus, care must be taken to inspect columns for signs of weakness or cracks when reconditioning. Reconditioning of columns includes removing Pt spheres, catalyst and mesh from the column, flushing the quartz column and all Pt contents with ultrapure water to remove salt, then combusting the quartz column and Pt contents at 450°C

to dry and then re-packing with flushed contents. The Pt mesh and spheres can be reused for 4-6 weeks if cleaned properly; i.e., soak in water and agitate to remove the salt buildup. Pt alumina catalyst should last approximately 12-16 days of analysis. Always let the column and its contents cool prior to reconditioning or repacking columns. Appendix D provides a step-by-step description of column reconditioning.

3.4 Detectors

Per the Shimadzu user manual, the NDIR cell in the TOC-V and L series achieves a detection limit of 4 μg C L^{-1} (0.3 μmole C L^{-1}), the highest level for the combustion catalytic oxidation method. The Shimadzu TNM system uses a chemiluminescence detector to measure the excited NO_2 signal created by combining NO gas, generated through HTC at 720ºC, with O_3 inside the detector. Per the manufacturer the chemiluminescence detection limit for TN is ≤ 0.05 mg L^{-1} (3.57 μmole N L^{-1}).

3.5 Software

The TOC analyzer includes Shimadzu chromatographic software designed to enable PC control of the entire system; it includes programming the auto-sampler, calibration curves, acquire and display output in real time, peak area integration and quality control flags for raw data. Raw area counts are exported as a tab delimited text file for further processing and calculation of carbon and/or nitrogen concentration.

4. Operational Procedures

The procedures outlined below are recommendations based on HTC method conducted with a Shimadzu TOC- V_{CSH} or TOC-L_{CSH} system. Operations on other commercial or homemade instruments will vary.

Daily operation and procedures:
1) Instrument preparation and maintenance; system blanks
2) Standard curve preparation
3) Reference materials
4) Sample unknowns
5) Export raw data/calculate sample concentration

4.1 Instrument preparation and daily maintenance; system blanks

System readiness is assessed each day prior to running samples. Instrument baseline should sit at 0 mV prior to starting, indicating electrical noise is minimal and no immediate issues with the NDIR or gas generator are evident. Shimadzu software provides a general “background monitor” to indicate instrument readiness (baseline position and stability, furnace and dehumidifier temperatures). If baseline position or fluctuations exceed the presets, the instrument automatically indicates a not ready state.

The system blank is assessed by injecting a volume of low carbon water (LCW) identical to the volume used during sample analysis (100µL) and measuring peak area. This blank represents the background CO_2 signal from the system (catalyst and combustion tube) and should be subtracted from each sample analyzed. True blank water should have DOC below the limit of detection. Shimadzu recommends that blanks be sampled from the internal pure water trap to achieve this but in our experience this operation changes the "dead space volume" within the analytical system, altering the peak shape and affecting the machine blank over the course of the run. It is recommended that blank water be generated using a commercial ultrapure water system coupled with UV oxidizing kit (i.e., 18.2 MΩ resistance Nanopure™ systems with ultralow organics cartridge, UV sterilization and 0.2 µm filter or MilliQ® systems). The Hansell Lab at University of Miami provides LCW (0-1 µmole C L^{-1}) as part of their consensus reference material (CRM) program, and in house LCW concentrations can be cross checked against this. The Y-intercept of the standard curve (made in the same LCW) provides an independent assessment of C or TDN content in the blank water plus the "machine blank".

UV-oxidized blank water is generated daily and placed into pre combusted Pyrex® bottles (500 - 1000 mL). On Shimadzu TOC-V and L systems, plumbing a Teflon™ tube from the blank reservoir to port # 1 on the 8-port valve of the syringe / injector assembly will allow unlimited sample draws from the reservoir, which is necessary for column conditioning and numerous blank analyses throughout any given run. To draw sample from the blank reservoir, assign a sample to vial zero in the Shimadzu sample table; this bottle may be sampled numerous times throughout an analytical run. System blank values will vary across TOC systems due to internal configurations and column use. See Appendix C for an example of typical blank values generated across multiple TOC systems. If the conditioning of a new column is sufficient, blank peaks will decrease and seawater peak areas will stabilize and be highly repeatable (Figs. C6 and C7). We typically spend most of the working day diagnosing the system's readiness. After daily maintenance tasks are completed (refer to Appendix D) a series of 15 blanks is run from port #1, followed by another 15 samples where blanks are then alternated with seawater (fill several vials with the same seawater and place on autosampler, draw several times out of each vial). Once blanks and seawater samples meet these criteria then the column and system are ready to run and a sample set along with standards, blanks and reference waters are prepared for an overnight analytical run.

4.2 Standard curve

DOC - Systems are standardized daily with a four-point calibration curve of either glucose or potassium hydrogen phthalate (KHP) made in LCW. The working standard concentrations are evenly distributed to bracket the dynamic range of oceanic DOC concentrations (typically 25, 50, 75, 100 µM C).

TDN – A five-point calibration curve of potassium nitrate (KNO_3) dissolved in LCW is used (typically 3, 8, 16, 24, and 48 µM N) to bracket oceanic concentration ranges.

Standards are analyzed at the start of each day's run, in advance of the samples, to monitor system response. Working standards are prepared gravimetrically each week. These are independent dilutions prepared from a concentrated primary stock, which is made monthly in

LCW. The resulting standard curve is used to calculate DOC and TDN concentrations in post processing steps. This daily response factor should be tracked for each system in use and rarely changes over the lifetime of a column. Alterations in flow through the columns and into the NDIR are also monitored as these will change the response factor. Refer to Section 5 for detailed standard preparation guidelines.

4.3 Reference material

A critical component for maintaining accuracy and inter comparability between laboratories and within laboratories through time is the routine use of seawater references. All samples should be systematically compared to a set of references that include or have been calibrated against consensus reference material (CRM) like that provided by University of Miami's CRM program (Hansell 2005). These CRMs include deep, mid and surface seawater as well as LCW references that are calibrated by independent international DOM analysts. For practical purposes, it is recommended that individual labs generate a set of "in house" reference materials in large volumes that are calibrated against the CRMs. An example of "in house" reference preparation could include the collection of 10- 20L each of filtered (GF/F) from a vertical DOM gradient i.e., surface, mesopelagic and bathypelagic seawater, acidification to pH 2-3, and partitioning into several hundred glass vials (35 mL) for each depth. Alternatively, if unable to access large volumes of seawater, a batch of artificial seawater can be prepared, organic carbon compound added and acidified, which can serve as an "in house" reference. Any "in house" reference water should be calibrated against CRMs regularly to ensure the carbon concentration remains stable (within +/- 3σ of the calibrated value). Stored properly, these references remain stable for at least a year. It is recommended that the set of "in house" references, which bracket the dynamic range of the sample set, be analyzed several times throughout a given analytical run (i.e., every 8 -10 samples) as a diagnostic of the system's stability and quality assurance of the data. This practice of using calibrated "in house" references over long spans of time proves especially useful to ensure run-to-run comparability.

4.4 Running samples (NPOC method)

Seawater collected into 40 mL glass vials, acidified at time of collection and stored in liquid form can be loaded directly onto the auto-sampler. It is customary in our labs to exchange only the septa prior to analysis, switching the unpierced septa used at collection for a pierced septa that is used (and re-used) only during analysis on the TOC instrument. When the TOC run is completed, the unpierced septa is returned to the same vial for placing samples back in storage. This sequence allows unpierced septa to be conserved for repeated collections after cleaning. Frozen samples must be: first fully thawed at room temperature (no ice should remain before proceeding), thoroughly mixed via vortex, and transferred to a glass vial if necessary. If sample transfer is required it is recommended that 1-2 mL aliquots of sample water be used to rinse a combusted vial 3 times prior to filling it to a minimum of 15 mL per vial. A sample volume of 15 mL allows for multiple runs on one sample if needed.

"Unknown" seawater samples should be analyzed using the Non-Purgeable Organic Carbon (NPOC) method on the Shimadzu TOC system. See the Shimadzu TOC user's manual for "Principles of NPOC Analysis" and "Analysis-Related Technical Information" (Peak area and

shape). Users may define settings to establish their own method; see Shimadzu TOC user manual for step-by-step details on software method set-up. For seawater samples, the "best 3 of 5" option in the software is commonly used. For this method, 3 mL of pre-acidified sample is drawn into the 5 mL injection syringe and sparged for 1.5 minutes at a flow rate of 100 mL min^{-1} with CO_2-free gas (sparge time should be tested empirically). 100µL aliquots of sample are injected into the combustion tube until at least three replicate injections meet the Shimadzu specified peak area standard deviation (SD) of 0.1 or a CV $\leq$2% , or until five injections are reached (replication criteria is applied separately to DOC and TDN). The resulting DOC or TDN peak area is integrated with Shimadzu chromatographic software. It is recommended that an analytical run be organized so that every 8-10 unknown samples are bracketed by a set of "in house" references (or CRMs) and blanks, and that the total number of unknowns be limited to a maximum of 30-36 per run (42-48 for large columns) to avoid clogging of the quartz column during the run. This set-up also allows ample space for standards, references, and blanks on the 68-place autosampler. See Appendix E for example run log-sheet.

4.5 Data export and processing

An example of post processing: corrections and calculation of concentrations

It is good practice to review the blank, reference and sample peaks after each run to look for anomalies. If the analytical run has proceeded without interruption or errors, then raw peak data are exported for final processing and QA/QC. If an error or interruption is noted then a run is aborted and samples are re-analyzed.

Files are saved as tab delimited text and exported from Shimadzu software for further processing offline. Raw peak data (area) are sorted by sample ID and all injections are grouped and averaged for blank, standard, reference and "unknown" samples. Injections flagged by Shimadzu software as outliers are excluded from area averages, maintaining 3 injections for any given sample. An average machine blank is determined for all blanks throughout a day's analytical run (typically this is an average of at least 10-20 blanks) and is subtracted from all samples, standards and references. A linear regression analysis is performed on the blank corrected calibration standards (4-point glucose or KHP for DOC or 5-point KNO_3 standards for TDN). Calibration curves are not forced through zero and should have a correlation coefficient $\geq$0.995. The slope is used to calculate sample concentrations from peak areas as below:

µmol C or N per L = (average sample area – average machine blank area) / (slope of standard curve)

It is recommended that blanks be analyzed frequently throughout a run as a diagnostic of the system's performance (refer to Appendix E for spacing of 10-20 blanks in a typical run). Blanks for each system should be assessed daily and values should remain within (+/- 3σ) throughout the course of a run. Systematic drift or a rapid shift in blank values outside this range within a given run or between runs over the lifetime of the combustion tube are indicative of a problem within the combustion tube, its packing material, the traps, or an obstruction in the gas flow. If drift or shifts in blank values are detected within a run then the run should be flagged as questionable and rerun as necessary. The flow rate should be checked to determine if clogging or

backpressure in the system has developed. If the problem persists then the combustion tube, packing material and traps should be replaced.

CRM's and / or "in house" references are also used to assess the performance of the analytical system. It is recommended that a set of "in house" references, calibrated against CRMs, be run 3-5 times throughout an analytical run and averaged. If references do not meet calibrated values or stability specifications (within +/- 3σ of the calibrated value, and daily CV for each reference should be ~ 2%) then a maintenance check should be performed on the analytical system, combustion tube and traps changed / reconditioned as necessary, and the run repeated. All references should remain stable over time and across systems. It is recommended that "in house" references be calibrated against CRM approximately every 6 weeks. It is also recommended that several sets of "in house" references be prepared and stored in order to maintain overlapping sets of calibrated material.

5. Standards

5.1 Supplies

It is critical to have accurate concentrations of standard solutions, and for DOC and TDN care must also be taken to avoid contamination during preparation of stocks. For this reason, glass bottles (heated to 450°C for ≥ 4 h) are used for preparing the primary stock solution. Note that volumetric glassware should not be used to prepare standards as high temperature will affect the accuracy of the volumetric graduation. Dry standard compounds should be kept in a desiccator under vacuum to ensure quality. Solutions are prepared gravimetrically at room temperature using analytical balances with 0.0001 g resolution. Larger volume working stocks can be prepared by diluting primary stock into combusted glass bottles using ultrapure water.

Pipettes used for any standard preparation should be DOC clean (use should be restricted to DOC only – never use a pipette that has been used with fixatives or volatiles). Additionally, the use of non-autoclaved pipette tips is suggested, as the sterilization process can result in leaching of organics from the plastic material. All pipette tips should be rinsed with 4 M HCl prior to standard preparation.

5.2 Primary standards

DOC - High grade (≥99.8% purity) potassium hydrogen phthalate (KHP) or glucose are the compounds typically used as a carbon standard. A 10 mmol L^{-1} C primary stock is prepared in ultrapure water as detailed in SOP3.

TDN – High grade (≥99.8% purity) potassium nitrate (KNO_3) is recommended as a nitrogen standard. A 10 mmol L^{-1} N primary stock is prepared as detailed in SOP4.

5.3 Working standards

Working standards are prepared by diluting the primary stock to the desired concentrations using room temperature LCW. At least four different concentrations of working standards are

appropriate (bracketing expected sample concentration range) and should be analyzed daily at the start of each sample run. Refer to SOP3 and 4 for a step-by-step guide to preparing standard solutions using glucose and potassium nitrate as examples.

6. Quality Control

To provide the community with standard measures for the analytical quality of the DOC and TDN HTC method in seawater, we here present guidelines for Quality Control (QC). This consists of an (1) an initial demonstration of laboratory capability (method validation) and (2) guidelines for assessing laboratory performance by the continued analysis of instrument blanks, calibration standards, and reference material analyzed as samples.

6.1 Method validation

All parameters are defined and calculated according to the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) in establishing a uniform approach for performance characteristics of the chemical measurement process (IUPAC 1995).

Critical Value (L_c) – Determined using blanks (for this method blanks are ultrapure water sourced from Nanopure™ systems with low TOC cartridge, UV light and 0.2µm final filter) according to IUPAC 1995 (Equation 11):

$$L_C = t_{1-\alpha,\nu}\, s_o$$

Blanks were analyzed in replicate over separate dates (a minimum of 30 blanks per day over 7 runs for DOC, and >20 blanks per day over 4 runs for TDN). See Appendix F for details.

Limit of Detection (L_D) - The method detection limit is established using a spiked water sample at low concentration as in IUPAC 1995 (Equation 14):

$$L_D = \delta_{\alpha,\beta,\nu}\, \sigma_o \approx 2t_{1-\alpha,\nu}\, \sigma_o$$

For this method 25 µmol C L^{-1} samples for DOC and 3 µmol N L^{-1} for TDN were prepared and analyzed over separate dates (5 individual batches over 7 runs for DOC and 4 batches across 4 runs for TDN). See Appendix F for details. The detection limit should be determined annually, or whenever there is a significant change in instrument configuration or response.

Limit of Quantification (L_Q) – Expressed using IUPAC default relative standard deviation (RSV) of 10% and using lowest calibration standard (IUPAC 1995, Equation 22):

$$L_Q = 10\, \sigma_Q = 10\, \sigma_o$$

For this method computed using lowest calibration standards (25 µmol C L^{-1} for DOC and 3 µmol N L^{-1} for TDN). See Appendix F for details.

Table 1: Method validation results for analysis of DOC and TDN in seawater using the HTC method.

Characteristic	**DOC (µmol C L^{-1})**	**TDN (µmol N L^{-1})**
Critical Value (L_C)	2.5	0.5
Limit of Detection (L_D)	4.3	0.9
Limit of Quantification (L_Q)	11.6	2.0
Range of typical seawater samples	32 – 86	3 – 50

Note – typical seawater DOC and TDN concentration ranges are well above the L_C, L_D and L_Q indicating the HTC method is appropriate for analyses of DOC and TDN at typical seawater concentrations.

6.1.2 Analytical quality limits

Accuracy – Evaluated by use of consensus reference material as a control (there is no national or international standard for seawater DOC). The community has accepted the CRM distributed by the Hansell Laboratory, Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami. Concentrations should remain within range of consensus values (as reported by Hansell Lab: https://hansell-lab.rsmas.miami.edu/consensus-reference-material/index.html) to within ± 2% (for DOC) and ± 2-6% for TDN (depending on the concentration range). See Appendix G for details.

Precision

Repeatability – Best achievable internal precision can be assessed through repeated observations of replicate sample vials over a short period of time. Conditions such as instrument type and operator should remain constant. See Appendix G for details.

Reproducibility – The external complement to repeatability, assessed by analyzing identical batches of samples with the same method among different laboratories to evaluate how reproducible results are. This method utilized intercomparisons performed on batches of reference waters between the Carlson and Hansell DOM labs from 2018-2019. See Appendix G.

Table 2: Summary of analytical quality limits for analysis of DOC and TDN in seawater using the HTC method

	DOC	**TDN**
Accuracy [range]	±2% [40-75 µmol C L^{-1}]	±2% [8-32 µmol N L^{-1}] ±6% [4-6 µmol N L^{-1}]
Precision - repeatability	±0.6 µmol C L^{-1}	±0.7 µmol N L^{-1}
Precision - reproducibility [low/mid/high range]	±0.6 µmol C L^{-1} @[39 µmol C L^{-1}] ±0.6 µmol C L^{-1} @[62 µmol C L^{-1}] ±1.6 µmol C L^{-1} @[72 µmol C L^{-1}]	±0.2 µmol N L^{-1} @[5-10 µmol N L^{-1}] ±0.3 µmol N L^{-1} @[20-30 µmol N L^{-1}] ±1.6 µmol N L^{-1} @[40 µmol N L^{-1}]

6.2 Assessing laboratory performance

As outlined in Section 4 and 5, the use of blanks, calibration standards and reference materials provide ongoing checks on instrument performance. Once validation exercises have been conducted and the method established in a laboratory ongoing assessment of data quality should occur on a frequent basis in order to maintain tight quality control. The table below presents a summary of recommendations for assessing DOC and TDN data runs using the HTC method as presented here.

Table 3: Suggested quality control criteria and guidelines

QC Indicator	Acceptance/Action Limits	Action	Frequency (per run)
Consensus Reference Material (CRM)	The value should fall inside the reported consensus range	If the CRM falls outside of the reported range, rerun	5 ampoules or vials of CRM per reference calibration run. Must overlap with new batches of in-house reference material.
In house Reference Material (calibrated against CRM)	within +/- 3σ of the calibrated value	If outside of the 3σ, rerun	Minimum 2 vials of each deep & surface reference per run, with 2-3 observations per vial.
Calibration Curve - Correlation Coefficient (R)	$\geq$0.995	If <0.995, rerun	4-5 point curve over the full analytical range (~25-100 µmol C L^{-1} for DOC and ~3-50 µmole N L^{-1} for TDN), analyzed at the start of each day's run prior to samples

6.3 Quality Assurance (QA)

If a run passes the QC specifications outlined above for analytical performance, then the data are accepted and further scrutinized in the context of collection and additional metadata available. If the run did not pass these initial requirements, the system is checked and the entire run is repeated.

6.3.1 GO-SHIP data compilation and assessment

For GO-SHIP, DOM data are compiled using shipboard logs and merged with bottle data files containing any other chemical and physical data available and then plotted in Ocean Data View (Schlitzer, R., Ocean Data View, https://odv.awi.de, 2021). Initial plots of vertical profiles and / or contour plots are helpful in identifying potential outliers. Any samples outside of a reasonable range for oceanic DOC/TDN values are flagged as potentially contaminated or suspected of handling error (values <30 or >90 µmol C kg^{-1}, <3 or >50 µmol N kg^{-1}).

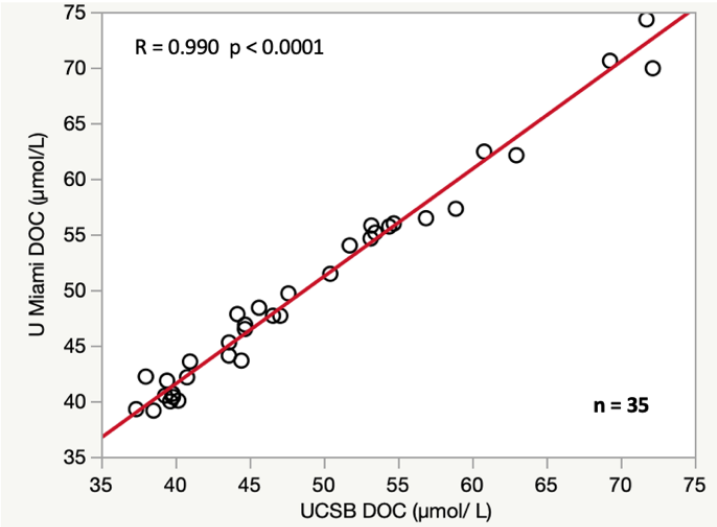
Flagged samples are either compared against replicates or re-analyzed to confirm. If analytical errors are suspected, entire profiles or sample subsets (including problematic value and surrounding samples) are re-analyzed. Upon re-analysis of sample, if analytical specifications are met and data remain anomalously high or low then the data are reported but flagged as questionable or bad according to WOCE quality flag codes (Table 4).

Table 4. WHP bottle parameter data quality codes
(https://www.nodc.noaa.gov/woce/woce_v3/wocedata_1/whp/exchange/exchange_format_desc.htm)

WHP bottle parameter data quality codes	Description
1	Sample for this measurement was drawn from water bottle but analysis not received. Note that if water is drawn for any measurement from a water bottle, the quality flag for that parameter must be set equal to 1 initially to ensure that all water samples are accounted for.
2	Acceptable measurement.
3	Questionable measurement.
4	Bad measurement.
5	Not reported.
9	Sample not drawn for this measurement from this bottle.

6.3.2 Interlaboratory data comparisons

It is recommended that samples and references be shared periodically between analytical groups to ensure interlaboratory comparability. Figure 1 is an example of intercomparisons between the University of Miami and UCSB DOM laboratories.



Intercept	Slope	LowerCL	UpperCL	Alpha
2.886968	0.96601	0.919523	1.014848	0.05000

Figure 1. Results of interlaboratory comparisons conducted between UCSB and University of Miami between 2017- 2018. Samples include comparisons of CRMs, in-house references, and field profiles collected from various locations in the Pacific and Atlantic Oceans. Samples were

shared equally between the groups for analysis. Correlation coefficient shows a strong relationship between UCSB and UMIAMI data ($R = 0.990$, $p < 0.001$). Orthogonal regression (univariate variances, prin comp) using JMP software (JMP®, Version <*15*>. SAS Institute Inc., Cary, NC, 1989–2021) gives a 0.919-1.015 confidence interval for the slope, which includes 1.0 and shows strong agreement between the values reported by each laboratory across a broad dynamic range, providing confidence in accurate and precise results for GO-SHIP data collected and analyzed as described in this best practices guide.

7. Documentation

7.1 DOM analysis reports

The following are examples of metadata included in GO-SHIP DOM cruise reports:

- Cruise designation and principal investigator(s)
- Names and affiliations of technicians who collected DOM samples at sea
- Number of stations occupied and samples collected (sampling frequency)
- Sampling and storage procedures
- Names and affiliations of technicians who analyzed DOM samples on-shore
- Number of samples analyzed
- Methods of analysis (equipment & methodology)
- Data processing procedures and Quality Control (calculations, accuracy, precision and detection limits, CRM information)
- Any details of problems or trouble-shooting that occurred with sampling or analysis
- Scientific references

7.2 Bottle data files

Data from DOM analysis (DOC and TDN) is merged with CCHDO bottle exchange files based on sample identifiers (station/cast/depth/bottle ID).

Once data are merged with other chemical parameters in the bottle file, dissolved organic nitrogen (DON) is calculated as the difference between TDN and DIN [$NH_4^+ + NO_3^- + NO_2^-$]. As DON is a derived variable, it is not reported (i.e., not included in the bottle file).

Final results are reported in units of μmol kg^{-1}. Where possible direct measure of sample salinity and analytical temperature are used to calculate average seawater density. In practice we have found that applying an average seawater density of 1.027 kg m^{-3} to open ocean water column DOM samples, compared to direct measure of sample density results in a difference of less than 0.01 μmole kg^{-1} (i.e., less than analytical resolution). However, when salinity and an average analytical lab temperature are available or in regions where salinity varies strongly, a more accurate density correction is determined and applied for each sample. Each parameter includes a field for quality control flags.

References

Aluwihare, L. I., and Meador, T. (2008). "Chemical composition of marine dissolved organic nitrogen," in *Nitrogen in the Marine Environment*, eds D. G. Capone, D. A. Bronk, M. R. Mulholland, and E. J. Carpenter (Burlington, MA: Academic Press), 95–140.

Benner R. and Strom, M. (1993). A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. *Mar. Chem*. 41, 153-160.

Bronk, D. A. (2002). "Dynamics of DON," in *Biogeochemistry of Marine Dissolved Organic Matter*, 1st ed., edited by D. A. Hansell and C. A. Carlson (Academic Press, San Diego, CA), 153–247.

Carlson, C.A., Ducklow, H.W. and Michaels, A.F. (1994). Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea. *Nature* 371, 405–408.

Carlson, C. A., Ducklow, H.W., Hansell, D.A., and Smith, W.O. (1998). Organic carbon partitioning during spring phytoplankton blooms in the Ross Sea Polynya and the Sargasso Sea. *Limnology and Oceanography* 43, 375-386.

Carlson, C.A., Hansell, D.A., Nelson, N.B., Siegel, D.A., Smethie, W.M., Khatiwala, S., Meyers, M.M., and Halewood, E. (2010). Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. *Deep-Sea Res. II* 57, 1433-1445.

Cauwet, G. (1978). Organic chemistry of seawater particulates. Concepts and developments. *Oceanologica Acta 1(1).* 99-105.

Eppley, R.W., Stewart, E., Abbott, M.R., and Owen, R.W. (1987). Estimating ocean production from satellite-derived chlorophyll: insights from the Eastropac data set. *Oceanologica Acta* SP, 109-113.

Fasham, M.J.R., Balino, B.M., Bowles, M.C., Anderson, R., Archer, D., Bathmann, U., Boyd, P., Buesseler, K., Burkill, P., Bychkov, A., Carlson, C., Chen, C.T.A., Doney, S., Ducklow, H., Emerson, S., Feely, R., Feldman, G., Garcon, V., Hansell, D., Hanson, R., Harrison, P., Honjo, S., Jeandel, C., Karl, D., Le Borgne, R., et al. (2001). A new vision of ocean biogeochemistry after a decade of the Joint Global Ocean Flux Study (JGOFS). *Ambio* 30 (SPEC. ISS. 10), 4-31.

Hansell, D. A. (1993). Results and observations from the measurement of DOC and DON using high temperature catalytic combustion techniques. *Mar. Chem.* 41, 195-202.

Hansell, D.A. (2005). Dissolved organic carbon reference material program. *Eos, Transactions, American Geophysical Union* 86 (35), 318.

Hansell, D.A., and Carlson, C.A. (1998). Deep ocean gradients in dissolved organic carbon concentrations. *Nature* 395, 263-266.

Hansell, D.A., Carlson, C.A., Repeta, D.J., and Shlitzer, R. (2009). Dissolved organic matter in the ocean: A controversy stimulates new insights. *Oceanography* 22, 202-211.

Hansell, D. A., Carlson, C. A., & Schlitzer, R. (2012). Net removal of major marine dissolved organic carbon fractions in the subsurface ocean. *Global Biogeochemical Cycles*, *26*(1), [GB1016].

International Union of Pure and Applied Chemistry. (1995). Nomenclature in evaluation of analytical methods, including detection and quantification capabilities (IUPAC Recommendations 1995). *Pure & Appl. Chem.*, 67 (10), 1699-1723.

Knap, A., Michaels, A., Close, A., Ducklow, H. and Dickson, A. (1996). Protocols for the joint global ocean flux study (JGOFS) core measurements. *JGOFS, Reprint of the IOC Manuals and Guides No. 29, UNESCO 1994***.**

Letscher, R. T., Hansell, D. A., Carlson, C. A., Lumpkin, R., & Knapp, A. N. (2013). Dissolved organic nitrogen in the global surface ocean: Distribution and fate. *Global Biogeochemical Cycles*, 27, 141-153.

MacKenzie, F. T. (1981). "Global carbon cycle: Some minor sinks for CO2." In *Flux of Organic Carbon by Rivers to the Ocean* (G. E. Likens, F. T. MacKenzie, J. E. Richey, J. R. Sedell, and K. K. Turekian, Ed.), 360-384. U.S. Dept. of Energy, Washington, DC.

Mopper, K., and Qian, J. (2006). Water Analysis: Organic Carbon Determinations. *Encyclopedia of Analytical Chemistry, Online © 2006 John Wiley & Sons, Ltd.*

Novak, M.G., Cetinić, I., Chaves, J.E., and Mannino, A. (2018). The adsorption of dissolved organic carbon onto glass fiber filters and its effect on the measurement of particulate organic carbon: A laboratory and modeling exercise. *Limnol. Oceanogr.: Methods.* 16, 356-366.

Peltzer, E.T., and Brewer, P.G. (1993). Some practical aspects of measuring DOC – sampling artifacts and analytical problems with marine samples. *Marine Chemistry* 41(1-3), 243-252.

Ridgwell, A., and Arndt, S. (2014). "Why Dissolved Organics Matter: DOC in Ancient Oceans and Past Climate Change," in *Biogeochemistry of Marine Dissolved Organic Matter*, 1st ed., edited by D. A. Hansell and C. A. Carlson (Academic Press, San Diego, CA), 1-19.

Schlitzer, R. (2021). Ocean Data View, https://odv.awi.de.

Sharp, J.H. (1993). The dissolved organic carbon controversy: an update. *Oceanography* 6, 45 – 50.

Sharp, J.H., Benner, R., Bennett, L., Carlson, C.A., Fitzwater, S.E., Peltzer, E.T., Tupas, L.M. (1995). Analyses of dissolved organic carbon in seawater: the JGOFS EqPac methods comparison. *Mar. Chem.* 48, 91– 108.

Sharp, J. H., Carlson, C. A., Peltzer, E. T., Castle-Ward, D. M., Savidge, K. B. and Rinker, K. R. (2002a). Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. *Mar. Chem.* 77(4), 239-253.

Sharp, J. H., Rinker, K. R., Savidge, K. B., Abell, J., Benaim, J. Y., Bronk, D., Burdige, D. J., Cauwet, G., Chen, W., and Doval, M.D. (2002b). A preliminary methods comparison for measurement of dissolved organic nitrogen in seawater. *Mar. Chem.* 78(4), 171-184.

Tappin, A. D., Nimmo, M. (2019). "Water Analysis | Seawater: Dissolved Organic Carbon." In *Encyclopedia of Analytical Science* (3rd ed.), Editors: Worsfold, P., Poole, C., Townshend, A., Miró, M., 345–352.

Turnewitsch, R., Springer, B.M., Kiriakoulakis, K., Vilas, J.C., Aristegui, J., Wolff, G., Peine, F., Werk, S., Graf, G., and Waniek, J.J. (2007). Determination of particulate organic carbon (POC) in seawater: The relative methodological importance of artificial gains and losses in two glass-fiber-filter-based techniques. *Marine Chemistry* 105, 208–228.

Walsh, T.W. (1989). Total dissolved nitrogen in seawater: a new high-temperature combustion method and a comparison with photo-oxidation. *Marine Chemistry* 26, 295-311.

Williams, P.M., and Druffel, E.R.M. (1987). Radiocarbon in dissolved organic matter in the central North Pacific Ocean. *Nature* 330, 246-248.

Williams, P.M., and E.R.M. Druffel. (1988). Dissolved organic matter in the ocean: Comments on a controversy. *Oceanography* 1(1), 14–17.

Appendix A

Experimental results: sample collection and storage. Effects of bottle type, handling, and storage conditions on DOM samples.

As stated in Section 2 of this manual, proper sampling techniques and handling are essential to provide high quality data. A series of tests were conducted at UCSB to highlight some common sources of error and provide insight into our methodological recommendations.

Sample bottle selection

Tests were conducted to compare dissolved organic carbon (DOC) samples stored frozen or acidified/at room temperature in glass or plastic bottles (PC = polycarbonate, HDPE = high density polyethylene). All glassware was pre-combusted, and all plastic bottles were acid washed prior to collection. Samples were collected via Niskin bottle in the Sargasso Sea in September 2012 and analyzed at UCSB within one month of collection. No systematic difference was resolved for bulk DOC measurement between bottle types when cleaned properly or whether a sample was stored frozen or acidified at room temperature (Figure A1). Because combustion of glass is the easiest method to ensure that storage vessels can be rendered organic free, and the 40 ml borosilicate vials can be loaded directly on to the Shimadzu autosampler (minimizing further handling), it is recommended to use glass borosilicate vials when logistically feasible. Properly cleaned HDPE and PC bottles are acceptable alternatives and can be more robust during transport of samples, but due to concerns with leaching of organics from plastic when in contact with acid over long periods, glass is the preferred bottle type.

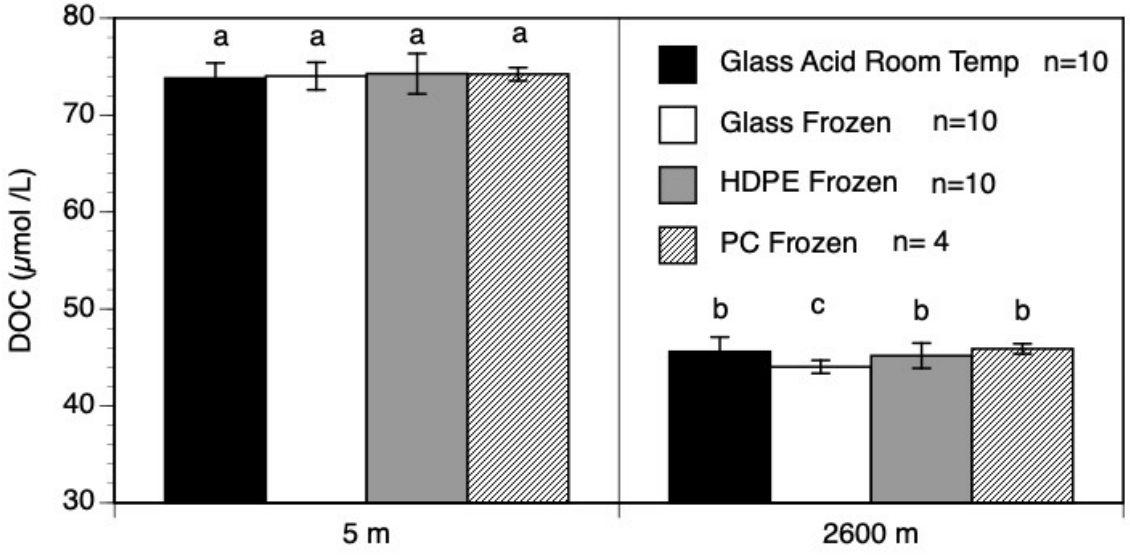


Figure A1. Comparison of DOC samples (GF/F filtered) stored frozen vs acidified/at room temperature in various bottle types. Error bars represent standard deviation. Letters that are different indicate statistical difference using a Tukey-Kramer test after analyses of variance were performed (α= 0.05).

Filtration

Filtration is a necessary step to ensure separation of particulate from dissolved organic matter. However, filtration adds an additional handling step that can result in contamination if not performed properly. Due to potential for contamination during sampling, some studies in oligotrophic systems forgo filtration and only total organic carbon (TOC) is analyzed as TOC is often indistinguishable from DOC within analytical precision (Mopper and Qian, 2006) in these systems. However, POC can become quantitatively important in surface waters of coastal or eutrophic systems that exhibit high productivity. Figure A2 presents vertical profiles of TOC and DOC measured in contrasting regions such as the Southern Ocean (A) vs. oligotrophic Sargasso Sea (B) and exemplifies method error versus the error that would be introduced by using TOC rather than DOC in such settings.

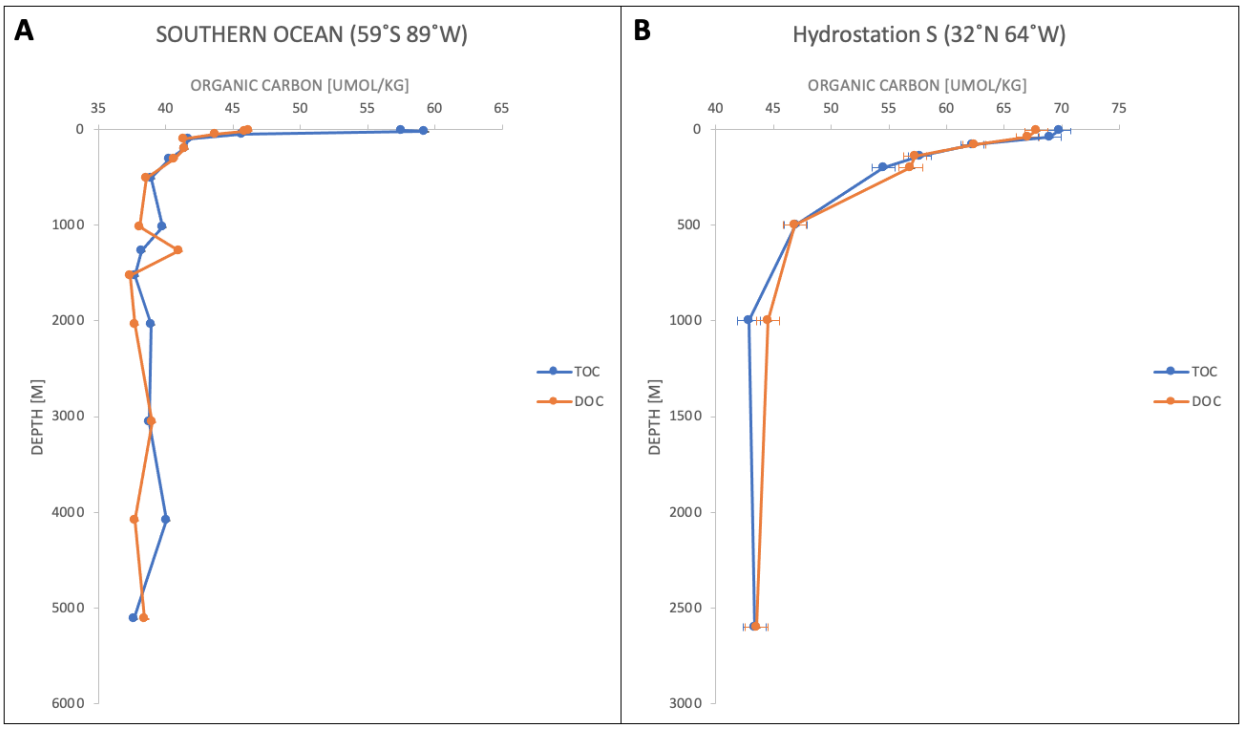


Figure A2. Vertical profiles of TOC and DOC in regions exhibiting high surface productivity (A) vs. an oligotrophic setting (B). In (B) error bars represent standard deviation of triplicate samples, while in (A) only single vials were collected due to sampling constraints.

Assessing potential DOC stripping by GF/F filtration- As described in the document, to minimize handling, we recommend filtration be done in-line via gravity filtration through a combusted glass fiber filter when possible. The combustion of glass fiber filters creates active sites that can absorb DOM, thus questions about whether stripping of organic matter from bulk DOC measurement have been raised (Novak et al., 2018; Turnewitsch et al., 2007). To test whether DOM sorption to the GF/F filter led to a resolvable removal (stripping) of organic carbon from bulk DOC filtrate an experiment was conducted at UCSB. First coastal seawater

was filtered through a pre-flushed 142mm 0.2um polyethersulfone (PES) filter membrane, to remove organic particles, and collected into an acid clean polycarbonate (PC) carboy. The filtrate was partitioned into fifteen vials and immediately acidified (*Initial DOC*). Then the 0.2 µm filtrate was filtered at various volumes from a carboy through a combusted 47mm GF/F filter (in PC filter holder) to assess whether DOC stripping could be resolved. Water flowed continuously from the carboy through the GF/F filter and the filtrate was collected into a series of 40 ml borosilicate vials (after rinses) after various volumes ranging from 60 – 730 mL had passed through the GF/F filter (*GF/F filtered DOC*). Total water volume that passed through the GF/F at each sampling point was accounted for. The process was conducted a total of three times with a fresh combusted GF/F each time. All GF/F filtered samples were acidified and stored at 14ºC alongside the Initial DOC controls until analysis was conducted within a week of collection. The results showed no resolvable difference in bulk DOC concentrations between 0.2 µm filtrate that had not been exposed to GF/F active sites (*Initial DOC*) and 0.2 um filtrate that had been exposed to GF/F filtered active sites after various filtration volumes (*GF/F filtered DOC*) (Figure A3). Thus, the results suggest that DOC sorption saturates the active sites on the glass fiber filters quickly (i.e., during the time of filter flushing and vial rinsing) and does not lead to resolvable stripping of bulk DOC filtrate (Figure A3).

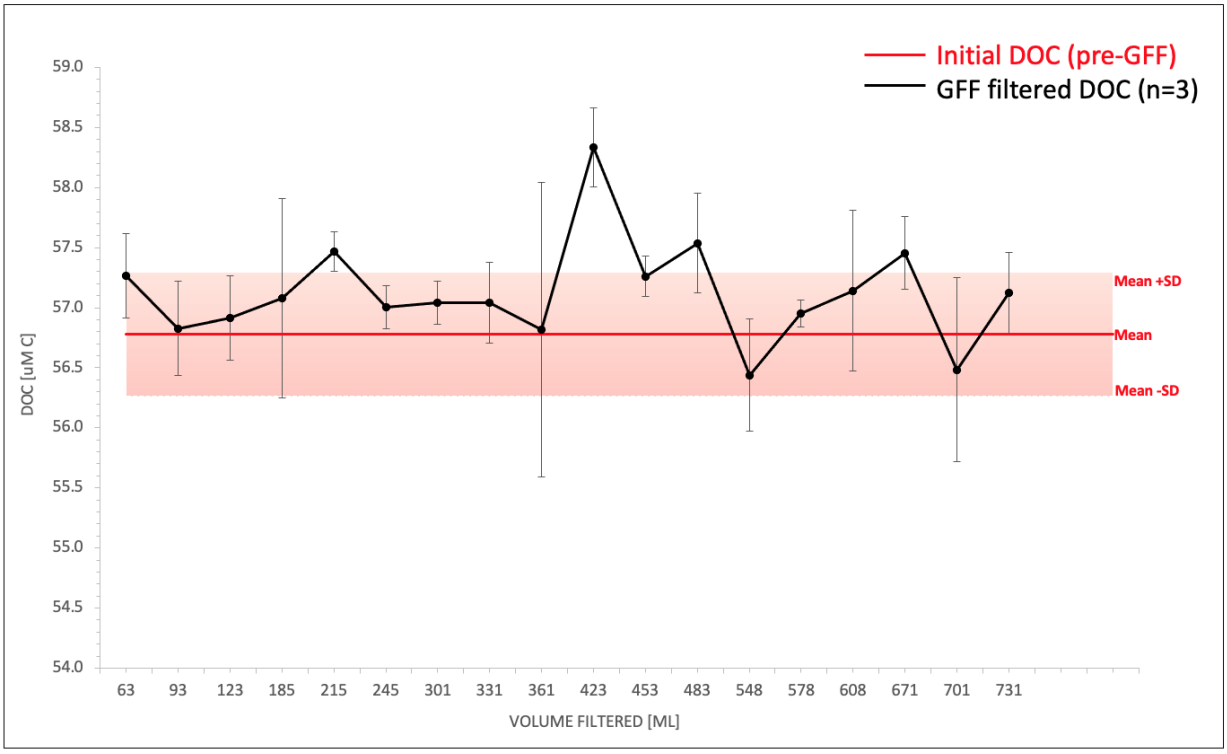
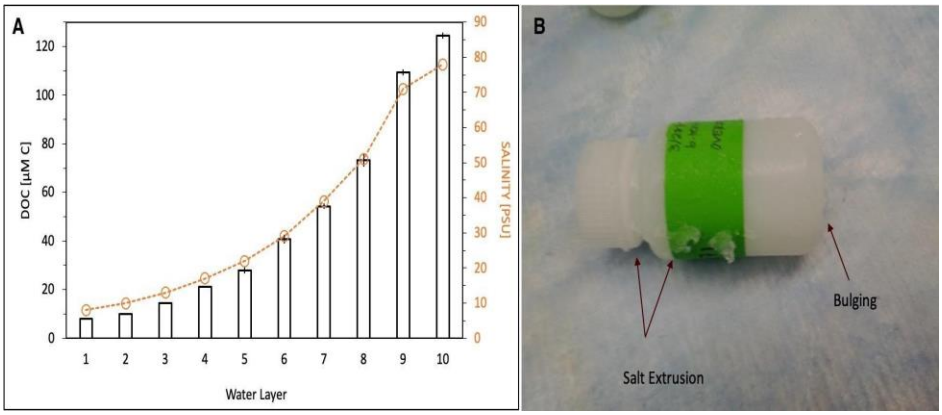


Figure A3. Filtration test showing no resolvable loss in DOC concentrations between 0.2 µm filtrate that had not been exposed to combusted GF/F active sites and 0.2 µm filtrate that had been exposed (filtered) to combusted GF/F filter active sites at filtration volumes from 60 – 730 ml. Error bars represent standard deviation of experimental results repeated three times with the same 0.2 µm filtrate and three different GF/F filters.

Bottle handling precautions

Figure A4 demonstrates the importance of not overfilling sample bottles. Freezing leads to stratification of DOC concentrations and salinity within the sample bottle (panel A). In cases where samples were overfilled (to greater than the ¾ full recommended) and frozen (panel B), a loss of DOC rich brine from the cap threads was observed (C); thus, diluting DOC concentrations in the collection bottle or vial (panel C). It is therefore critical to never overfill a sample container prior to freezing, and upon thawing it is equally important to thoroughly mix samples via vortex before analysis.



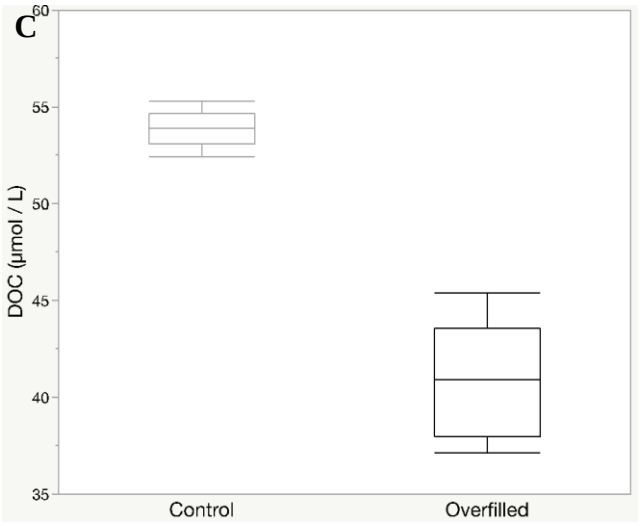


Figure A4. The effect of freezing on salinity and DOC gradients in frozen samples and the potential impact of overfilling frozen vials. (A) 2-liter bottles were filled with seawater and frozen overnight, then placed on the benchtop and allowed to thaw without mixing. Sequential 150 mL layers were gently aspirated from the top and salinity and DOC measurements for each layer were conducted. Orange circles indicate salinity of each layer and columns represent DOC concentration in each layer. Error bars represent standard deviation. (B) Example of an overfilled HDPE bottle, showing salt accumulated on the threads and along the side as high salinity DOC rich water was pushed out upon freezing. (C) Comparison of DOC concentration in bottles that were stored frozen ¾ full (control; n=5) and overfilled (as shown in panel B; n=5). Data demonstrates that overfilling can lead to displacement of brine from the storage container, carrying with it high DOC concentration and resulting in a low concentration within the remaining volume in the container.

Additional tests underscored the importance of small details such as proper sealing of glass vials. Figure A5 shows DOC concentrations for open ocean seawater samples that were received at our facility for analysis. Upon receipt it was noted that some of the glass borosilicate vials contained septa that had already been pierced (a), leaving the samples exposed during shipment and storage, or that the septa were upside down in the cap, leaving the septa silicone side facing the sample rather than the Teflon-coated side (b). Replicate samples with intact and correctly oriented septa were subsequently analyzed, revealing the level of contamination that can result with improper sealing of the sample bottles.

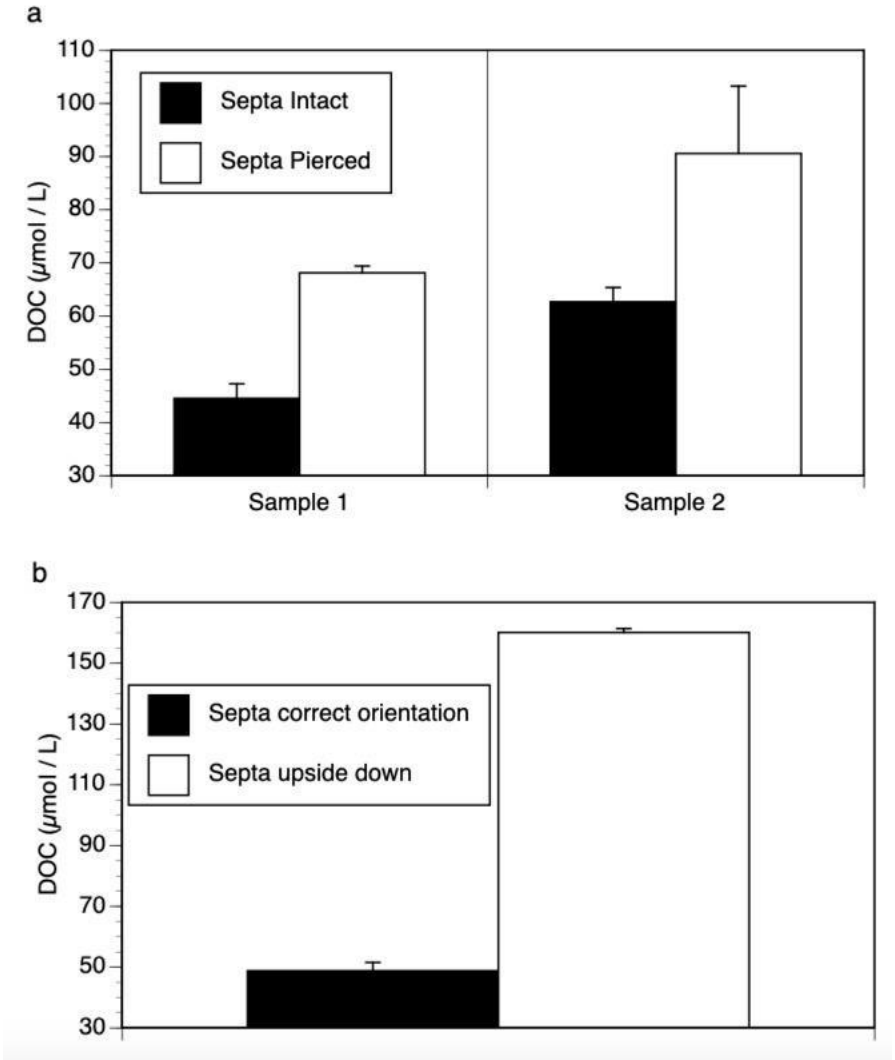


Figure A5. Example of DOC contamination that can result from the improper sealing of the sample bottle. (a) Contamination that can arise when samples are stored and shipped in caps that have pierced septa. (b) Potential contamination that can arise when samples are stored in bottles where the septa are placed upside down in the cap. Error bars represent standard deviation for replicate injections of a single sample.

Sample Storage

If care is taken seawater samples can be collected in the field, shipped internationally, and safely stored on the order of years. Figures A6 and A7 show results of long-term storage experiments on both frozen and acidified/room temperature samples.

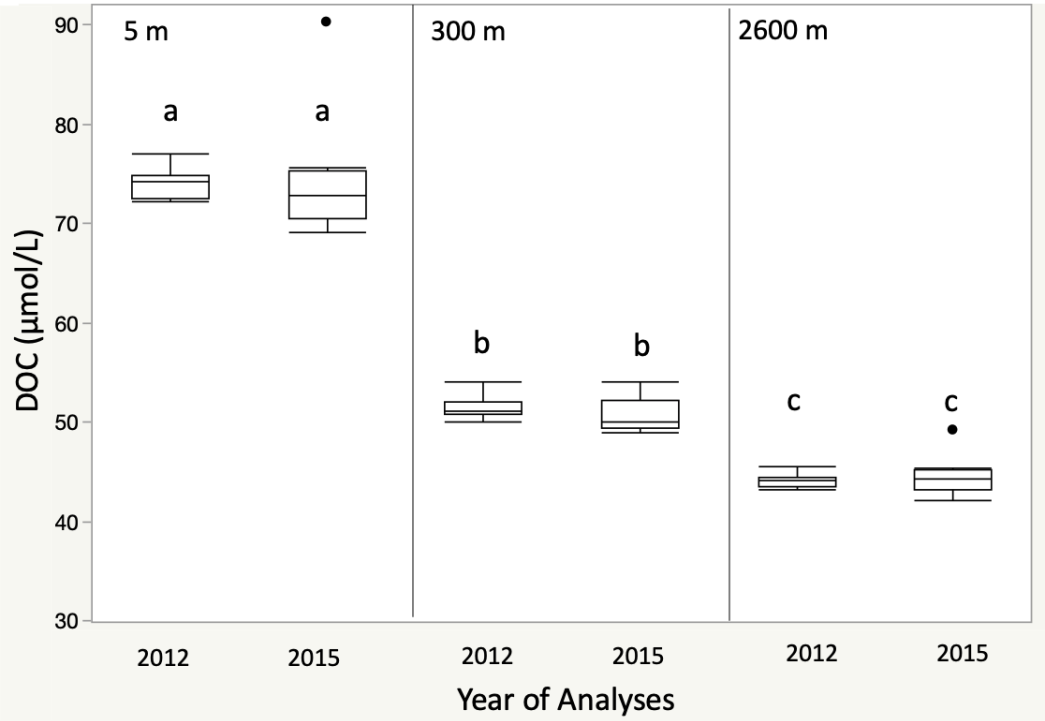


Figure A6. Box and whisker plot of long-term storage of frozen DOC samples. Seawater samples collected in the Sargasso Sea from 5, 300 and 2600 m in 2012 (glass borosilicate vials) and analyzed within a month of collection. Samples were returned to storage and re-analyzed again in 2015. n = 10 for all depths. Line in the box represents the median value, the bottom of the box represents the 2nd quartile value, top of the box represents the 3rd quartile of data range and whisker represents the data range (excluding outlier; dots). Letters that are different indicate statistical difference using a Tukey-Kramer test after analyses of variance was performed (α= 0.05). There was no significant difference in frozen samples analyzed three years apart.

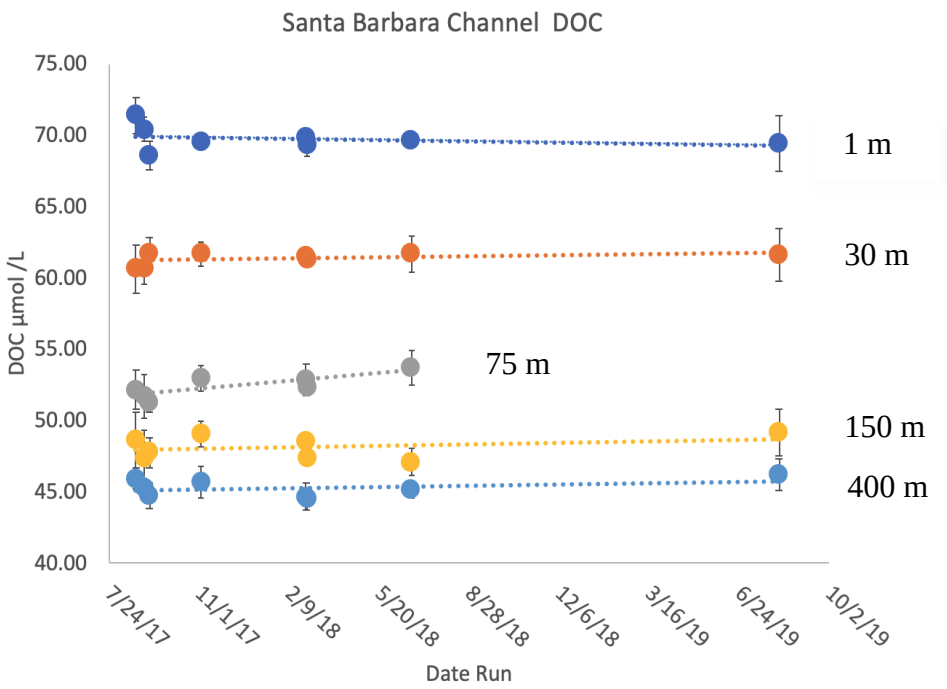


Figure A7. Storage stability test of acidified seawater. DOC samples collected at 1, 30, 75, 150 and 400 m from the Santa Barbara Channel were acidified to a pH of 2-3 and partitioned into numerous borosilicate vials and stored at room temperature. Replicate (3-5) samples from each depth were acidified and analyzed on various dates. Mean data are plotted for each depth over time. The data show that samples remained stable over the course of 2 years when stored acidified at room temperature. Error bars represent standard deviation. Dotted lines are linear regressions showing over the course of three months there was no statistically significant change at any sampling depth.

Appendix B

Recommended sampling equipment and consumables

Item	Manufacturer	Part No.
40 mL borosilicate vial, clear, with bonded septum (unprocessed)	Thermo Scientific	34040C/DB
Polycarbonate in-line filter holder, 47 mm	Pall Laboratory	1119
Binder-Free Glass Microfiber Filters GF/F Circles, 47 mm	GE Healthcare Whatman	1825047
60 mL HDPE bottle	Thermo Scientific	21040002
Hydrochloric Acid, Certified ACS Plus, 36.5 to 38.0%, Fisher Chemical	Fisher Scientific	A144SI-212
Pt-cured Silicone tubing	Cole Parmer	ZM-96410-15
Eppendorf™ Repeater™ M4 Manual Handheld Pipette Dispenser	Eppendorf	4982000322
Eppendorf™ Combitips advanced™ Standard Pipettor Tips	Eppendorf	0030089430
Special order field box, pizza style	Flexcon www.flexcontainer.com	HDPP versatote (16 x 8 3/8 x 3 7/8 OD)
9-hole foam insert vial shipper	Quality Environmental Containers Inc.	OF6000

Supplies & consumables for analysis

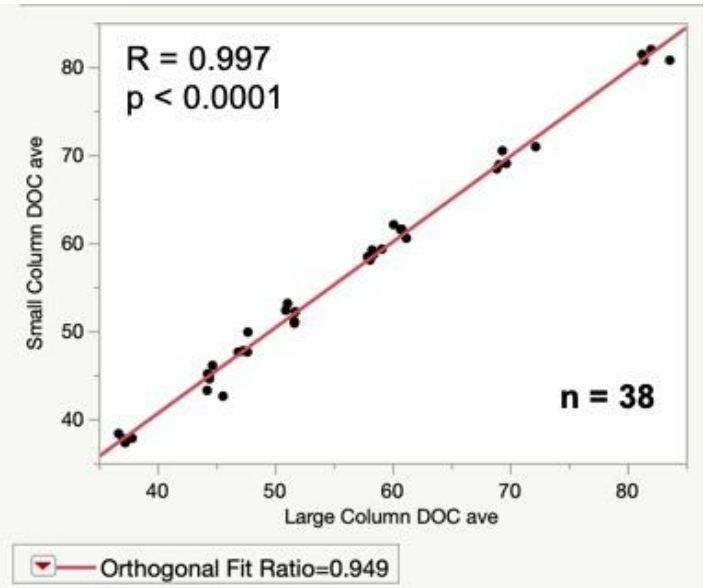
Item	Manufacturer	Part No.
Quartz Tubing for small tubes (18 mm ID x 20 mm OD) (4 mm ID x 6 mm OD)	Quartz Scientific Inc.	100018B, 100004B
Quartz Tubing for large tubes (6 mm ID x 8 mm OD) (17 mm ID x 20 mm OD) (27 mm ID x 30 mm OD)	Quartz Scientific Inc.	100006B,100017C,100027C
Premade TC Combustion Tube (small column)	Shimadzu Scientific Instruments Inc.	638-41323-00
Premade High Salt Combustion Tube (large column)	Shimadzu Scientific Instruments Inc.	638-42076-00
Special Order Platinum Gauze (45 mesh 145 x 145 mm square)	Exeter Analytical Inc.	0240-1147D
Normal Sensitivity Platinum Catalyst (support 5/64" alumina balls)	Shimadzu Scientific Instruments Inc.	638-60116-00
Platinum Catalyst Large beads for High Salts Kit (support alumina balls)	Shimadzu Scientific Instruments Inc.	638-60193-00
Magnesium Perchlorate Anhydrous (Certified ACS)	Fisher Scientific	M54212
Polyethylene Tube (drying traps) 16 mm I.D. x 19 mm O.D. (0.675 x 0.75 in.) 6"	Bel-Art™ SP Scienceware	F199610000
Copper wool reel, FINE - 5LB	Palmer Engineered Products	7165010
Membrane Filter, 0.45 µm	Shimadzu Scientific Instruments Inc.	046-00042-12
Polyprowool, 150 g	Shimadzu Scientific Instruments Inc.	630-00325-00

Appendix C

Shimadzu HTC system setup and maintenance

Combustion tube configuration tests

One Shimadzu TOC system at UCSB was configured in the small column (TOC-V) format and one in the large column (TOC-L) format; DOC outputs were compared. The results demonstrate excellent agreement between the two system configurations for DOC analyses (Figure C1). Either configuration is acceptable for seawater samples.



Intercept	Slope	LowerCL	UpperCL	Alpha
1.6185	0.974049	0.947435	1.001411	0.05000

Figure C1. Results of an orthogonal regression using JMP software (JMP®, Version <*15*>. SAS Institute Inc., Cary, NC, 1989–2021) comparing DOC data generated under small and large column configurations using a Shimadzu TOC-V and TOC-L, respectively. GF/F filtrate was collected from surface to 400 m from the Santa Barbara Channel.

Combustion tube dimensions and setup

As noted in Section 3, it is possible to procure quartz materials to fabricate combustion tubes in-house if a glass blowing shop is accessible. Figure C2 provides quartz tube dimensions and shows combustion tube packing as described in Section 3.2. Refer to Appendix B for parts needed.

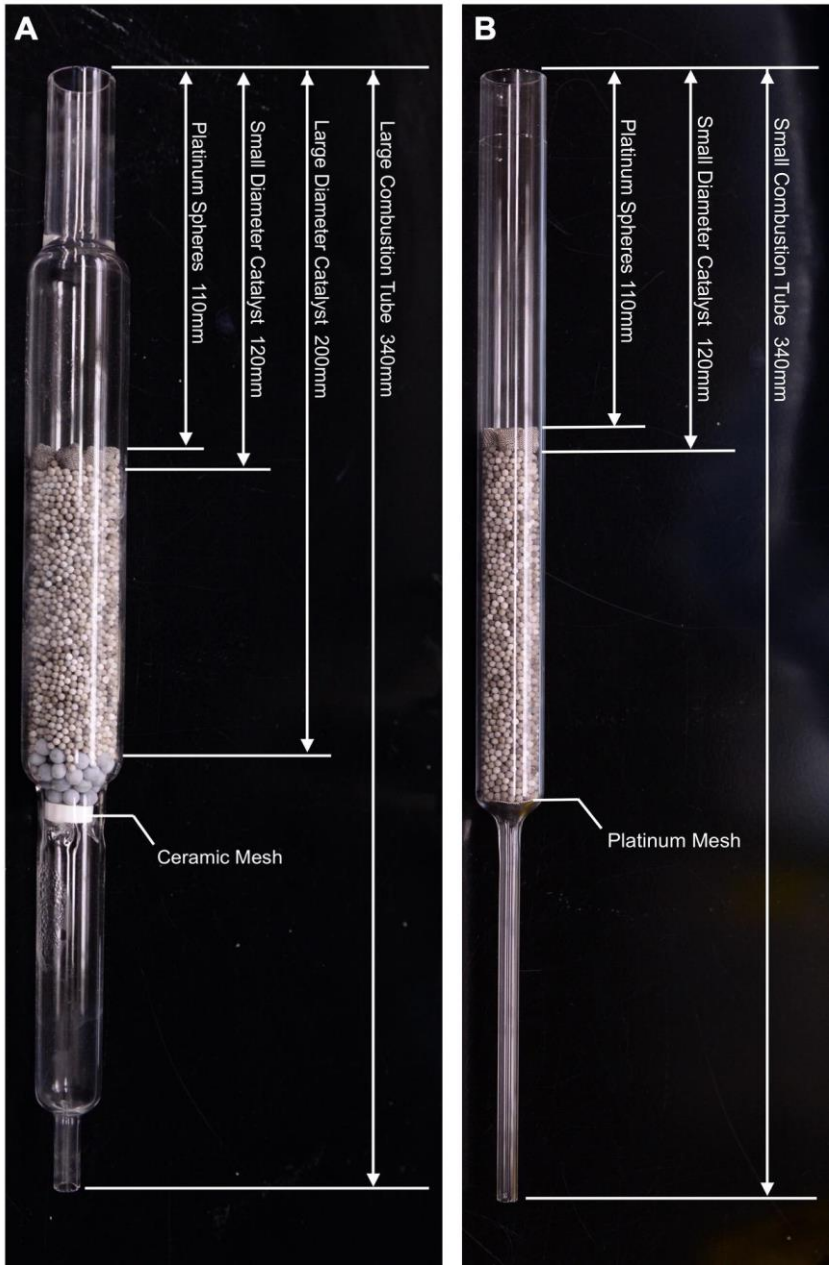


Figure C2. Dimensions and packing for large (A) and small (B) combustion columns.

Modification of the Shimadzu TOC systems

Figure C3 provides a diagram showing the modifications for signal optimization discussed in Section 3. An in-line trap- $Mg(ClO_4)_2$, has been added for removing water vapor from the gas stream. Daily inspection of the system should include a check of the flow rate at various points as indicated. Figure C4 gives a view of the added perchlorate trap as well as the orientation of the pure water trap inside a TOC-V system. As discussed in Section 3, the pure water trap has been tilted slightly (such that the gas stream passes just over the surface of the water and bubbles are observed, but no water is being drawn into the line). This modification reduces the headspace of the trap and maintains constant volume in the system while also retaining the trap as a catchment for particulates (grey colored sediment can be observed collecting in the base of the trap in Figure C4, this is a breakdown product of the platinum catalyst beads). As shown in panel (C) of Fig. C4, we have reduced the system dead-space by removing the condensation coil and replacing it with a smaller section of tubing such that the connection is as follows: small combustion tube with 6mm base tube -> PTFE tube fitting elbow 6mm to 6mm (such as Swagelok® T-6M0-9) -> glass or PTFE tube 6mm OD, 4mm ID, 50mm long (UCSB glass shop homemade) -> PTFE tube fitting reducing union 6mm to 3mm (such as Swagelok® T-6M0-6-3M) -> PTFE tubing 3mm OD, 2mm ID, 500mm long -> Pure water trap.

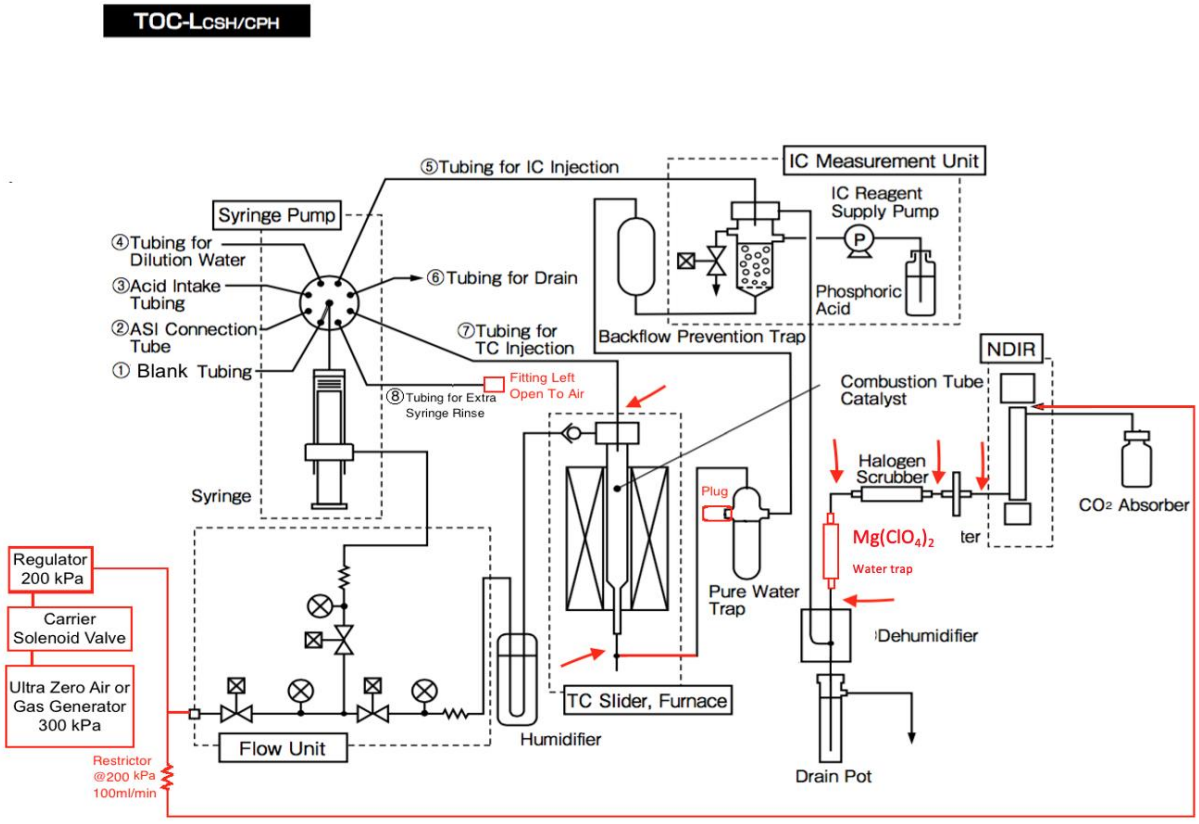


Figure C3. Schematic of the UCSB Shimadzu systems, adapted from the Shimadzu TOC-L manual and showing added in-line $Mg(ClO_4)_2$ trap. Red arrows indicate recommended points for daily flow checks.

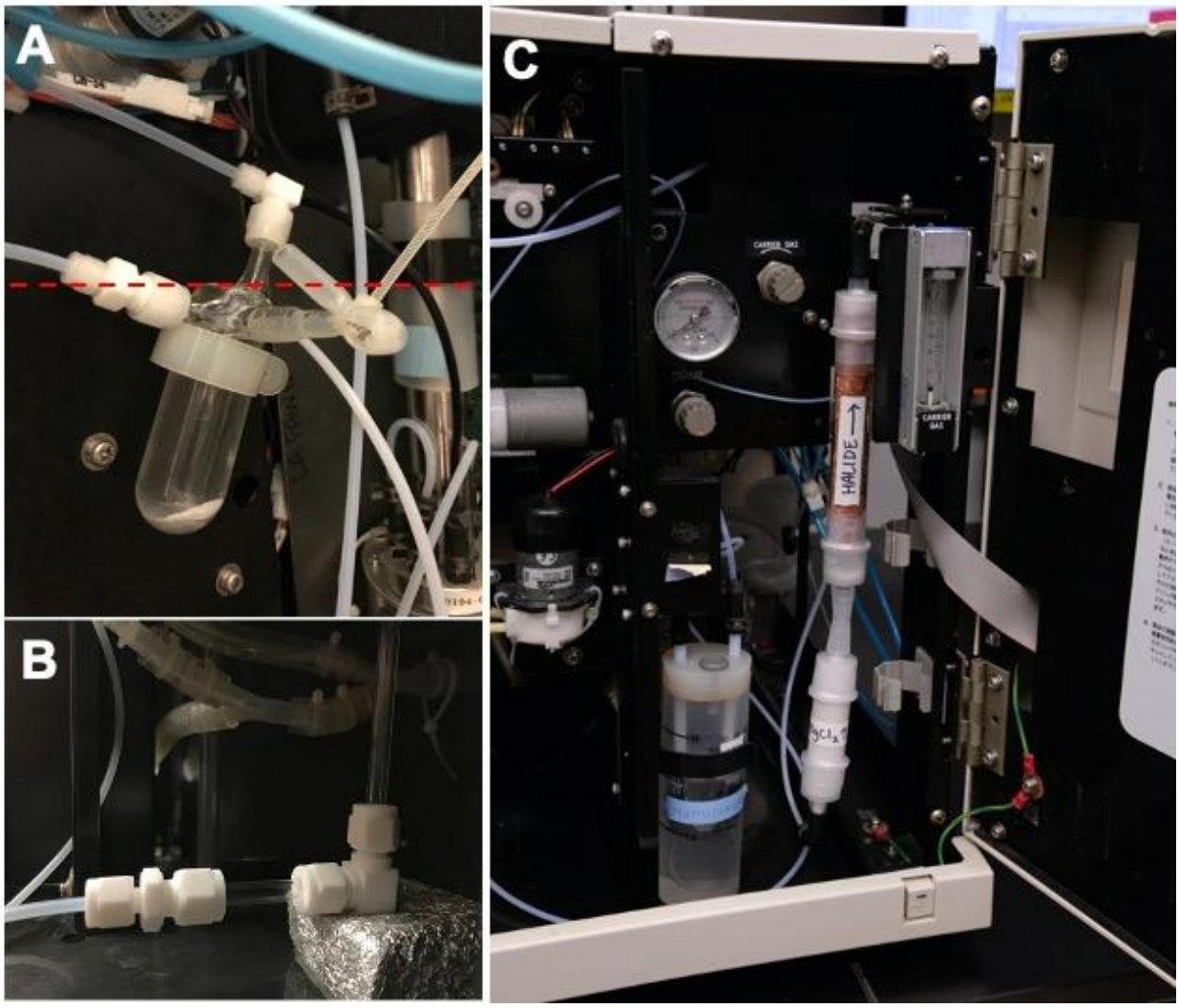


Figure C4. System modifications. Orientation of the pure water trap (A), view of combustion tube outlet with condensation coil removed (B), and halide and $Mg(ClO_4)_2$ traps (C) within a TOC-V system. Note the pure water trap has been tilted slightly. Sediment from pulverized catalyst can collect in this trap; thus, we do not recommend sampling it as a pure water blank. It is recommended that the $Mg(ClO_4)_2$ and halide traps be placed vertically to minimize void spaces and so that gas flow is up through the bottom of the traps.

Daily system assessment

As described in section 4, it is critical to assess system performance daily before beginning a sample run. System readiness is indicated by criteria such as a stable baseline, low blanks and reproducible seawater peaks. Figures C5-C7 provide examples of system assessment.

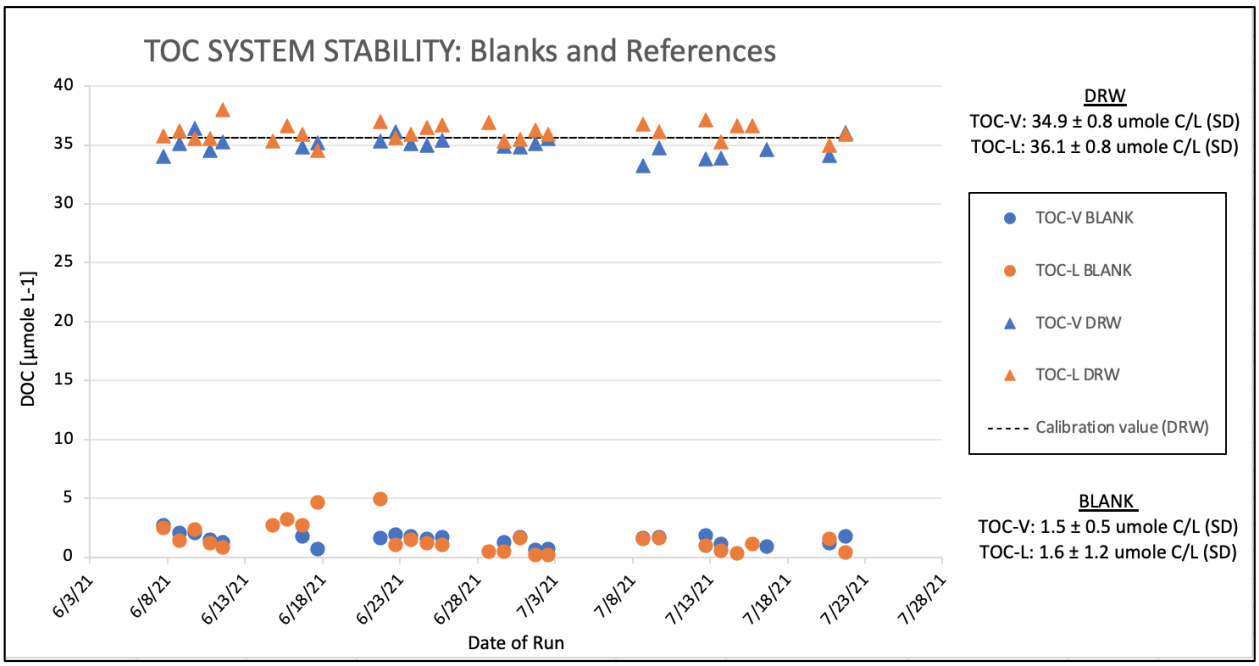


Figure C5. Example of typical blank concentrations and stability across Shimadzu TOC-V and TOC-L systems. Values for system blanks on either system configuration remain on average below 2 µmole C L^{-1} on a long-term basis, resulting in good reference stability when samples are blank corrected.

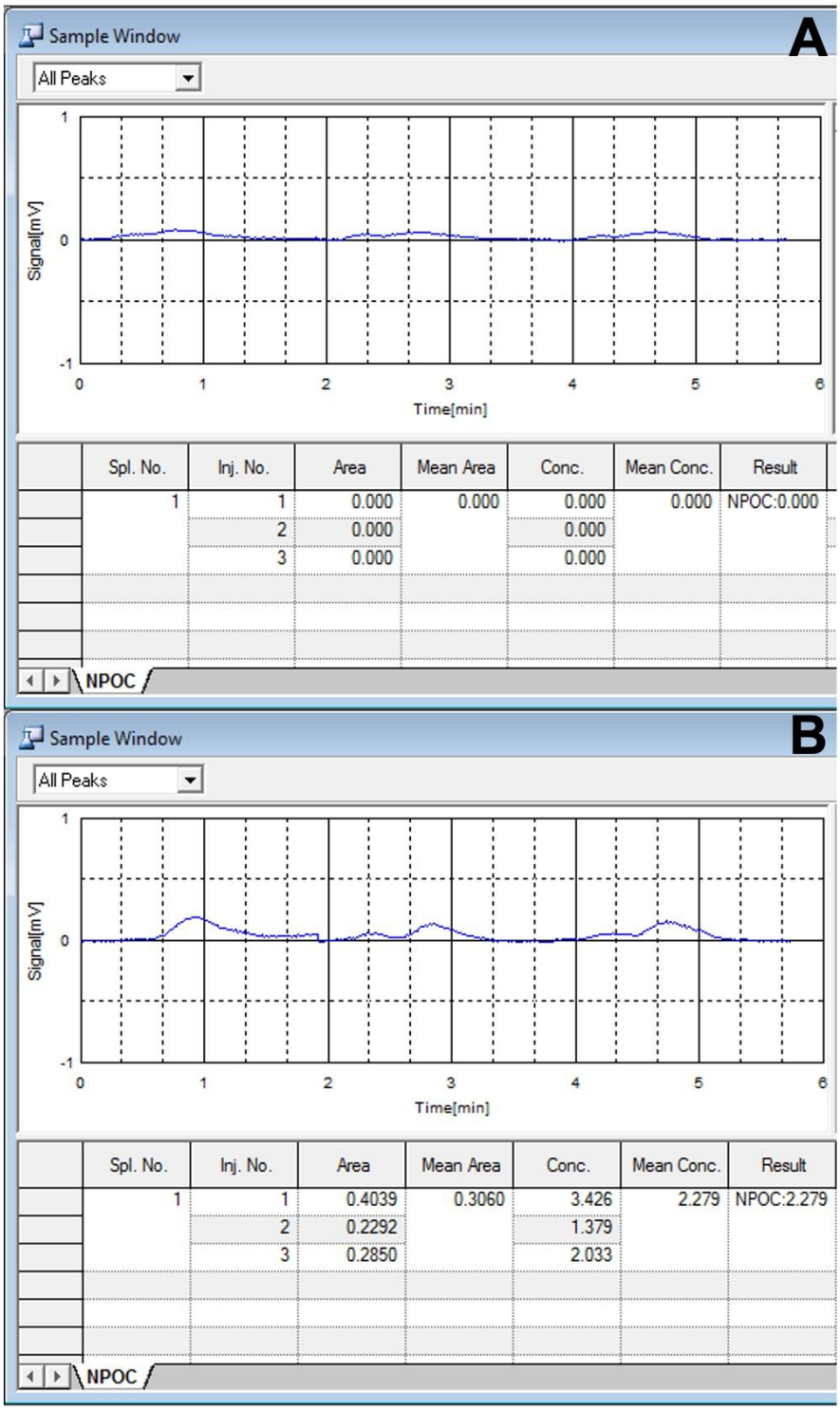
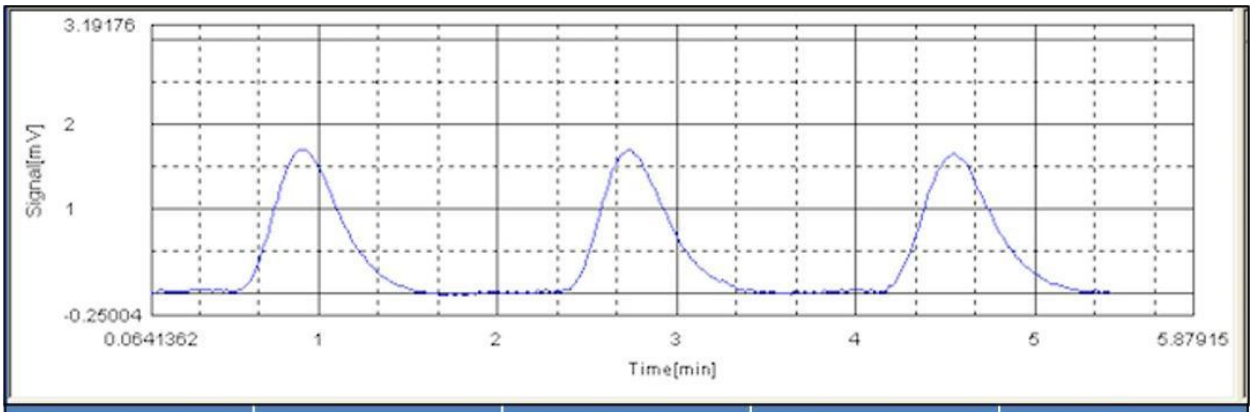


Figure C6. System readiness – blank assessment. (A) Baseline position is near 0 mV and stable, and blank water peaks are below detection and reproducible, resulting in a zero value. System is ready to run. (B) Example of bad blank peaks- reproducibility is poor and baseline shifts are evident. Recommend additional conditioning of column.



Injection No.	Area	Mean Area	SD Area	CV Area
1	4.425	4.421	0.04362	0.99
2	4.376			
3	4.463			

Figure C7. System readiness – seawater sample showing 3 injections that are highly reproducible.

Appendix D

Shimadzu HTC system: recommended maintenance for seawater sample analysis

DAILY TASKS

1. **Fill TOC reservoirs:**

Fill all the following reservoirs with ultrapure water. If using a transfer vessel (2.5-liter glass bottle), be sure to rinse with 20mls 2x before filling.

- ☐ Humidifier (carrier gas is humidified for high sensitivity analysis)
- ☐ Drain Pot (level is critical to prevent carrier gas from being released from drain tubing).
- ☐ ASI Rinse
- ☐ Ultrapure water Vial "0"

2. **Clean injection slider:**

- ☐ Pull injection block slider out by removing screw
- ☐ Spray with ultrapure water to remove salt buildup and wipe dry with Kimwipes®
- ☐ Check injection block for salt as well, use wet Kimwipe® to remove excess salt
- ☐ Re-assemble slider

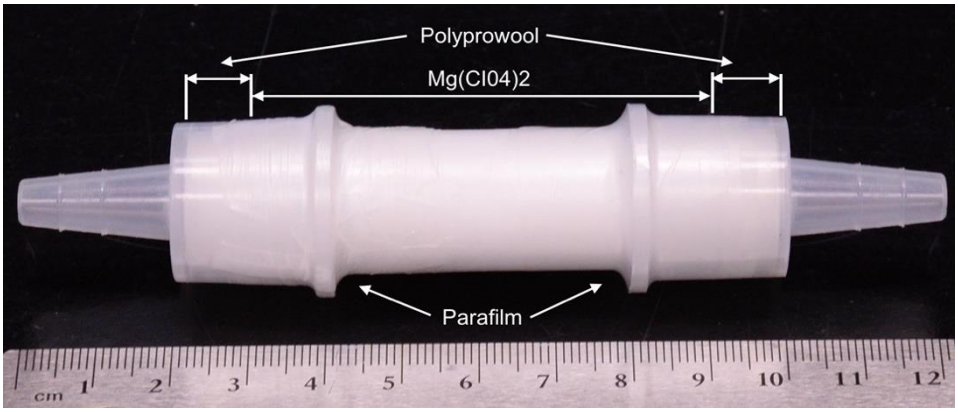
3. **Regenerate IC chamber (only on CSH/CPH models):**

- ☐ Under Instrument menu > Maintenance > Regeneration of the IC Solution
- ☐ Select Start
- ☐ Select Close once completed

4. **Change perchlorate trap:**

- ☐ To be changed every 2-3 days, or after every column break-in
- ☐ Recommended assembly:

  - ☐ 80mm polyethylene tube with barbed caps
  - ☐ With one side capped, pack in 10mm of polyprowool
  - ☐ Fill loosely with $Mg(ClO_4)_2$ leaving 10mm at end of tube
  - ☐ Pack remaining end with 10mm of polyprowool and cap
  - ☐ Wrap both ends tightly with parafilm

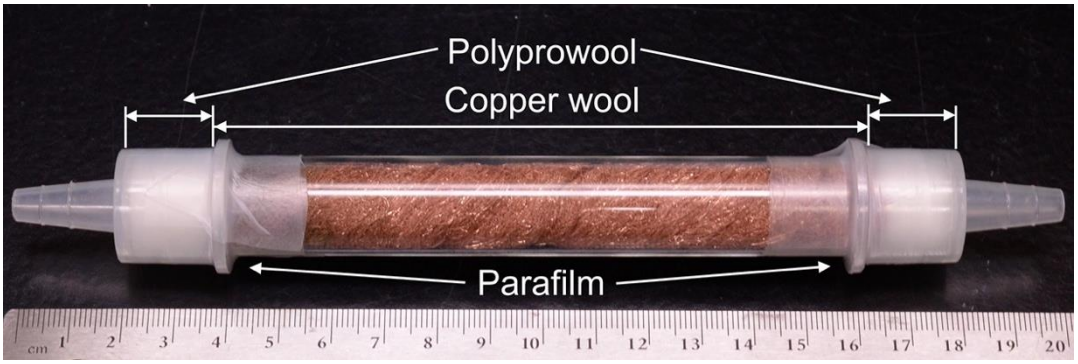


5. Change halide scrubber:

❏ To be changed once the copper discolors within 2cm of the trap outlet

❏ Recommended assembly:

- ❏ 160mm glass tube (polyethylene tube can also be used) with polyethylene barbed caps
- ❏ With one side capped, pack in 15mm of polyprowool
- ❏ Pack in copper wool leaving 15mm at end of tube
- ❏ Pack remaining end with 15mm polyprowool and cap
- ❏ Wrap both ends tightly with parafilm



6. Check injection spray:

☐ Once carousel is loaded and sample tables are connected, start the run with an Untitled Vial "0" and change method to "10 out of 10" injections. This allows you enough time to check the injection spray and correct if needed.

☐ Check the spray by viewing the top of the column from the left of the instrument AND from the front of the instrument. Spray should be straight down the center of the combustion tube. A flashlight helps with viewing the spray.

☐ If spray is shifted to left or right, back or forward, slightly adjust the base of the column to correct the spray.

☐ Once corrected, let the instrument continue to run.

7. Flow check:

☐ Using a flow meter, check the carrier gas flow rate is constant through the system. See Appendix C (Figure 10) for typical check points:

- ☐ Going into the injection port
- ☐ At the base of the combustion tube
- ☐ Before and after the perchlorate and halogen traps
- ☐ Before and after the particle filter

☐ If there is a drop in the flow rate by more than 3 ml min^{-1} (from where the flowrate enters the column) the filter should be replaced.

WEEKLY TASKS

1. **Reconditioning of the combustion column:**
   - ☐ The quartz column will devitrify as salt infuses into the quartz matrix and will become "chalky" and fragile after several heating and cooling cycles; thus, care must be taken to inspect columns regularly. Columns should be removed weekly or at any time poor data quality indicates degradation of the platinum catalyst has occurred.
   - ☐ From TOC software, turn furnace off and allow combustion tube to cool completely before handling
   - ☐ At top of column, remove injection block screw and remove slider
   - ☐ Also remove TC injection tubing from side of injection block
   - ☐ At base of column, disconnect tubing and elbow fitting
   - ☐ Carefully pull column out of TOC and remove injection block
   - ☐ Empty contents of column (Pt mesh, catalyst and spheres) into a glass beaker
   - ☐ Inspect column carefully for signs of weakness or cracks. If quartz appears very devitrified (chalky appearance), it should be disposed of and a new column prepared. If column remains glassy and shows no sign of cracks, it can be rinsed thoroughly with ultrapure water, combusted at 450°C to dry and re-packed.
   - ☐ Inspect the mesh and spheres for wear. The mesh will become darkened and mesh will contract (spheres will appear to shrink) as the salt and heat degrade the platinum. These can be soaked in ultrapure water, vortexed to remove salt, and combusted at 450°C to dry for re-use several times (one batch of Pt screen and spheres should last 4-6 weeks, under continuous operation and will be dependent on sample throughput).
   - ☐ Inspect the Pt catalyst beads. If heavily degraded (also appear excessively "chalky" and surfaces rough or cracking) catalyst should be disposed of and column should be packed with fresh matrix. If spheres appear in good shape, they can be flushed with ultrapure water to remove salt, combusted at 450°C to dry and re-packed into a column. (Pt catalyst beads should last 12-16 runs depending on sample load through column)
   - ☐ If preparing fresh matrix for a column, note that the catalyst comes with a heavy layer of grey Pt "dust" from the manufacturing process and should be flushed extensively with ultrapure water before packing into a column to save time when conditioning the TOC system.
   - ☐ Refer to Appendix C (Figure A8) for repacking of column with reconditioned or fresh matrix and Pt support screens.

2. **Empty TOC waste**
   - ☐ Each week, or when full, dispose of properly according to environmental health and safety guidelines (water is acidic; will need to be neutralized).

MONTHLY TASKS

1. **Syringe and plunger tip**
   a. The syringe plunger tip should be inspected periodically for wear, leaks, bubbles, salt and particulate accumulation.

b. If any wear, leaks, bubbles or accumulation are apparent, follow the procedure in the Shimadzu manual for removing the syringe. Clean the syringe and plunger tip with ultrapure water and replace, following the procedure in the manual.
c. If you see excess wear on the plunger tip after cleaning, or the plunger was leaking before removal, the plunger should be replaced. See Shimadzu manual for replacing the plunger tip.
    i. If the plunger still leaks after it is replaced, the glass on the syringe may be worn and the syringe should be replaced.
    ii. If there are still excess bubbles, the syringe or rotor may need to be replaced. Follow the Shimadzu manual for troubleshooting.

2. Injection slider and o-rings

a. Inspect the injection slider and o-rings for wear.
b. The top injection slider o-ring is made of Teflon and will eventually wear down. Inspect the o-ring for flatting or knicks and replace accordingly. The second black o-ring will flatten over time and should also be replaced when apparent.
c. Inspect the injection slider for any deep knicks or scrapes and replace if any are found.

3. Combustion tube o-ring

a. Periodically inspect the combustion tube o-ring.
b. If there are any cuts on the o-ring, the combustion tube no longer fits snugly into the injection block, or leaks are apparent from the top of the combustion tube, the combustion tube o-ring should be replaced.

YEARLY TASKS

1. Gas Generator

a. If you are operating with a CO_2/Hydrocarbon free gas generator, it is important to keep up with the yearly maintenance.
b. For a Parker Balston® TOC-1250 model, which is used by UCSB, the filters are replaced yearly and the catalyst module is replaced every three years. Depending on your compressed air source, this maintenance may occur more or less often.

Appendix E

Example of a daily DOC run

EXAMPLE DOC RUN

Sample #	Rosette Position	Sample name	Sample #	Rosette Position	Sample name
1	0	*Blank*	46	31	Sample #24
2	0	*Blank*	47	0	*Blank*
3	0	*Blank*	48	0	*Blank*
4	1	25 uM C Standard	49	55	Surface Seawater Reference
5	2	50 uM C Standard	50	56	Mid Seawater Reference
6	3	75 uM C Standard	51	57	Deep Seawater Reference
7	4	100 uM C Standard	52	32	Sample #25
8	0	*Blank*	53	33	Sample #26
9	0	*Blank*	54	34	Sample #27
10	5	Surface Seawater Reference	55	35	Sample #28
11	6	Mid Seawater Reference	56	36	Sample #29
12	7	Deep Seawater Reference	57	37	Sample #30
13	8	Sample #1	58	38	Sample #31
14	9	Sample #2	59	39	Sample #32
15	10	Sample #3	60	0	*Blank*
16	11	Sample #4	61	0	*Blank*
17	12	Sample #5	62	0	*Blank*
18	13	Sample #6	63		
19	14	Sample #7	64		
20	15	Sample #8	65		
21	0	*Blank*	66		
22	0	*Blank*	67		
23	55	Surface Seawater Reference	68		
24	56	Mid Seawater Reference	69		
25	57	Deep Seawater Reference	70		
26	16	Sample #9	71		
27	17	Sample #10	72		
28	18	Sample #11	73		
29	19	Sample #12	74		
30	20	Sample #13	75		
31	21	Sample #14	76		
32	22	Sample #15	77		
33	23	Sample #16	78		
34	0	*Blank*	79		
35	0	*Blank*	80		
36	5	Surface Seawater Reference	81		
37	6	Mid Seawater Reference	82		
38	7	Deep Seawater Reference	83		
39	24	Sample #17	84		
40	25	Sample #18	85		
41	26	Sample #19	86		
42	27	Sample #20	87		
43	28	Sample #21	88		
44	29	Sample #22	89		
45	30	Sample #23	90		

References bracket every 6-8 samples (note that 2 sets of vials are included for each reference water, and these are repeatedly drawn from throughout the run (at least twice and up to 3 draws per vial)

Standard curve is run at the start

Blanks are interspersed throughout run

Maximum sample load is about 30-36 for seawater to avoid clogs in column

Appendix F

Method validation data

Method validation is an essential component a laboratory should implement to ensure it is capable of providing quality data. To maintain a uniform and internationally recognized approach, we have followed the recommendations and nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) in establishing a uniform approach for performance characteristics of the chemical measurement process (IUPAC 1995) and provide several parameters that characterize the analysis of DOC&TDN in seawater by the Shimadzu HTC method presented here.

Critical Value (L_c)- the minimum significant value of an estimated net signal or concentration, applied as a discriminator against background noise.

default alpha=	0.05
deg freedom (v)=	256
S_0 (µM C)=	1.525
t=	1.651
L_C (µM C)=	2.5

Table F1. Calculation of critical value (Lc) for DOC analysis using the Shimadzu HTC method for seawater samples. An analysis of 257 blanks across 7 independent TOC runs over the course of a year (Jan-Dec 2020) was used.

default alpha=	0.05
deg freedom (v)=	128
S_0 (µM N)=	0.3
t=	1.657
L_C (µM N)=	0.5

Table F2. Calculation of critical value (L_C) for TDN analysis using the Shimadzu HTC method for seawater samples. 4 independent system runs with 129 blank samples in total over the course of a year (Jan-Nov 2020) were analyzed.

Limit of Detection (L_D) – Minimum detectable (true) value of the chemical variable (measure of the inherent detection capability of a chemical measurement process).

Limit of Quantification (L_Q) – Minimum quantifiable (true) value (inherent quantification capability of a chemical measurement process).

default alpha=	0.05
deg freedom (v)=	6
σ_0= S_0 (µM C)=	1.16
t=	1.943
corr factor 2t=	0.96
L_D (µM C)=	4.3
L_Q (µM C)= 10*σ_0 =	11.6

Table F3. Limit of Detection (L_D) and Limit of Quantification (L_Q) for DOC analysis using the Shimadzu HTC method for seawater samples. Spiked samples (25 µmol C L^{-1}) were prepared and analyzed over separate dates (5 individual batches over 7 runs, Jan-Dec 2020). As recommended in (IUPAC 1995), due to smaller degrees of freedom ($v \leq 25$)a correction factor (4v/(4v+1)) for 2t is applied to take into account bias in S.

default alpha=	0.05
deg freedom (v)=	3
σ_0= S_0 (µM N)	0.2
t=	2.353
corr factor 2t=	0.92
L_D (µM N)=	0.9
L_Q (µM N)= 10*σ_0 =	2.0

Table F4. Limit of Detection (L_D) and Limit of Quantification (L_Q) for TDN analysis using the Shimadzu HTC method for seawater samples. Spiked samples (3 µmol N L^{-1}) were prepared and analyzed over separate dates (4 batches across 4 runs, Jan-Nov 2020). As recommended in (IUPAC 1995), due to smaller degrees of freedom ($v \leq 25$)a correction factor (4v/(4v+1)) for 2t is applied to take into account bias in S.

Appendix G

Analytical quality limits

Accuracy- evaluated by use of Consensus Reference Material as a control.

Date of Run	SYSTEM	Vial #	Hansell CRM batch			
			DSR 07-15	DSR 10-19	MSR 10-19	SSR 10-19
1/9/20 19:37	UCSB TOC-V	1	42.0	41.7	60.4	72.6
1/9/20 19:37		2	42.5	41.5	61.2	72.5
1/10/20 18:43	UCSB TOC-L	1	41.4	41.5	59.8	72.3
1/10/20 18:43		2	43.2	42.0	60.6	71.5
8/11/20 18:29	UCSB TOC-V	1	42.8	42.1	61.4	73.7
8/11/20 18:29		2	no data	41.7	59.5	72.7
8/12/20 18:28	UCSB TOC-V	1	42.4	42.5	59.9	72.8
8/12/20 18:28		2	no data	41.0	60.2	72.7
11/10/20 19:04	UCSB TOC-V	1	41.5	41.9	62.6	72.6
11/10/20 19:04		2	no data	41.2	59.3	72.2
12/1/20 17:24	UCSB TOC-V	1	41.3	41.7	61.7	72.5
12/1/20 17:24		2	no data	41.6	60.2	71.9
		n	8	12	12	12
		Measured Mean	42.2	41.7	60.6	72.5
		Measured Std Dev	0.7	0.4	1.0	0.5
		Reported consensus value	42.5	42.0	61.0	71.0
		Accuracy (%rel error)	***0.8***	***0.7***	***0.7***	***-2.1***

Table G1. Accuracy determination for DOC. All data are reported in µmole C L^{-1}. CRM values are typically reported as a range of values. To calculate accuracy as percent relative error we have taken the mean of the reported consensus values for each batch as provided by the Hansell lab (https://hansell-lab.rsmas.miami.edu/_assets/pdf/table1-2021.pdf)

Date of Run	SYSTEM	Vial #	Hansell CRM batch			
			DSR 07-15	DSR 10-19	MSR 10-19	SSR 10-19
1/10/20 18:43	UCSB TOC-L	1	31.20	32.86	9.04	5.01
1/10/20 18:43		2	31.17	31.65	9.84	7.94
1/10/20 18:43		3	31.78	no data	no data	no data
8/13/20 15:34	UCSB TOC-L	1	30.87	31.29	7.99	4.20
8/13/20 15:34		2	31.02	29.79	8.95	4.36
11/5/20 17:28	UCSB TOC-V	1	31.57	31.89	8.35	4.13
11/5/20 17:28		2	31.71	31.99	8.72	4.02
7/28/21 17:31	UCSB TOC-V	1	31.02	no data	no data	4.44
7/28/21 17:31		2	31.17	no data	no data	3.57
		n	9	6	6	8
		Measured Mean	31.3	31.6	8.8	4.7
		Measured Std Dev	0.3	1.0	0.6	1.4
		Reported consensus value	31.5	31.0	9.0	5.0
		Accuracy (%rel error)	***0.7***	***-1.9***	***2.0***	***5.9***

Table G2. Accuracy determination for TDN. All data are reported in µmole N L^{-1}. Consensus values are typically reported as a range of values. To calculate accuracy as percent relative error we have taken the mean of the reported consensus values for each batch as provided by the Hansell lab (https://hansell-lab.rsmas.miami.edu/_assets/pdf/table1-2021.pdf)

Precision (Repeatability) – reflects best achievable internal precision. Observations are mutually independent and conditions are kept constant (same instrument, skilled operator and analysis over a short time frame).

Date analyzed	System ID	Operator	Vial #	[µmole C L-1]	Std Dev	Date analyzed	System ID	Operator	Vial #	[µmole C L-1]	Std Dev
2/7/21	Sys A	EH	1	56.1	0.9	2/8/21	Sys A	KO	35	56.9	0.4
2/7/21	Sys A	EH	2	57.2	0.3	2/8/21	Sys A	KO	36	57.1	0.7
2/7/21	Sys A	EH	3	56.8	0.9	2/8/21	Sys A	KO	37	55.5	0.3
2/7/21	Sys A	EH	4	57.8	0.9	2/8/21	Sys A	KO	38	58.1	0.5
2/7/21	Sys A	EH	5	57.0	0.6	2/8/21	Sys A	KO	39	57.4	0.5
2/7/21	Sys A	EH	6	57.6	0.8	2/8/21	Sys A	KO	40	57.9	1.0
2/7/21	Sys A	EH	7	56.8	0.6	2/8/21	Sys A	KO	41	56.1	0.7
2/7/21	Sys A	EH	8	57.2	0.9	2/8/21	Sys A	KO	42	57.1	0.9
2/7/21	Sys A	EH	9	57.2	0.7	2/8/21	Sys A	KO	43	57.7	0.4
2/7/21	Sys A	EH	10	57.6	0.5	2/8/21	Sys A	KO	44	57.2	1.0
2/7/21	Sys A	EH	11	57.0	0.4	2/8/21	Sys A	KO	45	55.7	0.6
2/7/21	Sys A	EH	12	57.3	0.7	2/8/21	Sys A	KO	46	56.7	0.2
2/7/21	Sys A	EH	13	56.7	0.3	2/9/21	Sys A	KO	47	56.9	0.7
2/7/21	Sys A	EH	14	57.2	0.1	2/9/21	Sys A	KO	48	57.2	0.1
2/7/21	Sys A	EH	15	58.2	0.4	2/9/21	Sys A	KO	49	57.2	0.4
2/7/21	Sys A	EH	16	57.3	0.8	2/9/21	Sys A	KO	50	57.0	0.3
2/7/21	Sys A	EH	17	57.1	0.1	2/9/21	Sys A	KO	51	56.7	0.4
2/7/21	Sys A	EH	18	57.0	0.3	2/9/21	Sys A	KO	52	57.3	0.8
2/7/21	Sys A	EH	19	56.9	0.5	2/9/21	Sys A	KO	53	57.2	0.7
2/7/21	Sys A	EH	20	56.4	0.5	2/9/21	Sys A	KO	54	56.5	0.4
2/7/21	Sys A	EH	21	57.8	0.4	2/9/21	Sys A	KO	55	57.9	0.6
2/7/21	Sys A	EH	22	57.3	0.4	2/9/21	Sys A	KO	56	57.3	0.8
2/7/21	Sys A	EH	23	57.3	0.3	2/9/21	Sys A	KO	57	57.2	0.7
2/8/21	Sys A	KO	24	55.6	0.9	2/9/21	Sys A	KO	58	56.9	0.6
2/8/21	Sys A	KO	25	56.7	0.4	2/9/21	Sys A	KO	59	57.3	0.8
2/8/21	Sys A	KO	26	56.5	0.9	2/9/21	Sys A	KO	60	57.8	0.4
2/8/21	Sys A	KO	27	56.3	0.9	2/9/21	Sys A	KO	61	58.7	0.7
2/8/21	Sys A	KO	28	56.6	0.2	2/9/21	Sys A	KO	62	57.1	0.8
2/8/21	Sys A	KO	29	56.9	0.5	2/9/21	Sys A	KO	63	57.5	0.4
2/8/21	Sys A	KO	30	56.5	0.5	2/9/21	Sys A	KO	64	56.3	0.5
2/8/21	Sys A	KO	31	57.1	0.2	2/9/21	Sys A	KO	65	56.9	0.3
2/8/21	Sys A	KO	32	56.2	0.6	2/9/21	Sys A	KO	66	57.3	0.2
2/8/21	Sys A	KO	33	57.6	0.2	2/9/21	Sys A	KO	67	57.4	0.2
2/8/21	Sys A	KO	34	56.8	0.9	2/9/21	Sys A	KO	68	56.5	1.0
						2/9/21	Sys A	KO	69	57.3	0.7
									AVE [µmole C L-1]:	**57.0**	
									STDEV [µmole C L-1]:	**0.6**	

Table G3. DOC precision (repeatability). For this exercise, a total of 69 independently filled vials of the same water (GFF filtered seawater) were analyzed over 3 days on the same TOC-V system by skilled operators. Standard deviation provides a measure of repeatability and was 0.6 µmole C L^{-1} for seawater DOC samples using the Shimadzu HTC method.

Date analyzed	System ID	Operator	Sample ID	[µmole N L-1]	Std Dev
8/24/21	Sys C	KO	Hansell DSR 07-15	31.5	0.5
8/24/21	Sys C	KO	Hansell DSR 07-15	30.9	0.7
8/24/21	Sys C	KO	Hansell DSR 07-15	31.3	0.4
8/24/21	Sys C	KO	Hansell DSR 07-15	30.6	0.4
8/24/21	Sys C	KO	Hansell DSR 07-15	32.1	0.2
8/27/21	Sys C	KO	Hansell DSR 07-15	32.1	0.5
8/27/21	Sys C	KO	Hansell DSR 07-15	32.2	0.4
8/27/21	Sys C	KO	Hansell DSR 07-15	31.7	0.4
8/27/21	Sys C	KO	Hansell DSR 07-15	30.7	0.3
8/27/21	Sys C	KO	Hansell DSR 07-15	30.3	0.3
			AVE [µmole N L-1]:	**31.3**	
			STDEV [µmole N L-1]:	**0.7**	

Table G4. TDN precision for seawater samples using the Shimadzu HTC method. Multiple observations of deep seawater CRMs (5 ampoules per run) over two runs (same TOC system and operator) were used to assess standard deviation as a measure of repeatability. IUPAC recommends at least 4 observations per set of references.

Precision (Reproducibility) – external complement to repeatability. The goal is to evaluate how reproducible results are across laboratories utilizing the same method.

Range (µmole L^{-1})	Reference Batch	UCSB Repeatability Test			UMIAMI Repeatability Test			**(REPRODUCIBILITY)**
		AVE [µmole L^{-1}]	STDEV	n	AVE [µmole L^{-1}]	STDEV	n	**STDEV [µmole L^{-1}]**
DOC Low (39)	EXPORTS 08-18 DRW	39.8	0.7	79	40.7	1.1	39	**0.6**
DOC Mid (62)	CRM 04-17 MID	63.0	6.0	4	62.1	2.4	8	**0.6**
DOC High (72)	CRM 08-18 SUR	72.2	1.8	79	69.9	1.8	39	**1.6**
TDN Low (5-10)	PB290 SUR	5.4	0.6	12	5.1	0.5	22	**0.2**
TDN Mid (20-30)	PB290 MID	22.6	0.4	8	22.1	0.7	22	**0.3**
TDN High (40)	PB290 DRW	39.3	1.5	12	37.1	1.2	23	**1.6**

Table G5. Interlaboratory comparisons were conducted between the Carlson and Hansell labs using identical batches of reference materials across the full measurement range for seawater samples. The reproducibility is the standard deviation of multiple repeatability test results where the conditions of measurement have been changed (in this case, the lab is the changing condition but the same method/instrument type/and skilled operators were utilized.