#### **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 for one month after collection 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 ( $\alpha$ = 0.05).

#### Filtration

Filtration is a necessary step to ensure separation of particulate from DOM. 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 142 mm 0.2 µm 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 47 mm 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 14 °C 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 μm 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  $\mu$ m filtrate that had not been exposed to combusted GF/F active sites and 0.2  $\mu$ m 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  $\mu$ m 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 <sup>3</sup>/<sub>4</sub> 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 bench top 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 <sup>3</sup>/<sub>4</sub> 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 reanalyzed again in 2015. n = 10 for all depths. Line in the box represents the median value, the bottom of the box represents the 2<sup>nd</sup> quartile value, top of the box represents the 3<sup>rd</sup> 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 ( $\alpha$ = 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 (no 75 m samples were available in summer 2019). 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
GF75 filter	Advantec MFS, Inc.	GF7525MM
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 <sup>™</sup> Combitips advanced <sup>™</sup> 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	Quartz Scientific Inc.	100018B, 100004B
(18 mm ID x 20 mm OD)		
(4 mm ID x 6 mm OD)		
Quartz Tubing for large tubes	Quartz Scientific Inc.	100006B,100017C,100027C
(6 mm ID x 8 mm OD)		
(17 mm ID x 20 mm OD)		
(27 mm ID x 30 mm OD)		
Premade TC Combustion	Shimadzu Scientific	638-41323-00
Tube (small column)	Instruments Inc.	
Premade High Salt	Shimadzu Scientific	638-42076-00
Combustion Tube (large	Instruments Inc.	
column)		
Special Order Platinum Gauze	Exeter Analytical Inc.	0240-1147D
(45 mesh 145 x 145 mm		
square)		
Normal Sensitivity Platinum	Shimadzu Scientific	638-60116-00
Catalyst (support 5/64"	Instruments Inc.	
alumina balls)		
Platinum Catalyst Large beads	Shimadzu Scientific	638-60193-00
for High Salts Kit (support	Instruments Inc.	
alumina balls)		
Magnagium Darahlarata		
A phydroug (Contified ACS)	Fisher Scientific	M54212
Annyarous (Certified ACS)		
Polyethylene Tube (drying	D-1 A ATM CD	
traps) 16 mm I.D. x 19 mm	Bel-Art <sup>IM</sup> SP	F199610000
O.D. (0.675 x 0.75 in.) 6"	Scienceware	
Copper wool reel, FINE - 5LB	Palmer Engineered	7165010
	Products	
Membrane Filter, 0.45 µm	Shimadzu Scientific	046-00042-12
	Instruments Inc.	
Flow meter	Alltech	Digital Flow Check <sup>TM</sup> 4700
Polyprowool, 150 g	Shimadzu Scientific	630-00325-00
	Instruments Inc.	

#### 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.



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 inhouse 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

#### 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 cooling coil and replacing it with a smaller section of tubing such that the connection is as follows: small combustion tube with 6 mm base tube -> PTFE tube fitting elbow 6 mm to 6 mm (such as Swagelok® T-6M0-9) -> glass or PTFE tube 6 mm OD, 4 mm ID, 50 mm long (UCSB glass shop homemade) -> PTFE tube fitting reducing union 6 mm to 3 mm (such as Swagelok® T-6M0-6- 3M) -> PTFE tubing 3 mm OD, 2 mm ID, 500 mm long -> Pure water trap.

#### TOC-LCSH/CPH



Figure C3. Schematic of the UCSB Shimadzu systems, adapted from the Shimadzu TOC-L manual (©Shimadzu 2010, Kyoto, Japan) and showing added in-line Mg(ClO<sub>4</sub>)<sub>2</sub> 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 cooling 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  $\mu$ mol C L<sup>-1</sup> 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.



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 20 mL 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:
- □ 80 mm polyethylene tube with barbed caps
- □ With one side capped, pack in 10 mm of polyprowool
- $\Box$  Fill loosely with Mg(ClO<sub>4</sub>)<sub>2</sub> leaving 10 mm at end of tube
- $\Box$  Pack remaining end with 10 mm of polyprowool and cap
- □ Wrap both ends tightly with parafilm



#### 5. Change halide scrubber:

- □ To be changed once the copper discolors within 2 cm of the trap outlet
- □ Recommended assembly:
  - □ 160 mm glass tube (polyethylene tube can also be used) with polyethylene barbed caps
  - □ With one side capped, pack in 15 mm of polyprowool
  - □ Pack in copper wool leaving 15 mm at end of tube
  - □ Pack remaining end with 15 mm 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.
- $\Box$  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
    - $\hfill\square$  Before and after the particle filter
- □ If there is a drop in the flow rate by more than  $3 \text{ mL min}^{-1}$  (from where the flow rate 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 repacked.
  - □ 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<sub>2</sub>/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

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	22	Sample #16	78		
34	0	Blank	70		
35	0	Blank	80		
36	5	Surface Seawater Reference	81		
37	6	Mid Seawater Reference	82		
38	7	Deen Seawater Reference	82		
30	24	Sample #17	60		
39	24	Sample #19	95		
40	25	Sample #10	96		
41	20	Sample #19	65		
42	2/	Sample #20	8/		
43	28	Sample #21	88		
44	29	Sample #22	89		
45	30	Sample #23	90		

#### EXAMPLE DOC RUN

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

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

Blanks are interspersed throughout run

## 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 <sub>0</sub> (μM C)=	1.525
t=	1.651
L <sub>C</sub> (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 <sub>0</sub> (μΜ N)=	0.3
t=	1.657
L <sub>C</sub> (μΜ 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
<b>σ</b> ₀= S₀ (μM C)=	1.16
t=	1.943
corr factor 2t=	0.96
L <sub>D</sub> (μM C)=	4.3
L <sub>Q</sub> (μM C)= 10* <b>σ</b> <sub>0</sub> =	11.6

Table F3. Limit of Detection (L<sub>D</sub>) and Limit of Quantification (L<sub>Q</sub>) for DOC analysis using the Shimadzu HTC method for seawater samples. Spiked samples (25  $\mu$ mol C L<sup>-1</sup>) 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≤ 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
$\sigma_{o}$ = S <sub>o</sub> ( $\mu$ M N)	0.2
t=	2.353
corr factor 2t=	0.92
L <sub>D</sub> (μM N)=	0.9
L <sub>Q</sub> (μM N)= 10* <b>σ</b> ₀ =	2.0

Table F4. Limit of Detection (L<sub>D</sub>) and Limit of Quantification (L<sub>Q</sub>) for TDN analysis using the Shimadzu HTC method for seawater samples. Spiked samples (3  $\mu$ mol N L<sup>-1</sup>) 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≤ 25)a correction factor (4v/(4v+1)) for 2t is applied to take into account bias in S.

# Appendix G

Analytical quality limits

			Hansell CRM batch				
Date of Run	SYSTEM	Vial #	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	
	Me	asured Mean	42.2	41.7	60.6	72.5	
	Mea	sured Std Dev	0.7	0.4	1.0	0.5	
	Reported cor	nsensus value	42.5	42.0	61.0	71.0	
	Accurac	y (%rel error)	0.8	0.7	0.7	-2.1	

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

Table G1. Accuracy determination for DOC. All data are reported in  $\mu$ mol C L<sup>-1</sup>. 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)

Data of Rup	SVSTENA	Vial #	Hansell CRM batch					
Date of Kull	STSTEIVI	Vidi #	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		
	Measu	ured Mean	31.3	31.6	8.8	4.7		
Measured Std Dev		0.3	1.0	0.6	1.4			
F	Reported conse	nsus value	31.5	31.0	9.0	5.0		
	Accuracy (9	%rel error)	0.7	-1.9	2.0	5.9		

Table G2. Accuracy determination for TDN. All data are reported in  $\mu$ mol N L<sup>-1</sup>. 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)

Date analyzed	System ID	Operator	Vial #	[µmol C L <sup>-1</sup> ]	Std Dev	Date analyzed	System ID	Operator	Vial #	[µmol C L <sup>-1</sup> ]	Std Dev
2/7/21	Sys A	EH	1	56.1	0.9	2/8/21	Sys A	ко	35	56.9	0.4
2/7/21	Sys A	EH	2	57.2	0.3	2/8/21	Sys A	КО	36	57.1	0.7
2/7/21	Sys A	EH	3	56.8	0.9	2/8/21	Sys A	КО	37	55.5	0.3
2/7/21	Sys A	EH	4	57.8	0.9	2/8/21	Sys A	ко	38	58.1	0.5
2/7/21	Sys A	EH	5	57.0	0.6	2/8/21	Sys A	ко	39	57.4	0.5
2/7/21	Sys A	EH	6	57.6	0.8	2/8/21	Sys A	ко	40	57.9	1.0
2/7/21	Sys A	EH	7	56.8	0.6	2/8/21	Sys A	ко	41	56.1	0.7
2/7/21	Sys A	EH	8	57.2	0.9	2/8/21	Sys A	ко	42	57.1	0.9
2/7/21	Sys A	EH	9	57.2	0.7	2/8/21	Sys A	ко	43	57.7	0.4
2/7/21	Sys A	EH	10	57.6	0.5	2/8/21	Sys A	ко	44	57.2	1.0
2/7/21	Sys A	EH	11	57.0	0.4	2/8/21	Sys A	ко	45	55.7	0.6
2/7/21	Sys A	EH	12	57.3	0.7	2/8/21	Sys A	ко	46	56.7	0.2
2/7/21	Sys A	EH	13	56.7	0.3	2/9/21	Sys A	ко	47	56.9	0.7
2/7/21	Sys A	EH	14	57.2	0.1	2/9/21	Sys A	ко	48	57.2	0.1
2/7/21	Sys A	EH	15	58.2	0.4	2/9/21	Sys A	ко	49	57.2	0.4
2/7/21	Sys A	EH	16	57.3	0.8	2/9/21	Sys A	ко	50	57.0	0.3
2/7/21	Sys A	EH	17	57.1	0.1	2/9/21	Sys A	ко	51	56.7	0.4
2/7/21	Sys A	EH	18	57.0	0.3	2/9/21	Sys A	ко	52	57.3	0.8
2/7/21	Sys A	EH	19	56.9	0.5	2/9/21	Sys A	КО	53	57.2	0.7
2/7/21	Sys A	EH	20	56.4	0.5	2/9/21	Sys A	КО	54	56.5	0.4
2/7/21	Sys A	EH	21	57.8	0.4	2/9/21	Sys A	КО	55	57.9	0.6
2/7/21	Sys A	EH	22	57.3	0.4	2/9/21	Sys A	КО	56	57.3	0.8
2/7/21	Sys A	EH	23	57.3	0.3	2/9/21	Sys A	КО	57	57.2	0.7
2/8/21	Sys A	КО	24	55.6	0.9	2/9/21	Sys A	ко	58	56.9	0.6
2/8/21	Sys A	КО	25	56.7	0.4	2/9/21	Sys A	КО	59	57.3	0.8
2/8/21	Sys A	КО	26	56.5	0.9	2/9/21	Sys A	КО	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	KU	32	56.2	0.5	2/9/21	Sys A	KU KO	67	57.3	0.2
2/8/21	Sys A	KO	33	56.8	0.2	2/9/21	Sys A Svs A	KO KO	68	56.5	0.2
2/0/21	JYSM	ĸo	34	50.0	0.5	2/9/21	Sys A	KO	69	57.3	0.7
					1	2/3/21	5931	NO	05	57.5	0.7
								AV	F [umol C I <sup>-1</sup> ]:	57.0	
								STDE	/ [umol C L <sup>-1</sup> ]:	0.6	

*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).* 

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  $0.6 \mu mol C L^{-1}$  for seawater DOC samples using the Shimadzu HTC method.

Date analyzed	System ID	Operator	Sample ID	[µmol N L⁻¹]	Std Dev
8/24/21	Sys C	КО	Hansell DSR 07-15	31.5	0.5
8/24/21	Sys C	КО	Hansell DSR 07-15	30.9	0.7
8/24/21	Sys C	КО	Hansell DSR 07-15	31.3	0.4
8/24/21	Sys C	КО	Hansell DSR 07-15	30.6	0.4
8/24/21	Sys C	КО	Hansell DSR 07-15	32.1	0.2
8/27/21	Sys C	КО	Hansell DSR 07-15	32.1	0.5
8/27/21	Sys C	КО	Hansell DSR 07-15	32.2	0.4
8/27/21	Sys C	КО	Hansell DSR 07-15	31.7	0.4
8/27/21	Sys C	КО	Hansell DSR 07-15	30.7	0.3
8/27/21	Sys C	КО	Hansell DSR 07-15	30.3	0.3
			AVE [µmol N L <sup>-1</sup> ]:	31.3	
			STDEV [ $\mu$ mol N L <sup>-1</sup> ]:	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.* 

$\mathbf{P}_{\mathbf{r}} = \mathbf{r} + (\mathbf{r}_{\mathbf{r}} + \mathbf{r}_{\mathbf{r}} + \mathbf{r}_{$	Defense Detek	UCSB Repeatability Test			UMIAMI Repeatability Test			(REPRODUCIBILITY)
Range (µmor L)	Reference Batch	AVE DOC [µmol C L <sup>-1</sup> ]	STDEV	n	AVE DOC [µmol C L <sup>-1</sup> ]	STDEV	n	STDEV [µmol L <sup>-1</sup> ]
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.

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