




# LIMNOLOGY and OCEANOGRAPHY: METHODS

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## 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

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### Abstract

Particulate organic carbon (POC) represents a small portion of total carbon in the ocean. However, it plays a large role in the turnover of organic matter through the biological pump and other processes. Early on since the development of the POC measurement technique in the 1960s, it was known that dissolved organic carbon (DOC) adsorbs and is retained both on and in the filter. That retained DOC is measured as if it was part of the particulate fraction, an artifact that can cause significant overestimates of POC concentration. We set out to address the long-standing question of whether the magnitude of the DOC adsorption is affected by the quantity and quality of the dissolved organic matter in the sample. However, our results precluded an unequivocal answer to that question; nevertheless, the experimental data generated did allow us to develop and test predictive models that relate the mass of carbon adsorbed to the volume of sample filtered. The results indicate that the uptake of DOC can be predicted using an exponential model and that a saturation point is approached when approximately a half-liter of water is filtered. This model can be a valuable tool for correcting existing POC data sets that did not account for DOC adsorption. Nonetheless, this approach should not be regarded as a substitute for collecting in situ filter blanks in parallel with POC samples to properly correct for this artifact.

While not the largest pool of carbon in the ocean, particulate organic carbon (POC) attracts considerable attention in ocean carbon research as a vector for atmospheric CO<sub>2</sub> export to the deep ocean through the biological pump (Siegel et al. 2016 and references therein). A variable portion of the POC pool is living phytoplankton biomass (Graff et al. 2015), which is crucial for understanding plankton dynamics and its relationship to changes in the oceanic environment.

Since the 1960s, laboratory methods have been developed to directly measure POC in discrete seawater samples (e.g., Menzel and Vaccaro 1964; Knap et al. 1996), which rely on the filtration of a small (i.e., <1–8 L) volume of water to retain particles onto a glass fiber filter, such as Whatman GF/F with a 0.7 μm nominal pore size. Sample filters, after frozen storage (< –20°C), are dried, acidified to remove inorganic carbon, and then packed into tin foil squares or cups before analysis. Once prepared, the samples are measured using high-temperature combustion with subsequent detection of the CO<sub>2</sub> produced from the oxidation of the organic carbon. Due to the lack of a unified, community-supported

protocol for POC measurements, numerous variations exist in the steps of the sample collection and analysis. These differences in methodology can cause large variability within final POC concentrations (King et al. 1998; Gardner et al. 2003), differences that are very often hard to separate from the natural variability in the POC pool (Cetinić et al. 2012). Other methods exist that make use of large volume (i.e., >100–1000 L) in situ pumps; however, those are not addressed here (e.g., Bishop et al. 2012).

Several studies have suggested that GF/F filters adsorb and retain dissolved organic carbon (DOC). Once it is adsorbed onto a filter, the DOC ends up being measured as part of the POC fraction, which can lead to a significant overestimation of the POC concentration (Menzel 1967; Moran et al. 1999; Gardner et al. 2003). For example, in oligotrophic waters, DOC can account for up to 35% of the final POC concentration (Abdel-Moati 1990). Moran et al. (1999) first suggested that DOC adheres to reaction sites on the GF/F filters, and that the number and ability of these sites to hold DOC may vary from filter to filter. The idea that the filter adsorption sites are saturated with the filtration of only 1 L of seawater was challenged by Feely (1974). Yet, even though this

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problem has been identified for several decades, relatively few researchers are addressing the DOC retention issue or attempting to correct for it while measuring POC (Moran et al. 1999; Gardner et al. 2003; Behrenfeld and Boss 2006; Turnewitsch et al. 2007; Cetinić et al. 2012).

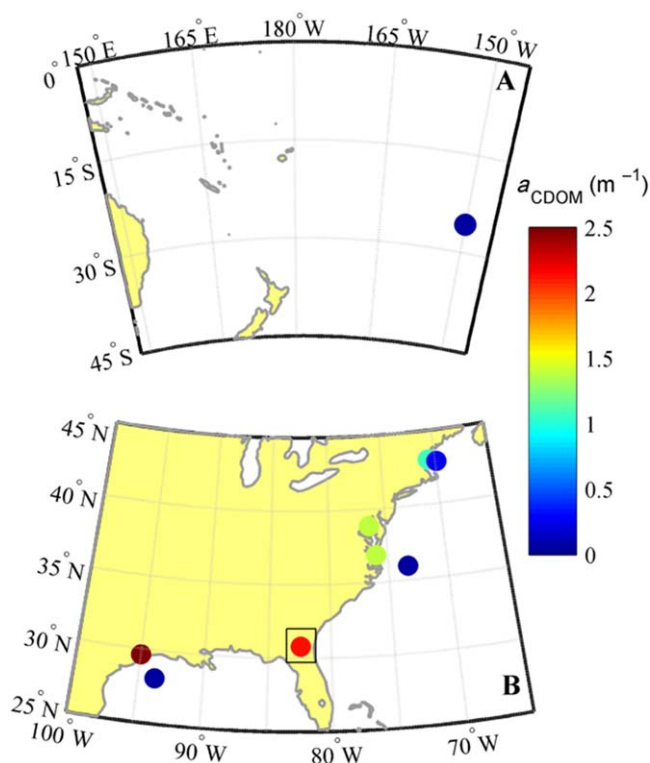
The heterogeneous distribution of POC in oceanic waters makes it impractical to collect enough in situ discrete water measurements to accurately assess the role of POC in the carbon cycle. Proxies have been developed based upon the relationship between water column particulates and optical properties (Gardner et al. 1993; Cetinić et al. 2012; Boss et al. 2015). For in situ observation, optical proxies based upon attenuation and backscattering measurements have been used for decades to measure POC pools at various spatial scales (e.g., Gardner et al. 2006), and from various types of research platforms (Briggs et al. 2011; Alkire et al. 2014; Estapa et al. 2017; Omand et al. 2017). The relationship of POC concentration to remote sensing reflectance band ratios has been also applied to develop algorithms to measure POC from satellites (Stramski et al. 2008). However, the efficacy of these technologies for the retrieval of POC concentration may be constrained by the quality of the in situ POC data derived from discrete samples used to develop the underlying relationships given the lack of an uniform consensus to carry out the measurement (Gardner et al. 2003; Cetinić et al. 2012).

The purpose of this study was to ascertain, using diverse natural and synthetic water samples, whether the quantity and the quality of DOC, as well as the volume of water filtered play a role in the magnitude of DOC that filters adsorb and predict the retention of DOC. Two models were developed to test hypotheses on whether (1) DOC retention increases linearly with filtration volume or (2) DOC retention reaches an asymptote with greater filtration volume as adsorption sites on the glass fiber filters become saturated. The outcome of this analysis was used to develop a correction procedure that can be used to estimate DOC retention on filters for existing POC datasets, in the hopes that such an approach can help improve the accuracy of the datasets including those employed for POC proxy and remote sensing algorithm development (Gardner et al. 1993; Cetinić et al. 2012; Boss et al. 2015).

## Materials and procedures

### DOC retention experiments

Water samples for DOC retention experiments were either collected in the field during various research cruises encompassing coastal to hyper-oligotrophic waters (Fig. 1; Table 1) or were produced in the laboratory using standard reference materials. Four different lots of Whatman GF/F filters 25 mm in diameter were used throughout the experiments and were combusted for 6 h at 450°C. To minimize the filter variability, for each water type, filters from the same lot were used. The “lot” pertain to the number printed on the side of each GF/F box. Multiple boxes of 100 count filters can have the same lot



**Fig. 1.** Locations of natural water sample collection employed in filter DOC retention experiments. Color-coded symbols depict CDOM absorption ( $a_{\text{CDOM}}$ ) at 355 nm measured for each of the samples (color bar). CDOM was not concurrently measured for the Chesapeake Bay sample. The value here is from a measurement near the time and location of the sample used in this experiment. The square denotes the region where the Suwanee River Fulvic Acid source material was collected (see methods).

number. For this experiment lot numbers 40716, 58823, 83948, and 96995 were used and will be referred to as lot 1, 2, 3, and 4, respectively, throughout the rest of the text.

Natural water samples were acquired during field campaigns following different modes of collection described in Table 1. All natural samples were prefiltered to remove particles immediately or within a day of collection using a 0.2  $\mu\text{m}$  Whatman Polycap capsule filter TC series 0.2  $\mu\text{m}$  poly ether sulphone (PES) membrane (in Table 1 as 0.2 poly), except for samples collected during CLIVAR that were prefiltered using GF/F filters and samples collected on the KORUS-OC cruise that were prefiltered with a ZENPURE capsule filter (SZL series 0.2  $\mu\text{m}$  PES membrane).

Immediately following the prefiltration, multiple volumes of the resulting filtrate, ranging from 0.05 L to 2 L, were refiltered through the combusted GF/F filters. In each experiment, triplicates were generated for each volume, with the exception of those using seawater from the CLIVAR P16S and KORUS-OC campaigns (Table 1), for which duplicates were generated due to seawater collection constraints. One other exception pertained to the Gulf Stream water. In the field, the sample was prefiltered after collection and then

**Table 1.** List of geographical locations on which water was collected for this study, with associated geographical location, in parenthesis is the abbreviation for sample group used through the article. Synthetic sample, Suwan. River Ref. II, is marked with an asterisk.

Campaign, location	Collection date (YYYY/MM/DD)	Latitude (°)	Longitude (°)	Mode of collection (depth)	Prefilter used	Frozen	<i>n</i>
CLIVAR P16S, South Pacific ( <b>CLIVAR P16S</b> )	2014/04/18	-25.66	-150.00	Peristaltic pump (1 m)	GF/F	Yes	16
GEO-CAPE, Gulf of Mexico offshore ( <b>GoMEX</b> )	2013/09/15	28.09	-93.72	Peristaltic pump (1 m)	0.2 poly	Yes	23
GEO-CAPE, Gulf of Mexico, Galveston Bay ( <b>Galv. Bay</b> )	2013/09/18	29.61	-94.96	Peristaltic pump (1 m)	0.2 poly	Yes	24
CLiVEC, ( <b>Gulf Stream</b> )	2013/02/11	35.99	-73.33	Flow-through system (~ 5 m)	0.2 poly	No	21
CLiVEC, New Hampshire Isle of Shoals, ( <b>NH Shoals</b> )	2013/06/03	43.02	-70.63	Surface via carboy	0.2 poly	No	23
CLiVEC, Wilkinson Basin, Gulf of Main ( <b>Wilk. Basin</b> )	2013/03/08	42.86	-69.86	Flow-through system (~ 5 m)	0.2 poly	No	22
CLiVEC, Virginia Beach ( <b>Virg. Beach</b> )	2013/05/22	36.88	-75.98	Surface via carboy	0.2 poly	No	23
CLiVEC, Chesapeake Bay ( <b>Ches. Bay</b> )	2013/12/03	38.86	-76.45	Surface via carboy	0.2 poly	No	22
KORUS-OC, South Korea: Sea Of Japan/East Sea, station 34 ( <b>East Sea</b> )	2016/05/25	37.82	129.56	Flow-through system (~ 5 m)	ZEN-PURE	Yes	12

stored for several months in the dark at room temperature. Before carrying out the volume filtration experiment, the water was refiltered through a 0.2 micron polycap filter.

All filters were initially placed in combusted (6 h at 450°C) aluminum foil packages. If not processed immediately (Table 1), filters were stored in liquid nitrogen and then transferred to a -80°C freezer until analysis (within 12 months of collection). The potential effect of the filter storage was tested with “dry filter blanks” (unused filters) that were subjected to the same analysis as the filters containing samples.

A synthetic DOC sample (Suwan. River Ref. II) was made by dissolving Suwannee River Fulvic Acid reference material in ultrapure water (18.2 MΩ Millipore Milli-Q ultraviolet oxidized water). Suwannee River Fulvic Acid II is a humic standard with well-defined chemical and chemical composition isolated and prepared by the International Humic Substances Society (IHSS; Thurman and Malcolm 1981; <http://humic-substances.org>). The solution was prepared after drying the material for 24 h at 50°C and weighed to a precision of 0.001 mg; the solution was mixed for several hours with a sterilized stir bar prior to use. The final solution was not pre-filtered but directly divided into the experimental treatment volumes and filtered through the GF/F filters.

Samples for the analysis of DOC concentration and measurement of chromophoric dissolved organic matter (CDOM) spectral absorption ( $a_{\text{CDOM}}$ ) from each experiment were collected in amber glass vials after the prefiltration step. CDOM samples were stored in the dark and refrigerated (~ 4°C), and

DOC samples were frozen (-20°C) until analysis. DOC was measured in duplicate or triplicate using high temperature combustion oxidation with a Shimadzu TOC auto analyzer. Accuracy of analyses was monitored by running DOC consensus reference material (CRM) standards (Hansell Laboratory, University of Miami RSMAS); CRM measurements must maintain an analytical error to within ± 5% of the consensus values.

CDOM samples were allowed to warm to room temperature before analysis and then refiltered through 0.2 μm disc filters (Polycarbonate or PES). The absorbance spectra of CDOM was measured using a Cary 100 dual-beam spectrophotometer with suprasil quartz 10 cm path length cells and ultraviolet oxidized Milli-Q reference water. The samples were scanned using a 4 nm slit width from 250 nm to 800 nm. Spectral absorption coefficients were calculated using the following equation:

$$a_{\text{CDOM}}(\lambda) = 2.303A(\lambda)/L \quad (1)$$

where  $A(\lambda)$  is the absorbance measured at each wavelength and  $L$  is the path length of the quartz cell. The spectral slope coefficients ( $S$ ) for CDOM were calculated using a nonlinear fitting function to a single exponential function of the form:

$$a(\lambda) = A(\lambda)e^{-S(\lambda-\lambda_0)} \quad (2)$$

where  $a(\lambda)$  and  $a(\lambda_0)$  refer to the absorption coefficients at  $\lambda$  and a reference wavelength  $\lambda_0$  and fit over several different wavelength ranges (300–600 nm, 275–295 nm, and 350–

400 nm). The slope ratio (Sr) was calculated by dividing the slope value from the 275–395 nm fit by the slope value of the 350–400 nm fit (Helms et al. 2008). Protocols for the preparation and measurement of CDOM and DOC were described in Mannino et al. (2008, 2014, 2016).

### Biogeochemical measurements during CLIVAR P16S and CLiVEC

POC samples from the cruises were collected from subsurface waters by Niskin-style bottles secured to a CTD Rosette package, while near-surface water (~ 1 m) was collected with a peristaltic pump. The water was transferred to the lab in rinsed carboys, and sample volumes were measured with rinsed graduated cylinders and subsequently filtered through GF/F filters using glass funnels and frits. In addition, 1 L GF/F prefiltered POC blanks were prepared from the near-surface water at each CLIVAR P16S station.

Samples for the determination of phytoplankton pigments were collected concurrently with the POC samples. Water in the amount of 0.5–5 L were filtered through GF/F filters; then, the filters were folded and placed in precombusted foil pouches and stored in liquid nitrogen and later in a  $-80^{\circ}\text{C}$  freezer until analysis in the laboratory. Phytoplankton pigments were determined using high performance liquid chromatography following the procedures of Van Heukelem and Thomas (2001), as further described in Hooker et al. (2005).

### Filter carbon analysis

All samples (DOC retention filters and filters containing particulates) were placed in a drying oven for 24–48 h in 8 mL glass vials either immediately after filtration or after removal from the freezer (Table 1). After drying, the filters were placed in a sealed glass desiccator chamber and exposed to hydrochloric acid fumes (12 M HCl) for approximately 24 h to remove any inorganic carbon and then dried again for 24–48 h. Dry filter blanks from each lot used in these experiments went through the same process.

The filters were then folded in half and placed on  $30 \times 30 \text{ mm}^2$  tin foil squares, wrapped, and pelletized using a stainless-steel press. A CHNS elemental analyzer (Vario MICRO cube manufactured by Elementar analysysteme GMBH) was used to measure organic carbon mass (Garcia et al. 2011). Once the instrument reached operating temperature, a fine leak test was carried out to determine whether the system was completely sealed and operating within the proper pressure and flow rate conditions. Before each analysis, several Sulfanilamide standards (Elementar analysysteme GMBH, 16.25%N, 41.81%C) as well as a reference material (Buffalo River Sediment [BRS] NIST reference material NIST RM 8704;  $3.348\% \pm 0.016\%$  Carbon) were packed in  $3 \times 3 \times 11 \text{ mm}^3$  tin boats and measured to determine instrument accuracy and stability. Only when all tests were passed were samples loaded into the instrument carousel for analysis. Every five samples, another sulfanilamide standard was

measured to ensure stability. Three to four BRS samples were also measured for each run. While packing the GF/F filters, three  $30 \times 30 \text{ mm}^2$  foil squares were randomly selected at different times, pelletized, and analyzed to estimate the carbon content. The same was done for the tin boats in which the standards were measured.

Once the analysis was complete, the instrument software was used to compute a *daily factor* based on a comparison of the factory calibration to the sulfanilamide measurements before and during the run. Then, the *daily factor* corrected values for the percentage of carbon measured in each sample were used to calculate the mass of carbon in each sample. To account for the carbon content measured in the foil boats and squares that the standards and samples were packed in, respectively, a *tin blank* correction was developed. The carbon content of the tin boats that the standards were measured in was already accounted for in the daily factor. Hence, to calculate a *tin blank* correction value, the average mass of carbon measured in the tin boats was subtracted from the average mass of carbon measured in the tin squares. A single *tin blank* value was subtracted from all the samples measured within a sample run (50–60 samples plus standards, reference material, and air blanks). The average *tin blank* correction value was  $1.86 \pm 0.88 \mu\text{g C}$  for nine separate sample runs.

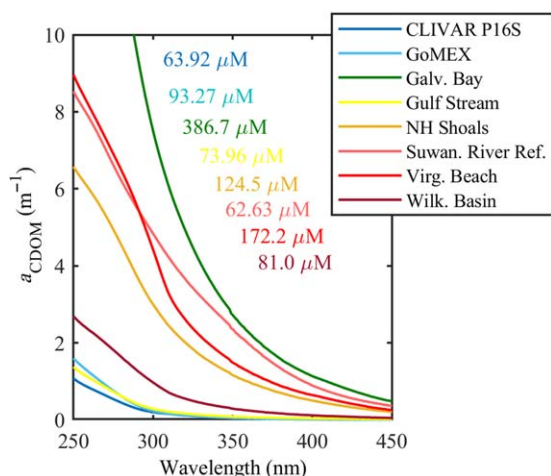
### Assessment

#### Spectral characteristics

The natural samples used for these filtration experiments to develop the models were collected from offshore, coastal, and estuarine waters along the U.S. East Coast and Gulf of Mexico (Table 1). The only exception was the water collected on the CLIVAR P16S line in the South Pacific near Tahiti. The intention was to use natural waters with different chemical and carbon makeup to ascertain whether there is a connection to DOC retention in GF/F filters (Fig. 1). The KORUS-OC DOC retention curves were not used in the development of the models and were only used as an independent assessment of how well the models work.

The in situ DOC concentrations and  $a_{\text{CDOM}}$  varied significantly for each set of samples. The range of the CDOM spectral slopes ( $S$ ) calculated from 300 nm to 600 nm was 0.0165–0.0314 with an average and standard deviation of 0.0210 and 0.0046, respectively. In general, the spectral slope increased with greater  $a_{\text{CDOM}}$  absorption and visually the spectral shapes were significantly different for high and low  $a_{\text{CDOM}}$  samples (Fig. 2). When the  $S$  value calculated over the 275–295 nm range is greater than the value for the 300–350 nm range, it is a good indication that the source of the DOM is mainly of marine origin. The slope ratio between the two ranges (Sr;  $S_{275-295}/S_{300-350}$ ) has proved to be a pragmatic and simple way to characterize CDOM (Helms et al. 2008). The Sr values from the experiments ranged from 0.646 to 2.29, with the greatest values coming from the

South Pacific Gyre (CLIVAR P16S) and the Gulf Stream, and the lowest values from the Suwan. River Ref. II and Virg. Beach measurements. The range of values encountered



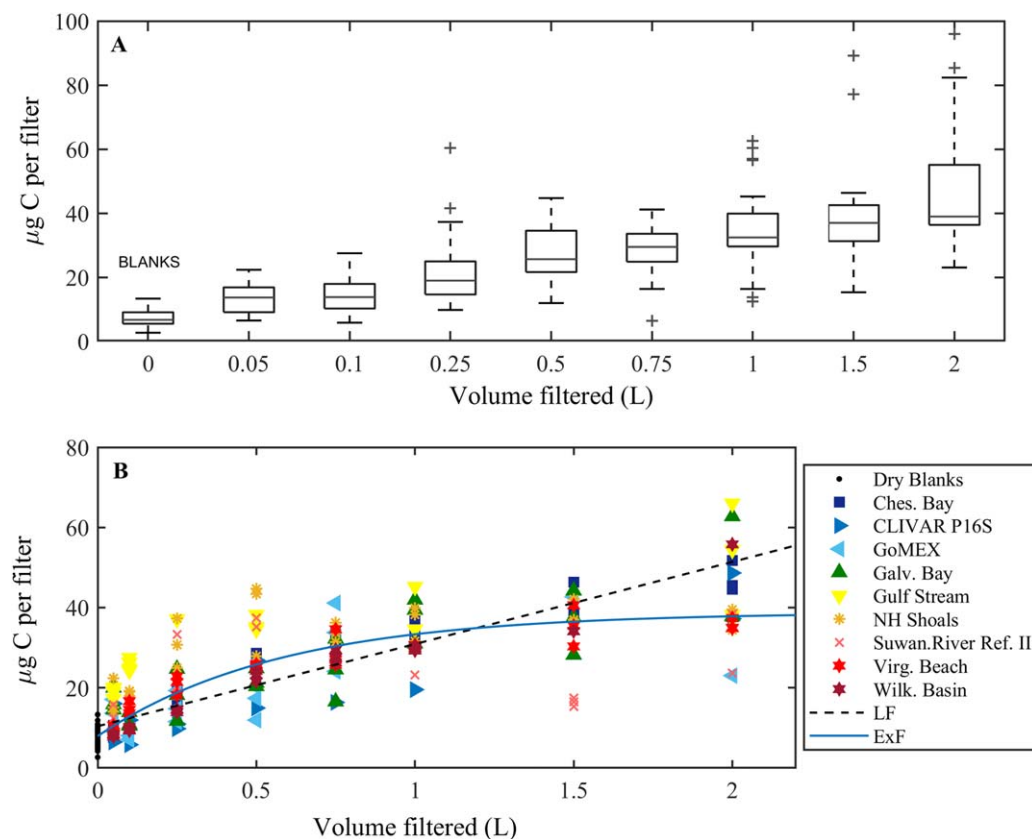
**Fig. 2.** CDOM spectral absorption (lines) and DOC concentrations given in  $\mu\text{M}$ . Both parameters varied significantly across the water types used to develop the models.

indicates that the DOM measured in these experiments represents a wide variety of compositions, spectral characteristics, and molecular weights.

### DOC retention experiments

DOC concentrations throughout the experiment ranged from  $62.63 \mu\text{M C}$  to  $386.7 \mu\text{M C}$ , and the increase in DOC concentration across the samples was directly proportional to spectral characteristics of  $a_{\text{CDOM}}$ , with the exception of the Suwan. River Ref. II sample that exhibited high  $a_{\text{CDOM}}$  and low spectral slope. The largest concentration of DOC was measured in Galveston Bay (Galv. Bay,  $386.7 \mu\text{M}$ ), while the lowest concentrations were measured from the South Pacific (CLIVAR P16S) and from the Suwan. River Ref. II which were  $63.92 \mu\text{M}$  and  $62.63 \mu\text{M}$ , respectively (Fig. 2).

The variation between replicates in the majority of the DOC retention samples was substantial (Fig. 3A). The average of all of the standard deviations computed for each volume and for each experiment was  $18.7 \pm 16.8 \mu\text{g C}$  with a range of  $0.11\text{--}81.3 \mu\text{g C}$ . Lower variability was observed for dry filter blanks (Fig. 3A, zero volume), where the average mass of carbon measured was  $7.3 \pm 2.4 \mu\text{g C}$  ( $n = 54$ ). The variability in average



**Fig. 3.** Distribution of carbon mass on filters with respect to volume filtered from all samples collected during DOC adsorption experiments (Panel A) and DOC adsorption models (Panel B). Outliers in Panel A, depicted with a plus symbol, were not used for the calculation of the global fits shown in Panel B linear model (dashed black line) or exponential model (solid blue line). Color/symbol coding in panel B depicts the samples from different experiments (see Table 1 legend).

**Table 2.** Comparison between the fit parameters of the linear and exponential models with associated statistics, coefficient of determination ( $r^2$ ) and root mean square error (RMSE), for each set of water samples. Experiment names are defined in Table 1. Experiments marked with an asterisk indicate that, based on statistics, behavior of samples was better or equally well explained by the linear model when compared to exponential model.

Experiment	Linear model				Exponential model					
	$a$ ( $\mu\text{g C L}^{-1}$ )	$b$ ( $\mu\text{g C}$ )	$r^2$	RMSE	DOC <sub>max</sub> ( $\mu\text{g C}$ )	$a$ ( $\mu\text{g C L}^{-1}$ )	$b$ ( $\mu\text{g C}$ )	$r^2$	RMSE	$V_k$ (L)
CLIVAR P16S*	15.01	7.22	0.77	6.14	5.49E+05	15.05	7.21	0.79	5.99	36,478
GoMEX	7.85	12.92	0.22	10.89	27.58	52.69	6.09	0.64	9.02	0.523
Gal. Bay*	17.31	13.70	0.74	3.59	52.55	36.62	7.08	0.70	7.57	4.239
Gulf Stream	15.5	23.34	0.71	7.07	31.24	293.2	7.26	0.90	4.55	0.107
NH Shoals	9.1	23.24	0.36	7.39	28.82	169.4	8.34	0.76	4.68	0.170
Wilk. Basin*	23.4	7.89	0.99	1.27	33.72	42.8	5.76	0.75	4.73	0.788
Virg. Beach	13.0	16.01	0.75	4.73	26.38	48.1	9.25	0.92	2.82	0.548
Ches. Bay	19.82	12.77	0.92	3.96	45.26	49.25	7.17	0.97	2.35	0.919
Suwan. River Ref. II	7.0	16.15	0.04	8.88	14.98	89.2	8.71	0.26	8.24	0.168

carbon per filter measured for the four individual lots used in these experiments was minimal and statistically not significant (lot 1 =  $7.9 \pm 3.9 \mu\text{g C}$ ,  $n = 7$ ; lot 2 =  $8.5 \pm 1.8 \mu\text{g C}$ ,  $n = 16$ ; lot 3 =  $6.29 \pm 1.6 \mu\text{g C}$ ,  $n = 6$ ; lot 4 =  $6.5 \pm 1.7 \mu\text{g C}$ ,  $n = 23$ ).

In all experiments, the mass of carbon measured when prefiltered water was filtered through glass fiber filters tended to increase with greater filtration volume. Samples from each experiment were used to evaluate two potential models: (1) a linear model that assumes a continuous increase of the DOC retention with an increase in volume, and (2) an exponential model that assumes an existence of a saturation point and the lack (or minimal) increase in DOC retention after this point. The first hypothesis was tested using a model II, least squares linear regression:

$$\text{DOC}_{\text{ret}}(V) = a \times V + b \quad (3)$$

where  $V$  is the volume used,  $a$  is the slope, and  $b$  is the offset on the  $y$ -axis, which accounts for the concentration of carbon measured in dry filter blanks. The second hypothesis was tested using a least squares exponential fit using the equation:

$$\text{DOC}_{\text{ret}}(V) = \text{DOC}_{\text{max}} * \left( 1 - \exp\left(\frac{-a * V}{\text{DOC}_{\text{max}}}\right) \right) + b \quad (4)$$

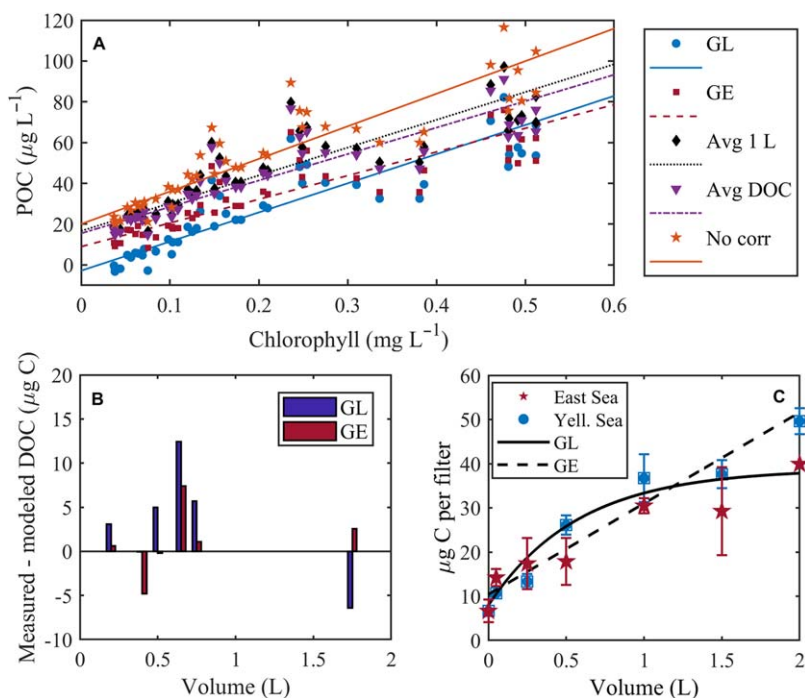
where  $\text{DOC}_{\text{max}}$  is the maximum amount of carbon that can be retained on a filter, and is the sum of carbon measured on filter blanks ( $b$ ) and the DOC originating from the sample. Same as above,  $a$  is the slope of the growth portion of the curve (Table 2). From Eq.4, we can calculate the minimal volume needed to achieve the maximum retention ( $V_k$ ):

$$V_k = \frac{\text{DOC}_{\text{max}}}{a} \quad (5)$$

Comparison of the retention experiments revealed that on average the exponential model more accurately explains

the behavior of the DOC retention on the GF/F filters (Table 2). Only three experiments (CLIVAR P16S, GoMEX, and Wilk. Basin) were better or equally well explained by the linear model (as suggested by the performance statistics). The term  $b$  in Eqs. 1, 2 represents the contribution that the dry filter blanks make to total carbon measured. The difference between  $b$ , calculated by the exponential model for each of the experiments (reported as average  $\pm$  SD  $7.43 \pm 1.09 \mu\text{g C}$ ), and the measured dry filter blanks ( $7.29 \pm 2.98 \mu\text{g C}$ ) is not statistically significant (two-tailed test,  $p > 0.05$ ). In contrast,  $b$  derived by the linear model ( $14.80 \pm 5.39 \mu\text{g C}$ , two-tailed  $t$ -test,  $p < 0.0001$ ) was significantly larger than the average content of carbon found on dry filter blanks. Due to the poor performance of the exponential model in some of the experiments (e.g., CLIVAR), the encountered range of  $\text{DOC}_{\text{max}}$  had a median of  $31.24 \mu\text{g C}$  and mean of  $61,029 \pm 172,524 \mu\text{g C}$ . For the same reason, we observed large differences between the mean ( $4054 \pm 11,463 \text{ L}$ ) and median value ( $0.548 \text{ L}$ ) of  $V_k$ . A comparison of the fit parameters with the sample's respective DOC concentration and spectral characteristics did not yield any statistically significant results.

In order to minimize the impact of the observed variability among the replicates (Fig. 3), and to consider the global variability in DOC concentrations and characteristics (and its potential impact on the model performance, Figs. 1 and 2), we combined all the samples and evaluated the retention of the DOC through above mentioned models. The exponential model outperformed the linear model ( $r^2$  of 0.99 vs. 0.65, and RMSE of 1.34 and 7.83, respectively). Slope for the linear model was found to be  $19.13 \pm 1.63 \mu\text{g C L}^{-1}$  and  $b$  of  $10.48 \pm 1.35 \mu\text{g C}$ . For the exponential model, slope ( $a$ ) was found to be  $53.42 \pm 2.37 \mu\text{g C L}^{-1}$ ,  $\text{DOC}_{\text{max}}$  of  $30.86 \pm 0.62 \mu\text{g C}$ , and  $y$ -axis offset ( $b$ ) of  $7.94 \pm 1.63 \mu\text{g C}$ . The resulting  $V_k$  value is  $0.58 \pm 0.05 \text{ L}$ .



**Fig. 4.** Evaluation of the global models. Relationship between Chl *a* and POC during CLIVAR cruise differs for different DOC corrections applied (panel **A**, see text for explanation of the sample groups). Difference between the modeled (GL: global linear model and GE: global exponential model) and actual DOC retention on filters over the different volumes filtered in the mid-Atlantic Bight (Panel **B**). Comparison of the DOC retention experiment performed on KORUS-OC samples from two optically diverse stations (Panel **C**, East Cell—red star, Yellow Sea—blue sea).

### Testing the global model

The performance statistics from the two evaluated models proved a significant outperformance of the exponential fit. However, evaluating the performance of these corrections on the retrieval of POC is a difficult task. Here, we assessed the impact of modeled DOC corrections on the final estimates of the POC via three different independent case studies. First, using the CLIVAR P16S dataset, we assessed the variability in chlorophyll *a* concentration (Chl *a*) to POC relationship as a function of the modeled DOC; second, we evaluated how efficiently modeled DOC retention replicated the variability observed in field-based DOC retention measurements collected during a CLiVEC cruise in February 2013; and third, we compared the models with two DOC retention experiments conducted during a KORUS-OC cruise (East Sea and Yellow Sea), at two locations characterized by distinct DOM properties.

In the first approach, the regression of POC concentrations with concurrent Chl *a* concentrations from CLIVAR P16S data was explored (Fig. 4). POC concentration was calculated by applying the following DOC retention corrections; (1) global linear mode (GL, Fig. 4A), (2) global exponential model (GE, Fig. 4A), (3) constant DOC correction of 14.64 µg C, representing the average value of all 1 L DOC filter blanks collected during the CLIVAR campaign (Avg 1 L, Fig. 4A), (4) average DOC blank, sensu Cetinić

et al. (2012), of 19.1 µg C (Avg DOC, Fig. 4A), and (5) no correction (essentially total organic carbon, no corr in Fig. 4A). The impact of chosen DOC correction proved to be significant in the oligotrophic environment of the southern Pacific Ocean (Fig. 4A). In this case, the global linear model performed the poorest, yielding a negative offset on the  $y$ -axis, and generating several negative POC concentrations for samples collected within lower Chl *a* waters ( $< 0.1 \text{ mg m}^{-3}$ ). Regardless of the correction, the relationship of the Chl *a* and derived POC was best described with the linear model (all  $r^2 > 0.76$ ), suggesting that the Chl *a* : POC relationship should not be used as a tool to estimate the validity of the DOC correction (n.b. nature of this relationship can be highly variable for other reasons, see Behrenfeld and Milligan 2012; Cetinić et al. 2015 and references therein).

For the second evaluation of the DOC global model performance, data collected at several stations along the mid-Atlantic bight were used, where DOC retention filters were collected in triplicates parallel to the POC measurements while using different filtration volumes. A comparison of the in situ DOC retention measurements with both global models demonstrates the outperformance of the global exponential model over the linear model, with an average absolute residual of an exponential model  $\sim 2.5$  times smaller than the one found for the linear model (2.78 µg C and 5.43 µg C per filter, respectively).

The final assessment of the performance of the global models was evaluated using data collected during the KORUS-OC cruise. The DOC retention experiments were conducted in two distinctly different sites: the first was in the oligotrophic water of East Sea (72.40  $\mu\text{M}$  DOC), and the second was in the turbid, DOM-rich (Kim et al. 2016) Yellow Sea (154.25  $\mu\text{M}$  DOC). In the East Sea, the exponential model performed slightly better than the linear model ( $r^2$  of 0.92 vs. 0.89 and RMSE of 3.84 vs. 4.09). The statistics also favored the exponential model for the optically complex Yellow Sea Station ( $r^2$  of 0.97 vs. 0.93 and RMSE of 3.46 vs. 4.63).

### Discussion

Previous studies have identified the issue of DOC retention in GF/F filters and the effect on the POC measurement (Moran et al. 1999; Gardner et al. 2003). Several studies have addressed the problem by using different techniques to derive a filter blank value that can be subtracted from the total mass measured regardless of the volume of the sample (Moran et al. 1999; Gardner et al. 2003; Behrenfeld and Boss 2006; Cetinić et al. 2012). Abdel-Moati (1990) carried out experiments with five 47 mm GF/C filters stacked in-line and found a relationship between volume-filtered and DOC-adsorbed, but not a linear one. Turnewitsch et al. (2007) also concluded that there is strong evidence for a nonlinear relationship between filter volume and adsorbed DOC. All of the DOC retention curves measured in this study support the hypothesis that DOC retention on glass fiber filters used for POC measurement is correlated to the volume of water passed through the filter (Fig. 3).

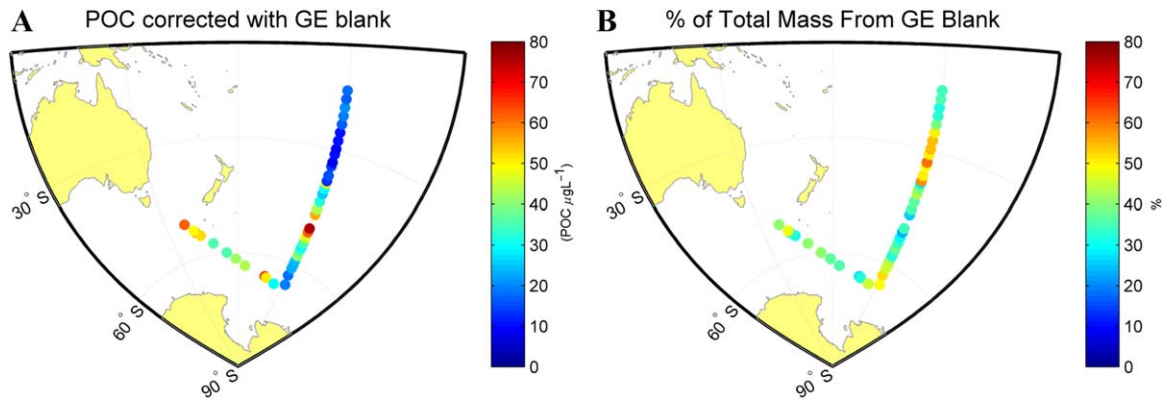
These data, collected across the oceanic realms, support the idea that there are a limited number of the “adsorption sites” within the GF/F filter that become saturated. However, based on these data, the saturation point is not reached almost instantaneously as suggested by Moran et al. (1999), and is dependent on the volume filtered. For most of our DOC retention experiments, regardless of the statistic used, exponential models outperform linear models in explaining the behavior of the DOC retention (Table 2; Fig. 3). No relationship between the model performance and encountered DOC concentration, spectral characteristics of the CDOM, or molecular mass (as inferred by the CDOM absorption slopes) was detected. This was surprising as Abdel-Moati (1990) found that the DOC retention saturation point is reached with different volumes in oligotrophic vs. coastal waters. Turnewitsch et al. (2007) also found evidence of surface waters exhibiting enhanced amounts of adsorbed organic carbon with respect to deeper ocean samples. The lack of a trend associated with DOC characterization might be due to the noise in our data caused by other factors, and not due to the lack of connection between the DOC characteristics and DOC retention. For example, King et al. (1998) performed an

inter-laboratory comparison of total and organic carbon measured from sediment trap samples. They found strong agreement between total organic measurements ( $\pm 3\%$  of the mean), yet outlying results elucidated that technical errors still existed in methodologies. When they compared samples that were acidified using different methods, the agreement decreased to  $\pm 8\%$  of the mean. All of the samples were acidified in these experiments, which may or may not have played a role in the variability seen in these measurements.

It is likely that the GF/F filters themselves are responsible for a large part of the variation in the measurements from these experiments, as suggested by previous studies (Gardner et al. 2003; Stramski et al. 2008; Cetinić et al. 2012), regardless of the fact that all GF/F filters used within each of our experiments came from the same lot. The only other available measure of the GF/F variability here was the carbon concentration measured on the dry filter blanks. The coefficient of variation for the dry filter blanks was 41%, suggesting that a portion of the noise observed within our dataset (average c.v. of triplicates was  $\sim 90\%$ ) could be attributed to the filters themselves, leaving a larger portion of the variability unexplained. Although dry filter blanks demonstrated less variable and lower carbon values, it is possible that the number of and potential of receptor sites to adhere to carbon in each filter play a role in the magnitude of DOC adsorption.

Additional sources of error, that we were not able to quantify, might have contributed to the observed variability. Previously studies have demonstrated that formation of the colloidal structures can occur spontaneously in the filtered water, additionally so if the balance between particles, colloidal matter and dissolved phase is disturbed (e.g., by removal of the particles via filtration, Liu et al. 2005 and references within). We had no way of quantifying these processes, except trying to minimize their impact by fast refiltration, minimal handling, and shaking. Second source of variability that we could not quantify is the effective area of filtration. While for the particulate samples that area is easily measurable so the impact of the change in filtration funnel diameter can be accounted for, for the dissolved filtration that number is hard to estimate. Development and testing of the global model allows for larger applicability of the correction, as well as the minimization of the potential error because of the large sample number ( $n=232$ , Fig. 3). For the global dataset, the exponential model outperformed the linear one, suggesting that indeed the DOC retention on the GF/F filter reaches a saturation point. Offset on  $y$ -axis,  $7.94 \pm 1.63 \mu\text{g C}$  is similar to average dry filter blank values  $7.29 \pm 2.98 \mu\text{g C}$ . Furthermore, this model suggests that the maximum amount of DOC that can be adsorbed on the filter is  $30.86 \pm 0.62 \mu\text{g C}$ . This amount is larger than previously reported (Moran et al. 1999; Gardner et al. 2003; Cetinić et al. 2012); however, the range of the  $\text{DOC}_{\text{max}}$  encountered during our study





**Fig. 5.** (A) Organic carbon mass measured during CLIVAR P16S cruise per volume filtered, calculated after the mass of DOC adsorbed based on the Global exponential model was subtracted from the total mass measured. (B) The percentage of the total carbon measured that is attributed to DOC adsorption based on the exponential model.

is well within the range of the previously reported values (Table 2). The higher  $\text{DOC}_{\text{max}}$  found in this study also could suggest there is bias regarding certain water types or some other source of error.

Across the three different ecosystems, performance evaluation confirms that the global exponential model is robust enough to successfully mimic the natural behavior of the DOC retention in filters, regardless of the DOC spectral or chemical characteristics (Fig. 4). A comparison with concurrently measured Chl *a* concentrations further suggests that in the open oligotrophic water, usage of the global linear model or a single-volume-based DOC value, could lead to “overcorrection” of the DOC, especially for samples where small volumes of water were filtered for POC analysis.

Our findings suggest that when using the global exponential model, the volume needed to reach maximum retention of DOC ( $V_k$ ) is  $0.58 \pm 0.05$  L. However, an analysis of the POC samples collected during the CLIVAR P16S cruise, calculated using exponential model (Fig. 5A), suggests the need of higher total filtration volumes in order to minimize the impact of the errors associated with the correction for DOC retention. In the ultra-oligotrophic regions of the southern Pacific Ocean, associated with Chl *a* lower than  $0.1 \text{ mg m}^{-3}$  and POC concentrations  $< 20 \text{ mg m}^{-3}$ , DOC can contribute up to 80% of the total carbon measured in the sample, even if the volume filtered was up to 5 L (Fig. 5B). Several studies have suggested that a large portion of the concentration of reported POC values may actually be from DOC retention (Abdel-Moati 1990; Moran et al. 1999). Using the same approach as above with uncorrected datasets collected in ultra-oligotrophic ocean and available in NASA’s SeaBASS dataset (<http://seabass.gsfc.nasa.gov/>), we estimate that DOC retained on the filters from ultra-oligotrophic waters might have contributed up to 50% of the reported POC concentration in publicly available data.

To the best of our knowledge, this is the first published data quantifying the mass of DOC adsorbed using a model

with the volume of sample filtered as a parameter. The model presented here is a powerful tool that could allow for correction of the previously collected data, and improvement of the existing in situ proxies and remote sensing algorithms. Regardless of the meticulous approach, we could not explain all the observed variability in the samples, or determine the drivers of the relationships between DOC and volume filtered found in different water types. The slightly higher  $\text{DOC}_{\text{max}}$  encountered here, when compared to the previous studies, suggests a potential bias that could lead to overcorrection in samples for which inadequate water volume was filtered. Finally, our study offers an easy and seemingly global model for the estimation of DOC retention on GF/F filters. However, these conclusions should be taken as a recommendation; the best approach to correcting the POC samples for DOC adsorption is to collect a concurrent DOC retention blank.

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### Conflict of Interest

None declared.

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