



METHOD ARTICLE

REVISED Uncertainties of particulate organic carbon concentrations in the mesopelagic zone of the Atlantic ocean [version 2; peer review: 2 approved with reservations, 1 not approved]

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Abstract

Measurements of particulate organic carbon (POC) in the open ocean provide grounds for estimating oceanic carbon budgets and for modelling carbon cycling. The majority of the published POC measurements have been collected at the sea surface. Thus, POC stocks in the upper layer of the water column are relatively well constrained. However, our understanding of the POC distribution and its dynamics in deeper areas is modest due to insufficient in POC measurements. Moreover, the accuracy of published POC estimates is not always quantified, and neither is it fully understood. In this study, we determined the POC concentrations of samples collected in the upper 500 m during an Atlantic Meridional Transect and described a method for quantifying its experimental uncertainties using duplicate measurements. The analysis revealed that the medians of the total experimental uncertainties associated with our POC concentrations in the productive and mesopelagic zones were $2.5(\pm 1.2)$ mg/m³ and $2.6(\pm 0.6)$ mg/m³, respectively. In relative terms, these uncertainties corresponded to ~14% and ~35% of POC concentrations, respectively. However, despite our best efforts, we could explain only ~21% of the total experimental POC uncertainty. The potential sources of this unexplained portion of uncertainty are discussed.

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REVISED Amendments from Version 1

All revisions have been listed in the responses to the Reviewers' Reports. Here, we summarise the most important ones.

- We clarified in the introduction the two different parts of the "uncertainty work" we have conducted: 1) the estimation of the POC uncertainty based on duplicate estimates, and 2) the modelled uncertainty and related uncertainty budget. We also improved the method section explaining what protocols were available at the time of sampling and what appeared later.
- We have added a new section discussing the need for a certified (or consensus) reference material for POC analyses. Where relevant, we have replaced the term "accuracy" with "uncertainty".
- We have added details to the method section (e.g., make of bottles and type of rosette frame; manufacturer and model number of the acetanilide used).
- We have added a part of the introduction that defines POC and places it in the context of the other carbon pools.
- We added a sentence to make the reader aware that broken cells release their inner DOC in the environment.
- We have replaced the word "coverage" with "measurements".
- We have added a paragraph at the beginning of [Section 2](#) in which we introduce the JGOFS and IOCCG POC protocols.
- We have written "sample delay" in quotes so that the reader is aware that the next part of the sentence explains what the adjustments were referring to.
- We have added a new figure plotting aDOC vs. POC and added related text with the correlation coefficients for the mesopelagic and productive zones.
- We have updated the [Table 1](#) and [Table 3](#) so that they contain all variables and symbols used.

Any further responses from the reviewers can be found at the end of the article

1. Introduction

Particulate organic carbon (POC) is operationally defined as the non-carbonate and combustible carbon matter in particulate form that is retained by a filter of a typical nominal pore size of $0.7 \mu\text{m}$ ([Kharbush et al., 2020](#), [IOCCG POC Protocols](#)). In the open-ocean, pelagic POC mainly includes suspended detrital matter (i.e., remains of dead organisms) and living organisms (i.e. phytoplankton, heterotrophic bacteria and zooplankton) ([Gardner et al., 2006](#); [Turnewitsch et al., 2007](#)).

POC constitutes the third most abundant carbon pool ($\sim 2 \text{ Pg C}$, [Brewin et al., 2021](#); [Stramska & Cieszyńska, 2015](#)) in the ocean, the others being the dissolved inorganic ($\sim 38,000 \text{ Pg C}$, [Hedges, 1992](#)) and dissolved organic ($\sim 662 \text{ Pg C}$, [Hansell & Carlson, 2013](#)) and the particulate inorganic carbon pools ($\sim 0.03 \text{ Pg C}$, [Hopkins et al., 2019](#)). POC has one of the highest turnover rates among the pools of carbon in the ocean ([Brewin et al., 2021](#); [Sarmiento & Gruber, 2006](#)) and the efficiency with which it is transferred from the upper to the deep ocean and sediments contributes to controlling atmospheric CO_2 concentrations ([Kwon et al., 2009](#); [Parekh et al., 2006](#)).

In the last decades, several studies have collected relatively extensive measurements of POC, leading to progress in understanding the nature of POC and the processes controlling its spatio-temporal distribution ([Aumont et al., 2017](#); [Bishop & Wood, 2008](#); [Bishop & Wood, 2009](#); [Cetinić et al., 2012](#); [Gardner et al., 2003](#); [Gardner et al., 2006](#); [Henson et al., 2012](#); [Kharbush et al., 2020](#); [Kiko et al., 2017](#); [Lam et al., 2011](#); [Moran et al., 1999](#); [Rasse et al., 2017](#); [Turnewitsch et al., 2007](#); [Wangersky, 1976](#)). However, even though much effort has been invested in developing methods for determining POC ([Gardner et al., 2000](#); [Gardner et al., 2003](#); [Gardner et al., 2006](#); [Liu et al., 2005](#); [Liu et al., 2009](#); [Moran et al., 1999](#); [Wangersky, 1974](#); [Wangersky, 1976](#)), we still have a relatively rudimentary understanding of the uncertainties associated with the different steps of the analysis and, therefore, with the resulting POC estimates. This is a problem because identifying and quantifying the uncertainties of POC will ultimately allow the community to further understand how and why POC varies in the ocean. In addition, by better characterising POC uncertainties and the major sources of these uncertainties, we might improve the method for determining POC.

The objectives of this study were 1) to present a method to experimentally quantify the uncertainties of POC measurements based on duplicate filters in both pelagic and deep ocean layers; 2) to model POC uncertainty based on assumed sources of uncertainty affecting the determination of POC; and 3) to compare modelled and experimental uncertainties. Our results show that modelled uncertainties accounted for only a small fraction of the

experimental POC uncertainties, suggesting that sources of uncertainty different from those considered in our analysis controlled the uncertainty of our POC determinations. Identifying, characterising and minimising these additional uncertainties will lead to improved measurements of POC and a better understanding of its variability and the role it plays in the ocean carbon cycle.

2. Data and methods

At the time when the data presented in this study were collected, the best known and accepted protocol for determining POC was the one established in the mid 90's for The Joint Global Ocean Flux Study (JGOFS) (Knap *et al.*, 1996). Studies that appeared after the JGOFS protocol identified additional potentially important sources of uncertainty in the POC determination, as for example the adsorption of DOC onto the POC filters or the volume of water needed to minimise uncertainties when POC values are low (Cetinić *et al.*, 2012; Gardner *et al.*, 2003; Gardner *et al.*, 2006; Moran *et al.*, 1999; Stramski *et al.*, 2008). In an attempt to quantify and minimize the uncertainties of the POC concentrations determined in the present study, we modified the sampling, processing and analysis described in the JGOFS protocol. Modifications, described in more detail in Section 2.1, Section 2.2 and Section 2.3, included the sampling of different volumes of water in accordance to expected in-situ concentrations, and using different types of blanks to quantify the dissolved organic carbon and any contamination due to the acidification step. Yet, minimising all uncertainties proved difficult. More recently, a NASA-led team has been developing a revised and considerably more detailed POC protocol (currently in draft version) to support the validation of ongoing and upcoming ocean-colour satellite missions (see Chaves *et al.*, 2021).

2.1 Sampling

Water samples were collected at 67 stations during the 24th Atlantic Meridional Transect (AMT-24) aboard the RRS James Clark Ross from September 25th to November 1st, 2014 (see Figure 1). Two casts were completed

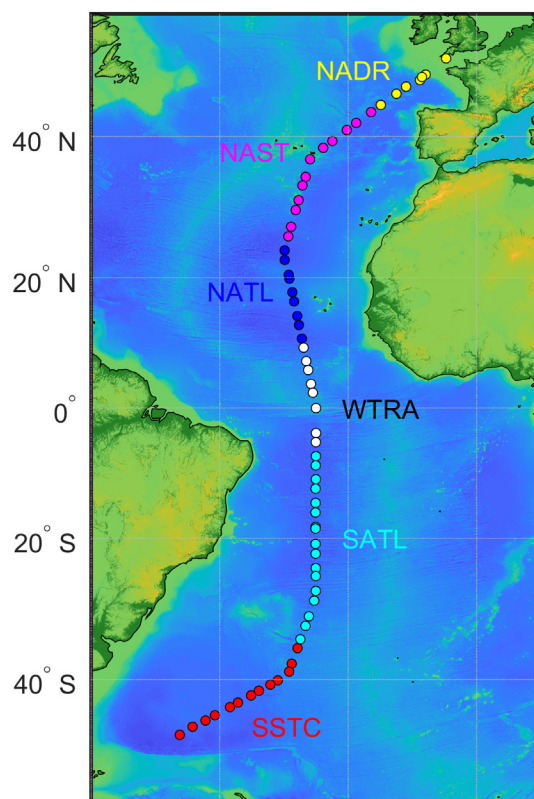


Figure 1. Track of AMT-24 cruise with the locations of 67 stations where samples for particulate organic carbon concentration were collected. Colour coding of the stations represents biogeographical provinces that were sampled: the North Atlantic Drift Province (NADR), the North Atlantic Subtropical Gyral Province (NAST), the North Atlantic Tropical Gyral Province (NATL), the Western Tropical Atlantic Province (WTRA), the South Atlantic Gyral Province (SATL), and the South Subtropical Convergence Province (SSTC).

every day (weather permitting): one pre-dawn and the other around solar noon. Different ecological provinces (Longhurst, 2007) were sampled: the North Atlantic Drift Province (NADR), the North Atlantic Subtropical Gyral Province (NAST), the North Atlantic Tropical Gyral Province (NATL), the Western Tropical Atlantic Province (WTRA), the South Atlantic Gyral Province (SATL), and the South Subtropical Convergence Province (SSTC).

At each station, water samples were collected from six depths in the upper 500 m using 20-l Niskin bottles (Ocean Test Equipment Inc., Standard ES, Model 115) that were installed on a bespoke stainless-steel frame. The sampling procedure did not account for dregs, the rare large particles that might have been under sampled by Niskin bottles (Gardner *et al.*, 2006). Water samples were transferred from the Niskin bottles into six 15-l HDPE carboys, which were pre-washed with 10% HCl and then with sample water prior to use. The carboys were taken to the on-board laboratory, where water samples were transferred into six ~2-l narrow-mouth amber bottles (Thermo Scientific Nalgene) and filtered through pre-combusted (450°C for five hours) 25 mm Whatman glass fiber filters (GF/F, nominal pore size 0.7 μm) at low vacuum (~125 mm Hg) by inverting the bottles into a standard funnel setup. Each of the bottles was also pre-washed with sample water prior to use. To keep the water samples homogenised, carboys were gently shaken before pouring into the bottles. The volume of sample water filtered for each POC measurement varied between ~1 and ~8 liters, and it was adjusted according to the expected concentration of POC in the respective environment. Thus, each 2-l bottle was typically re-filled (up to four times) during filtration to achieve the total volume for any given sample. No measures were taken to prevent atmospheric contamination during filtration.

We implemented the “double-filter” technique advocated by Banoub & Williams (1972); Feely (1974); Kinney *et al.* (1971); Loder & Hood (1972); Moran *et al.* (1999); Smith *et al.* (1996). These authors suggested using two stacked filters for each estimate of POC concentration. The upper filter was used to collect particles and adsorbed dissolved organic carbon (uncorrected POC sample, henceforth referred to as “uPOC”) while the lower filter quantified the dissolved organic carbon adsorbed onto GF/F filters (adsorbed DOC blank, henceforth referred to as “aDOC”) (see Table 1 for a list of variable names and their subscripts). After each filtration, uPOC and aDOC filters were removed from the filtration rig, wrapped into separate pre-combusted (450°C for five hours) aluminium foil envelopes, flash-frozen in liquid nitrogen, and then stored in a freezer at -80°C for post-cruise analysis.

At each cast, a duplicate sample from a randomly chosen depth was collected to assess the uncertainty of our method. Sample water for each pair of duplicate measurements was taken from the same Niskin bottle. In total, we collected 392 uPOC samples with their corresponding aDOC blanks and 57 duplicate uPOC samples with their related aDOC blanks. In theory, a larger number of replicates per sample will provide more robust estimates of the POC uncertainty. Yet, in our case duplicates were chosen as a compromise between statistical robustness, the water available from the rosette, and the time required to collect and analyse the samples.

2.2 Laboratory sample handling

All the filters were processed in 16 separate batches or CHN runs. Each run consisted of (1) uPOC filters and their corresponding aDOC blanks from multiple casts, (2) duplicate uPOC and aDOC filters from these casts used to estimate total experimental uncertainties, (3) empty tin capsules, acidified and non-acidified filter blanks (detailed below) used to estimate uncertainties related to the sample handling in the lab and systematic biases, and (4) standards used to calibrate the CHN analyser (see Section 2.3 for details).

Acidification at room temperature for a period of 12 to 16 hours was used to remove the inorganic carbon accumulated on the uPOC and aDOC filters. To do this, a crucible containing a small amount of 37% HCl (Sigma Aldrich, High Purity, 08256-500ml F) was located in the middle of a glass desiccator. The uPOC filters and aDOC blanks from each batch were removed from their aluminium envelopes, placed into individual acid-washed glass vials, and positioned around the crucible in the desiccators. In contrast to adding an aliquot of a dilute acid solution directly onto the filters, the technique of exposing them to acid fumes is expected to homogenize the effect of the acid on all the filters within a desiccator and to avoid losses of organic particles (Martin *et al.*, 1993). To minimise differences in contamination between corresponding uPOC and aDOC samples, paired uPOC and aDOC filters were acidified in the same desiccator. Duplicate filters were acidified in different desiccators.

To account for any potential contamination during acid fuming, we introduced an additional type of pre-combusted (450°C for five hours) 25 mm Whatman GF/F filter blank. Three of these filter blanks were placed into each desiccator, then acidified and processed along with uPOC filters and aDOC blanks, while three other filter blanks were kept clean and dry outside of the desiccators.

Table 1. List of variables names and their subscripts.

Subscript	Variable Name	Units
POC	Particulate Organic Carbon	N/A
uPOC	Uncorrected Particulate Organic Carbon	N/A
aDOC	Adsorbed Dissolved Organic Carbon	N/A
M_{uPOC}	Mass of organic carbon on uPOC	μg
M_{aDOC}	Mass of organic carbon on aDOC	μg
M_{cap}	Mass of organic carbon on tin capsules	μg
M_{ac}	Mass of organic carbon on acidified filters	μg
M_{nac}	Mass of organic carbon on non-acidified filters	μg
M_{uPOC}^*	Blank-corrected mass of organic carbon on uPOC	μg
M_{aDOC}^*	Blank-corrected mass of organic carbon on aDOC	μg
M	Mass of particulate organic carbon	μg
C	Concentration of particulate organic carbon	mg/m^3
D_1	Carbon concentration of the first duplicate	mg/m^3
D_2	Carbon concentration of the second duplicate	mg/m^3
\bar{D}	Average of POC concentration in the two duplicates	mg/m^3
Δ	Scaled arithmetic difference between duplicate pairs	mg/m^3
Δ_r	Scaled relative difference between duplicate pairs	dimensionless
σ_Δ	Uncertainty of each duplicate difference	mg/m^3
σ_{dr}	Relative experimental uncertainties of duplicate values	dimensionless
$\sigma_{\Delta r}$	Robust standard deviation of the relative duplicates pairs	dimensionless
σ_r	Median of σ_{dr}	dimensionless
σ_c	Total experimental uncertainty of POC estimates	mg/m^3
σ_v	Combined uncertainty of volume V	L
$\sigma_c(V)$	Total uncertainty explained by uncertainty in volume	mg/m^3
σ_M	Uncertainty of carbon mass predicted by the calibration equation (Prediction interval)	μg
σ_{Mp}	Uncertainty of carbon mass predicted by the calibration equation (standard law of propagation)	μg
$\sigma_c(M)$	Total uncertainty explained by uncertainty in regression models	mg/m^3
$\sigma_c(\eta)$	Total uncertainty explained by uncertainty in handling	mg/m^3
L_c	Critical value	μg
L_D	Detection limit	μg

After the acidification phase, all the filters, including the acidified uPOC filters, aDOC blanks, and acidified and non-acidified filter blanks were dried in an oven at 60°C for several hours. Subsequently, the acidified and non-acidified filters and blanks were wrapped into individual tin capsules (Pressed, Standard Clean, 10 × 10 by OEA Labs) and analysed for carbon. An increment on the acidified filter blank masses in comparison with the non-acidified ones would indicate contamination during sample acidification and drying.

2.3 Determination of POC

The mass of carbon contained on filters was determined by high-temperature combustion (Gordon & Sutcliffe, 1974; Menzel & Vaccaro, 1964; Sharp, 1974; Wheeler *et al.*, 1997) using a CHN analyser (FlashEATM 1112 Elemental Analyzer, with helium CP grade N5.0 as carrier gas). Filters were processed in accordance with the manufacturer's manual (ThermoQuest, 1999). In every CHN run, a new reaction tube was used. The extracted CO₂ was measured by a thermal conductivity detector.

As samples are analysed, the reaction tube of FlashEATM CHN analyser gradually fills up with the combusted tin capsules and filters. Thus, as the CHN run proceeds and the reaction tube fills up, a variation in the instrument combustion efficiency can occur with time. To stabilise the combustion efficiency of the instrument throughout each run, we performed constant adjustments to the “sample delay” parameter, which represents the time that it takes the CHN analyser to combust each sample, to deliver CO₂ released from the sample to the detector, and to run the analysis.

The CHN analyser was calibrated during each run using two sets of pre-weighted (Sartorius MC5 high-accuracy microbalance, calibrated yearly) acetanilide standards (C = 71.09%, N = 10.36%, OEA Labs, R66005) contained in tin capsules. The first set of 11 standards covered the entire range of expected masses of carbon on our uPOC and aDOC filters (5 – 300 µg) and it was analysed immediately prior to processing the sample filters. We will refer to this set of standards as the *calibration standards*. The second set of standards was processed alongside the filters (one standard after every six filters) to validate the initial calibration throughout filter processing. We will refer to the second set of standards as the *stability standards*.

In the absence of any instrumental drift, we expected the calibration coefficients derived from both sets of standards from the same CHN run to be statistically indistinguishable. However, during seven out of 16 runs the CHN analyser was unstable for unknown reasons and the calibration coefficients derived from the two sets of standards differed significantly. Thus, we decided to use for all CHN runs, both types of standards, *calibration* and *stability*, to develop the relationship between the response of the CHN analyser and the mass of carbon on processed filters.

The mass of carbon M on the i^{th} filter, processed during the k^{th} CHN run, was estimated by a linear regression model using a robust fitting method:

$$M_{ik} = m_k x_{ik} + b_k, \quad (1)$$

where x represents the output signal from the CHN analyser, m and b represent the slope and the intercept of the regression line, respectively. The intercept was removed from the model when it was not statistically significant (p-value > 0.05).

For each CHN run, we estimated the mass of organic carbon contained on uPOC (M_{uPOC}), aDOC (M_{aDOC}) filters, tin capsules (M_{cap}), and acidified (M_{ac}) and non-acidified (M_{nac}) GF/F filters using Equation 1. These M_{uPOC} and M_{aDOC} values, however, do not represent the true load of particulate organic carbon contained in the corresponding water samples as these values may be affected by biases, i.e., contamination during the acidification step, residual organic carbon on the tin capsules, and on the combusted GF/F filters). Therefore, the blank-corrected mass of organic carbon from the i^{th} uPOC and aDOC filters, which were acidified together in the j^{th} desiccator and processed in the k^{th} CHN run ($M_{\text{uPOC}_{ijk}}$ and $M_{\text{aDOC}_{ijk}}$, respectively), must be written as (notation as in Turnewitsch *et al.*, 2007):

$$M_{\text{uPOC}_{ijk}}^* = M_{\text{uPOC}_{ijk}} - \bar{M}_{\text{cap}_k} - (\bar{M}_{\text{ac}_{jk}} - \bar{M}_{\text{nac}_k})$$

$$M_{\text{aDOC}_{ijk}}^* = M_{\text{aDOC}_{ijk}} - \bar{M}_{\text{cap}_k} - (\bar{M}_{\text{ac}_{jk}} - \bar{M}_{\text{nac}_k}),$$

where $\bar{M}_{ac_{jk}}$ is the average carbon mass of the three filter blanks acidified in the same desiccator j^{th} as the i^{th} filter and \bar{M}_{nac_k} and \bar{M}_{cap_k} are the average carbon mass of the three non-acidified filter blanks and the average carbon mass of three tin capsules, respectively.

For each pair of uPOC and aDOC filters, the mass of POC, M , was determined as

$$M_{ijk} = M_{uPOC_{ijk}}^* - M_{aDOC_{ijk}}^* \quad (2)$$

We assumed that uPOC and aDOC filters had adsorbed the same amount of DOC, and their contamination due to sample handling during the CHN analysis was equal to the average mass of the three acidified filter blanks. Hence, the subtraction in Equation 2 removed various systematic biases from the final estimates of the mass of POC. Note that because we processed corresponding pairs of uPOC and aDOC filters in the same desiccator and during the same CHN run, Equation 2 is equivalent to:

$$M_{ijk} = M_{uPOC_{ijk}} - M_{aDOC_{ijk}} \quad (3)$$

To determine the POC concentration, C , for each water sample, we divided M by the volume of water, V , filtered for each sample:

$$C_{ijk} = \frac{M_{ijk}}{V} \quad (4)$$

2.4 Uncertainty analysis

The standard law of propagation of uncertainty (JCGM, 2008) was used throughout our uncertainty calculations and we recall it here for the reader:

$$\sigma_y^2 = \sum_{i=1}^N \left(\frac{\partial y}{\partial x_i} \right)^2 \sigma_{x_i}^2 + 2 \sum_{i=1}^{N-1} \sum_{j=i+1}^N \frac{\partial y}{\partial x_i} \frac{\partial y}{\partial x_j} \sigma_{x_i} \sigma_{x_j} r_{(x_i, x_j)}, \quad (5)$$

where σ_y^2 is the total (or “combined”) variance of the estimate y (POC, in our case), which is determined from the input quantities x_1, x_2, \dots, x_N through the functional relationship $y = f(x_1, x_2, \dots, x_N)$. The uncertainty of each of the input variables is denoted as σ_{x_i} and their inter-dependencies are represented by the correlation coefficients $r_{(x_i, x_j)}$. The total uncertainty is the positive square root of σ_y^2 .

2.4.1 Total experimental uncertainty. We first estimated the total *experimental* uncertainties associated with our POC concentrations by analysing the measurements from the duplicate samples. These total experimental uncertainties are expected to represent the uncertainties arising from all (or at least most of) the steps required to estimate POC concentrations (i.e., from sample collection in the field to sample analysis in the laboratory). Below we show that these uncertainties depend on the POC concentration and therefore we present them as relative uncertainties. The median values of the relative uncertainties estimated from all duplicates will then be used to estimate the uncertainty of any sample collected during the cruise. Thus, this last step uses the statistics from all duplicates to estimate *population* uncertainties, rather than *per-sample* uncertainties.

Experimental uncertainties were estimated from the scaled arithmetic differences between the POC concentrations of the duplicates, D_1 and D_2 :

$$\Delta = \frac{D_1 - D_2}{\sqrt{2}} \quad (6)$$

Since both D_1 and D_2 are uncertain and their uncertainties add in quadrature, to estimate the uncertainty in only one measurement, the difference of the duplicates was divided by $\sqrt{2}$ (Hyslop & White, 2009).

We found that the duplicate differences Δ were positively related to POC concentration and that this dependency was different between the productive and mesopelagic zones (Figure 2a). The correlation coefficient between Δ and the average POC concentration of duplicates was 0.4 in the productive zone and 0.54 in the mesopelagic zone. To remove this dependency on concentration, we expressed the differences in duplicate measurements as a relative difference ($\Delta_r = \Delta/\bar{D}$), where \bar{D} is the average of POC concentration in the two duplicates. Figure 2b confirms that, once normalised, the relative duplicate differences in the

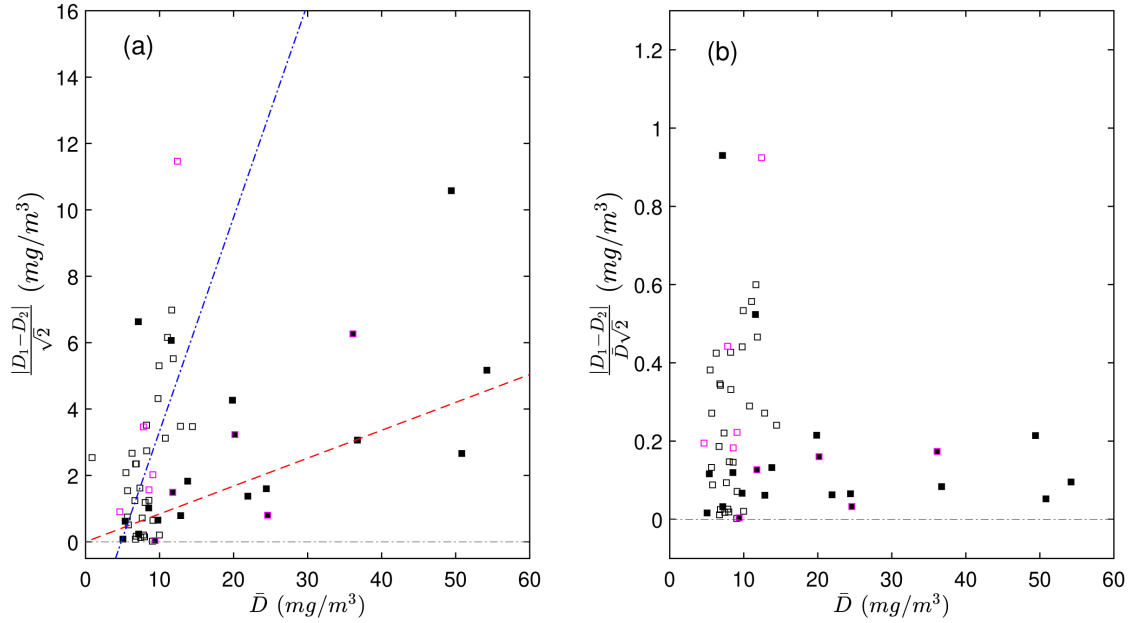


Figure 2. Scaled differences in duplicate particulate organic carbon (POC) measurements **(a)** as a function of the mean value of each pair of duplicates (\bar{D}). Scaled relative differences in duplicate POC measurements relative to \bar{D} **(b)**. The absolute values were used to more easily demonstrate the dependency of the duplicate differences on POC. Black and white points represent duplicate measurements from the productive and mesopelagic zones, respectively. Points with magenta borders represent samples processed during CHN runs with highly uncertain calibrations. The red dashed and blue dash-dotted lines are the linear fits to the data in the productive and mesopelagic zones, respectively.

productive and mesopelagic zones did not depend on POC anymore. These findings imply that estimates of POC concentration from a specific zone should have the same relative uncertainty, σ_r .

The total experimental uncertainty was computed by analysing the Δ values as follows. The uncertainty of each duplicate difference, σ_Δ , depends on the unknown uncertainties of the individual duplicate estimates, σ_{d_1} and σ_{d_2} . By applying the law of propagation of uncertainty (Equation 5) to Equation 6 and by assuming no correlation between the duplicate estimates, we find:

$$\sigma_\Delta^2 = \frac{\sigma_{d_1}^2 + \sigma_{d_2}^2}{2} = \frac{(\sigma_{d_r} D_1)^2 + (\sigma_{d_r} D_2)^2}{2} = \frac{\sigma_{d_r}^2 (D_1^2 + D_2^2)}{2}, \quad (7)$$

where σ_{d_r} is the relative uncertainty of the duplicate and we assumed that, as for the duplicate differences, also σ_{d_i} (with $i = 1, 2$) needs to be expressed in relative terms (i.e., $\sigma_{d_i} = \sigma_{d_r} D_i$).

By rearranging Equation 7, σ_{d_r} can be expressed in terms of the uncertainty of the difference, σ_Δ :

$$\sigma_{d_r} = \frac{\sigma_\Delta \sqrt{2}}{\sqrt{D_1^2 + D_2^2}}. \quad (8)$$

Expressing σ_Δ in relative terms yields:

$$\sigma_{d_r} = \frac{\sigma_{\Delta_r} \bar{D} \sqrt{2}}{\sqrt{D_1^2 + D_2^2}}. \quad (9)$$

σ_{Δ_r} was then estimated as the robust standard deviation of the relative duplicates in each zone:

$$\sigma_{\Delta_r} = \frac{P_{84}(\Delta_r) - P_{16}(\Delta_r)}{2}, \quad (10)$$

where P_{84} and P_{16} are the 84th and 16th percentiles of Δ_r (Hyslop & White, 2009).

Finally, the total experimental uncertainty of POC concentration was estimated as:

$$\sigma_C \approx \sigma_r C, \quad (11)$$

where σ_r is the median of σ_{d_r} , representing 14% and 35% uncertainties in POC concentrations for the productive and the mesopelagic zone, respectively.

2.5 Modeled uncertainty and uncertainty budget

In this section, we used a second method to model an independent estimate of the total uncertainty in POC concentrations. Specifically, the standard law of propagation of uncertainty was used to propagate the uncertainties associated with different steps of the POC determination. This independent model estimate of the POC uncertainty was then compared to the total experimental uncertainty derived from the duplicate measurements to assess the extent to which this latter theoretical calculation could reproduce the experimental uncertainties: the closer these estimates are, the more confident we can be in how we understand the measurement process and its uncertainties.

Equation 5 also allows one to estimate an uncertainty budget, that can be used to partition the total uncertainty into different contributions. Here, the relative contribution of each uncertainty source was computed as the ratio of the variance of each specific modeled uncertainty source divided by the total experimental variance (i.e., uncertainty squared). The sum of the modeled relative variances is an estimate of how well the modeled and total experimental uncertainties agree. The different relative uncertainty contributions can be ranked to identify the most uncertain steps in the methodology and prioritise improvements in the method. Table 2 presents the list of the sources of uncertainty that we were able to quantify.

2.5.1 Uncertainty in volume. Errors in measuring the volume of sample seawater translate into uncertainties in POC concentration. Since each POC sample required from one to five bottles of sample seawater, each with a volume V_n , the combined uncertainty of the total volume, V , of seawater used for a sample depended on the number of bottles, n , used and the uncertainty in volumetric measurements, σ_{V_n} , of each bottle. σ_{V_n} was set equal to half of a graduation mark of the measuring cylinder (uncertainty of 10 ml). The volume of each bottle (~2.2 l) was measured with a measuring cylinder multiple times.

Thus, the combined uncertainty of volume V can be expressed as

$$\sigma_V = \sqrt{\sum \sigma_{V_n}^2}. \quad (12)$$

Table 2. Sources of uncertainty that contribute to the total uncertainty of particulate organic carbon (POC) estimates and that we could quantify. More detailed explanation of calculations are given in Section 2.5. The median values of each uncertainty is given for the productive zone (PZ) and for the mesopelagic zone (MZ).

No	Source	Method of calculations	Values		Units
			PZ	MZ	
1	Volume	Volume uncertainty of each sample is equal to half graduation mark of the measuring cylinder used to estimate the volume of each bottle, multiplied by the number of bottles used during a given filtration	0.01	0.02	L
2	Calibration	Prediction intervals (PI) of the calibration curves of each CHN run	2.14	2.15	μg
3	Sample handling during CHN analysis	Quantify the uncertainty that could arise from acidification and processing of sample and blank filters.	0.37	0.34	μg
4	Total	Combined uncertainty calculated from POC duplicates	2.29	2.60	mg/m^3

We estimated the uncertainty in POC concentration that could be explained by the uncertainty in volume, $\sigma_c(V)$, by applying the propagation of uncertainty to Equation 4 and obtained:

$$\sigma_c(V) = \frac{M\sigma_V}{V^2}. \quad (13)$$

2.5.2 Uncertainty in mass predicted by the calibration equation. The uncertainty of carbon mass M_{ik} predicted using the calibration equation (Equation 1) was expected to be one of the largest contributors to the uncertainty budget because it determined the quality and reliability of the prediction of the masses of carbon in all components of the analysis. To estimate the uncertainties of the carbon masses predicted by our calibration equations, σ_M , we used 68% prediction intervals (PIs) as they serve in a normal distribution as one standard deviation. A PI is defined similarly to the better known confidence interval. However, the PI is more appropriate for quantifying the uncertainty of the calibration process because it represents the expected uncertainty of an individual future observation taking into account the uncertainties arising from all the regression parameters into the total uncertainty (Rawlings *et al.*, 1998).

For each CHN run, we estimated σ_M as stated in Altman (2000).

$$\sigma_{M_{ik}} = t_{1-\alpha/2} * \sigma_{\text{res}} \sqrt{1 + \frac{1}{n_s} + \frac{(x_{ik} - \bar{x})^2}{(n_s - 1)s_x^2}}, \quad (14)$$

where σ_{res} represents the robust standard deviation of the residuals of carbon mass about the regression line, x_{ik} is the instrument response (Equation 1), \bar{x} and s_x are the mean and the standard deviation of x_{ik} , n_s is the number of standards used to fit the model, and $t_{1-\alpha/2}$ is the value from the t distribution with $n_s - 2$ degrees of freedom and α equal to 0.32 corresponding to a 68% prediction interval.

The uncertainty of the regression model and the estimated masses depended on both the uncertainty of the weight of the standards and the sensitivity of the instrument to the carbon at the time when filters were processed. Therefore, σ_{res} and σ_M varied among CHN runs. Two CHN runs had significantly higher σ_{res} and therefore higher uncertainties of the regression models and mass estimates, compared to the rest of the runs. We believe that the cause of the poorer performance of the regression analysis in these instances was a less accurate weighting of the standards, and not an instability or low sensitivity of the CHN analyser. The differences of the duplicate POC estimates derived from these regression curves, on average, were not larger than those derived from regression curves with lower uncertainties (Figure 2). This means that there was no bias in the less accurate standards - they resulted in unbiased calibration coefficients, even though the random uncertainties around these coefficients were higher. Nevertheless, to avoid skewing our results, we did not include data derived from these two CHN runs when we determined our total experimental uncertainty (Equation 11).

The uncertainties of carbon masses estimated for uPOC and aDOC using the regression models were propagated into the uncertainties of POC masses by applying Equation 5 to Equation 2:

$$\sigma_{M_p} = \sqrt{\sigma_{M_{\text{uPOC}}}^2 + \sigma_{M_{\text{aDOC}}}^2 - 2\sigma_{M_{\text{uPOC}}} \sigma_{M_{\text{aDOC}}} r(M_{\text{uPOC}}, M_{\text{aDOC}})}, \quad (15)$$

where $r(M_{\text{uPOC}}, M_{\text{aDOC}})$ is the correlation coefficient between M_{uPOC} and M_{aDOC} .

Then, for each estimate of POC concentration, we calculated the contribution of this source of uncertainty to the total uncertainty of POC concentration, $\sigma_c(M)$:

$$\sigma_c(M) = \frac{\sigma_{M_p}}{V}. \quad (16)$$

2.5.3 Uncertainty due to sample handling during CHN analysis. The stages of acidification and drying of the filters may contaminate the samples, thus adding positive biases to the estimates of POC concentration and a higher variability between the duplicates (King *et al.*, 1998).

Considering that the uPOC samples, their corresponding aDOC blanks, and the filters blanks were acidified in the same desiccator and dried together in the oven, the amount of contamination that they received should have been approximately equal. Thus, the effect of the possible contamination should have been removed from the estimates of POC during the subtraction of M_{aDOC}^* from M_{uPOC}^* in Equation 2. However,

there was a degree of random uncertainty associated with the estimation of contamination as the average mass of carbon of the three acidified filter blanks for each desiccator, $\bar{M}_{ac,j}$, varied slightly.

The contribution of the uncertainty due to the handling of the filters to the total uncertainty, $\sigma_c(\eta)$, can be calculated by applying the standard law of propagation of uncertainty to Equation 4:

$$\sigma_c(\eta) = \frac{1}{V} \sqrt{\sigma_{\eta_1}^2 + \sigma_{\eta_2}^2}, \quad (17)$$

where σ_{η_1} and σ_{η_2} represents the uncertainties of contamination due to handling of uPOC and aDOC filters, respectively. These uncertainties were estimated as the standard error of the mean of the three corresponding estimates of acidified filter blanks, $\sigma_{\bar{M}_{ac}}$.

3 Results and discussion

3.1 Distribution of POC

The POC concentrations from the AMT-24 cruise were highly variable, ranging between 2 and 76 mg/m³ (Figure 3). The productive zone was characterised by relatively high POC concentrations, while low POC concentrations defined the mesopelagic zone. The overall median POC concentration from the productive zone was 18(±14) mg/m³, whereas that from the mesopelagic zone was 7(±3) mg/m³. We also observed latitudinal patterns of POC driven by the seasonality and differences between the biogeographical regimes sampled during the cruise (Figure 1 and Figure 3). In the productive zone, the highest POC concentrations were found at temperate latitudes and around the equator, whereas the most oligotrophic provinces were characterised by lower POC concentrations (Table 3). Such distribution in the POC concentration matched the typical latitudinal patterns encounter for the upper layer of the Atlantic ocean (Poulton *et al.*, 2006; Rasse *et al.*, 2017; Wangersky, 1976). Particularly, high POC concentrations were found in the sub-surface (50 m) of the South Subtropical Convergence Zone. In this province, Poulton *et al.* (2006) found relatively high POC concentrations deeper than 200 m as a result of sinking particles from the euphotic zone of the SSTC. Overall, POC concentration below the productive zone was less variable throughout the transect, but the latitudinal pattern remained (see Table 3). Even though our POC concentrations are comparable to those published in the literature, a direct comparison is complicated due to differences in methodologies, sampling times, and regions. The range of POC concentration estimated from bottle samples in the Atlantic ocean

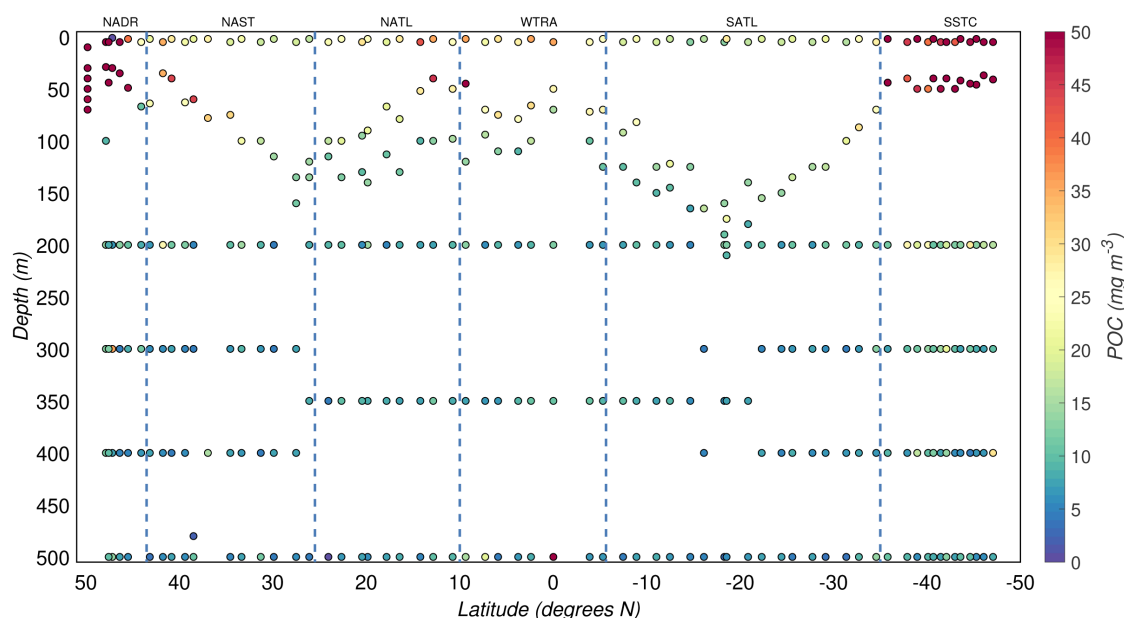


Figure 3. Depth-resolved distribution of particulate organic carbon (POC) concentration along the AMT-24 cruise. Borders of the sampled biogeographical provinces are marked by blue vertical lines.

Table 3. Particulate organic carbon distribution presented for productive and mesopelagic zones across the sampled biogeographical provinces: the North Atlantic Drift Province (NADR), the North Atlantic Subtropical Gyral Province (NAST), the North Atlantic Tropical Gyral Province (NATL), the Western Tropical Atlantic Province (WTRA), the South Atlantic Gyral Province (SATL), and the South Subtropical Convergence Province (SSTC). "Std" are robust standard deviations. Median and Std values are expressed in mg/m³.

No	Province	Productive zone			Mesopelagic zone		
		Samples	Median	Std	Samples	Median	Std
1	NADR	25	33	31	16	10	9
2	NAST	36	17	7	33	7	3
3	NATL	36	15	8	18	8	2
4	WTRA	32	19	10	16	9	6
5	SATL	57	14	5	44	7	2
6	SSTC	39	46	16	39	9	3
7	ALL	225	17	14	167	7	4

by numerous researchers spans from 5 to 350 mg/m³ (Balch *et al.*, 2010; Banoub & Williams, 1972; Cetinić *et al.*, 2012; Gardner *et al.*, 1993; Gardner *et al.*, 2003; Gardner *et al.*, 2006; Graff *et al.*, 2015; Marra *et al.*, 1995; Menzel & Goering, 1966; Mishonov *et al.*, 2003; Poulton *et al.*, 2006; Stramska & Stramski, 2005; Stramski *et al.*, 2008; Wangersky, 1974; Wangersky, 1976). A limited number of studies present POC concentrations measured in the Atlantic ocean between 100 and 500 m and spanning from ~0 to 35 mg/m³ (Carlson *et al.*, 2000; Cetinić *et al.*, 2012; Menzel, 1967; Poulton *et al.*, 2006; Wangersky, 1974; Wangersky, 1976).

3.2 Detection limits

To determine the limit of detection of our technique we used the approach recommended by The International Union of Pure and Applied Chemistry (IUPAC) and The International Organization for Standardization (ISO) (Analytical Methods Committee AMCTB No. 92, 2020). We calculated the detection limit, (L_D), by first computing the critical value, (L_C , Equation 18), which establishes the presence of the analyte (carbon in our case), and is defined as the minimum significant estimated value of an analytical result, which is used as to discriminate against background noise (Currie, 1995):

$$L_C = \bar{x}_0 + s_0 * t_{0.95;df}, \quad (18)$$

where \bar{x}_0 and s_0 are the mean and the standard deviation of a blank material free from carbon, in our case, the tin capsules. $t_{0.95;df}$ is the one-tailed 95% quantile for Student's t with degrees of freedom df , according to the number of values used to estimate \bar{x}_0 and s_0 .

Given L_C , we estimated L_D , which is defined as the lowest carbon mass that our analytical method is reliably capable of detecting 95% of the time:

$$L_D = L_C + s_0 * t_{0.95;df} = \bar{x}_0 + 2s_0 * t_{0.95;df}. \quad (19)$$

The median of the carbon mass from all the tin capsules used in the 16 CHN runs was 1.7(±1.3) µg. Thus, the estimated L_C and L_D were 3.2 and 4.9 µg C, respectively (Figure 4). The vast majority of mesopelagic aDOC filters collected and analysed during this study were above L_D , with just four filters fell between the estimated L_D .

3.3 Correction for biases

We corrected additional sources of bias in the estimates of POC concentration by subtracting aDOC blank measurements from the corresponding uPOC measurements (see Section 2.3 and Equation 2). Since the carbon mass determined on an aDOC blank includes the mass of the adsorbed DOC, the carbon masses detected

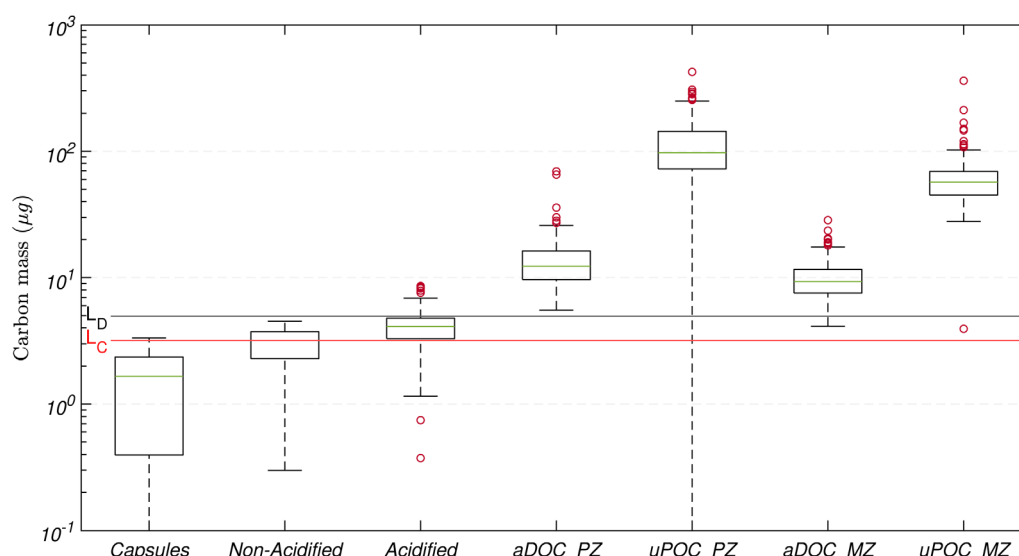


Figure 4. Distribution of carbon mass determined on tin capsules, non-acidified and acidified blank filters (GF/F) used for sample processing, and adsorbed dissolved organic carbon (aDOC) and uncorrected particulate organic carbon (uPOC) filters. In each element of the box plot, the central rectangle spans from the first quartile (25th percentile) to the third quartile (75th percentile). The green line inside each rectangle shows the median value and whiskers below and above the box show the locations of the 5th and the 95th percentile, respectively. Red circles represent outliers. Red and black horizontal lines represent the critical value (L_c) and detection limit (L_d), respectively.

on empty tin capsules, clean GF/F filters and any contamination occurring during filter acidification and handling, it represents the cumulative bias for which the carbon mass on a corresponding uPOC filter needs to be corrected. Hence, to minimise biases introduced by any potential contamination and mass predicted by the calibration equation, we processed pairs of uPOC and aDOC filters together during sampling, acidification, handling, and processing stages, i.e., acidified in the same desiccator and analysed during the same CHN run.

The magnitude of the masses on these additional components in comparison with the magnitude of the masses of aDOC and uPOC filters are presented in Figure 4. The median of the carbon mass from all the tin capsules used in the 16 CHN runs was $1.7(\pm 1.3) \mu\text{g}$, whilst the medians of the mass corresponding to non-acidified and acidified filter blanks were $3.2(\pm 0.8) \mu\text{g}$ and $4.1(\pm 1.1) \mu\text{g}$, respectively, indicating that our method minimised contamination during acidification.

On average, the acidification and handling of the filters resulted in contamination of $1(\pm 1) \mu\text{g}$. Nevertheless, when comparing corresponding sets of acidified and non-acidified filter blanks, the carbon masses from the acidified filters could be up to twice as large the amount of those from the non-acidified filters. Finally, the cumulative effect of all the biases and potential contamination that composed our aDOC blanks were $12(\pm 4) \mu\text{g}$ in the productive zone and $9(\pm 3) \mu\text{g}$ in the mesopelagic zone.

The range in carbon masses of our aDOC (Figure 5) is comparable with those from the Atlantic ocean reported by Cetinić *et al.* (2012): their average mass of DOC adsorption was $10.9 \mu\text{g}$ with 95% of the masses of their aDOC blanks within the range of 8.5 to $40.5 \mu\text{g}$. Also, our aDOC values are within the findings of Abdel-Moati (1990), who reported varying amounts of DOC adsorption between 9.2 and $15.0 \mu\text{g}$ and 3.5 and $6.5 \mu\text{g}$ for eutrophic and oligotrophic waters, respectively. The aDOC concentrations (carbon mass on aDOC filters normalized by volume and corrected for biases) in the productive zone had a median value of $2.2(\pm 1.3) \text{ mg/m}^3$, and $1.0(\pm 0.5) \text{ mg/m}^3$ in the mesopelagic zone. The distribution of these concentrations was correlated with POC concentrations ($r = 0.7$, Figure 8 and Figure 9). This correlation was particularly strong in the productive zone ($r = 0.87$ vs. $r = 0.38$ in the mesopelagic) and in regions characterized by high surface concentrations of POC: NADR ($r = 0.8$) and SSTC ($r = 0.9$).

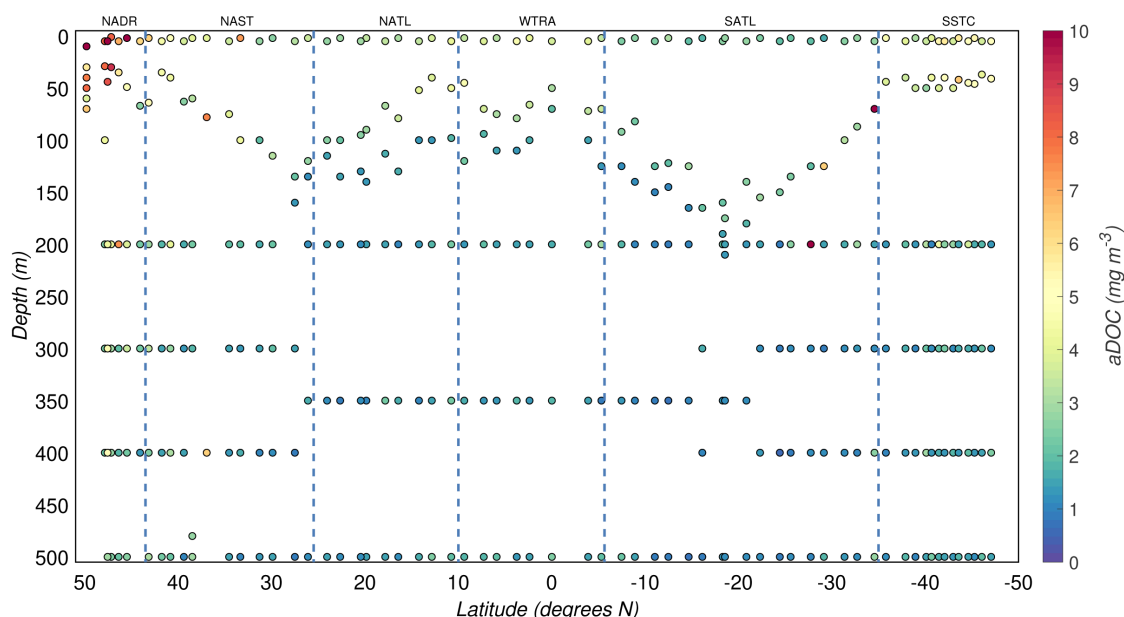


Figure 5. Depth-resolved distribution of adsorbed dissolved organic carbon (aDOC) estimates (mass of carbon on aDOC filters normalized as a function of volume and corrected for biases) along the AMT-24 cruise. Borders of the sampled biogeographical provinces are marked by blue vertical lines.

Since the filtered volumes were adjusted according to the expected POC concentration, and all GF/F filters were from a single manufacturer and treated identically during the cruise, the aDOC concentration should be relatively constant across the samples and not correlated with POC concentration. However, POC could be present on the aDOC filters, increasing the aDOC concentration due to fragmentation of particles through uPOC filters (Bishop & Edmond, 1976), perhaps due to the increased pressure differential caused by the two stacked filters (IOCCG POC Protocols). This fragmentation of larger particles from the uPOC filters into smaller particles has been observed (Wangersky, 1974) to contaminate aDOC filters and, perhaps, causing the higher aDOC values found in our study. For instance, Banoub & Williams (1972) filtered multiple samples of seawater through four stacked GF/C filters and found particles as evidence of contamination on the second filter from top to bottom. Abdel-Moati (1990) carried out a similar experiment with similar conclusions, suggesting the sum of masses from the first two filters should be used as a POC mass, while using the third filter, from top to bottom, as a true blank for aDOC. Cetinić *et al.* (2012) also pointed to the high variability of their aDOC values and potential contamination from the overlying uPOC filters. Finally, we note that when cells break, the DOC they contain is released in the environment and not retained on filters, potentially introducing biases in the POC determination.

If a higher than usual aDOC value is due to the contamination of an aDOC filter with particles filtered through the uPOC filter, particle loss from the uPOC filters should be higher in productive areas, thus explaining the observed strong positive correlation between POC and aDOC concentrations (Figure 9; see also Zhou *et al.*, 2016). Assuming that particles contaminated the aDOC filters, we expect that the typical mass of organic carbon adsorbed onto our GF/F filters should be better represented by the aDOC measurements at depth, where particles are less abundant. Thus, we estimated the loss of particles from uPOC to aDOC filters by subtracting from the carbon mass of all aDOC blanks (corrected for biases), the median carbon mass of aDOC filters collected in the mesopelagic zone (≥ 200 m). Then, we added this difference to our POC masses and found that POC concentrations increased by $3(\pm 4)\%$ in the productive zone and by $0(\pm 5)\%$ in the mesopelagic zone.

We cannot prove which mechanism determined the correlation between aDOC and uPOC concentrations. Nonetheless, the carbon mass ratio ($M_{\text{aDOC}}^*/M_{\text{uPOC}}^*$, corrected for biases, ranged from 3.5 to 58.2% with medians of $9.4(\pm 2.2)\%$ and $12.0(\pm 3.4)\%$ in the productive zone and the mesopelagic zone, respectively, indicating that DOC adsorption was important in the open ocean.

An additional reason that aDOC and POC concentrations showed a positive correlation pattern could be because DOC and POC have a similar decreasing pattern as a function of depth in the open ocean (Dai *et al.*, 2009).

3.4 Relative and total experimental uncertainties

The relative experimental uncertainty of POC concentrations, σ_{d_r} (Equation 9), was on average $\sim 14\%$ and $\sim 35\%$ in the productive zone and the mesopelagic zone, respectively. Higher σ_{d_r} were estimated for the mesopelagic zone where POC concentrations were lower and biases might have had a greater effect on the estimates. The resulting total experimental uncertainties of the estimates of POC concentration (σ_c) are shown in Figure 6. The median of σ_c for the productive and mesopelagic zones were $2.5(\pm 1.2)$ mg/m^3 and $2.6(\pm 0.6)$ mg/m^3 , respectively.

3.5 Uncertainty budget

The modeled uncertainty for POC is presented in Table 4 for each component, and compared to the estimated total experimental uncertainty of POC derived from the duplicate measurements. The uncertainty in volumetric measurements $\sigma_c(V)$ typically contributed about 1% to the total uncertainty of POC concentrations in the mesopelagic zone. However, since the volume of water filtered in the productive zone was smaller, the contribution of volumetric uncertainties was greater ($\sim 2\%$). Overall, the contribution of this source of uncertainty was insignificant, except when POC concentration was particularly high and, as a consequence, the volume of the water sample was ~ 2 L.

The uncertainty due to the calibration equation $\sigma_c(M_p)$, after excluding the unstable CHN runs, explained a median of $16(\pm 20)\%$ of the total experimental uncertainty of POC concentrations (Figure 7). Since unstable CHN runs were characterised by greater residual errors in the regression analysis, their contribution to the total experimental uncertainty was significantly higher with a median of $65(\pm 36)\%$.

Our acidification method and handling during the CHN analyses allowed us to minimise the effect of contamination of POC estimates. The median contribution of this source of uncertainty, $\sigma_c(\eta)$, in productive waters was $3(\pm 3)\%$, while in the mesopelagic zone attained $2(\pm 2)\%$.

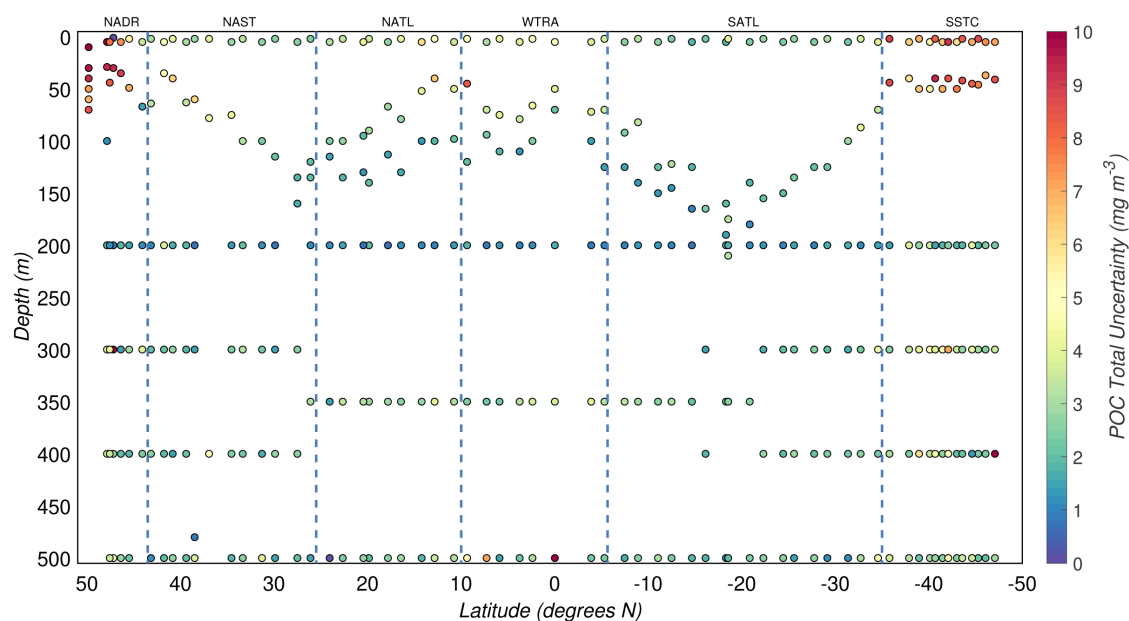


Figure 6. Depth-resolved distribution of the total uncertainty associated with the estimates of particulate organic carbon (POC) concentration derived during the AMT-24 cruise. Borders of the sampled biogeographical provinces are marked by blue vertical lines.

Table 4. Uncertainty budget presenting the contributions of each source of uncertainty that we could quantify, $\sigma_c(x_i)$, relative to the total experimental uncertainty of particulate organic carbon, σ_c . Median values are given for the productive and mesopelagic zones.

No	Source	$\sigma_c(x_i)/\sigma_c$	
		Productive zone	Mesopelagic zone
1	Volume	0.02	0.01
2	Calibration	0.17	0.12
3	Laboratory sample handling	0.03	0.02
4	Unquantified	0.78	0.85

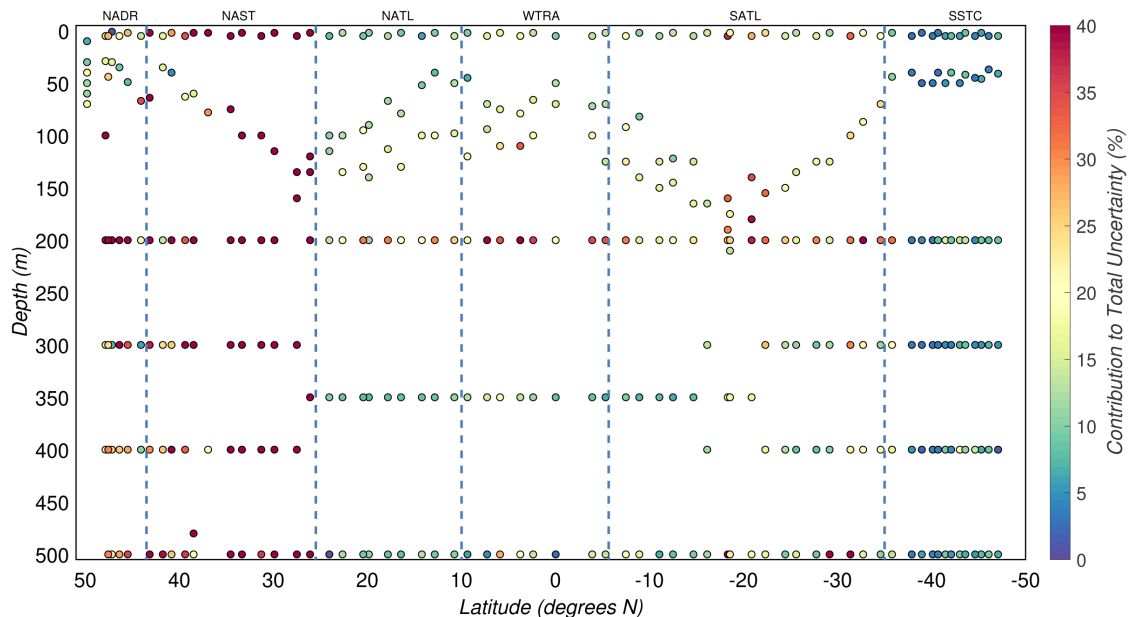


Figure 7. Portion of the total uncertainty of particulate organic carbon concentration that can be explained by the uncertainty of regression analysis. Borders of the sampled biogeographical provinces are marked by blue vertical lines.

Overall, the three sources of uncertainty described above explained only $21(\pm 22)\%$ of the total experimental uncertainty in POC. Thus, other sources of uncertainty must be responsible for the relatively large and unexplained part of the estimated experimental uncertainty (Figure 8).

3.6 Missing sources of uncertainty

In this section we discuss potential sources of uncertainty that could explain the missing part of the uncertainty budget.

3.6.1 Rare particles. Patchiness is ubiquitous in the ocean, ranging from microscale thin layers in the water column, to sub-mesoscale and mesoscale fronts, and could have contributed to our total experimental uncertainties in POC. For instance, [Bochdansky et al. \(2016\)](#) analysed the dynamics and abundances of particles at depth using a custom-made digital inline holographic microscope. They found that the concentration of patchy marine snow (large particles $>500 \mu\text{m}$) was 100 times higher than expected in comparison with the concentration of smaller particles. Additionally, [Ohman et al. \(2012\)](#) analysed the concentration and vertical distribution of suspended particulate matter and mesozooplankton at a deep-water

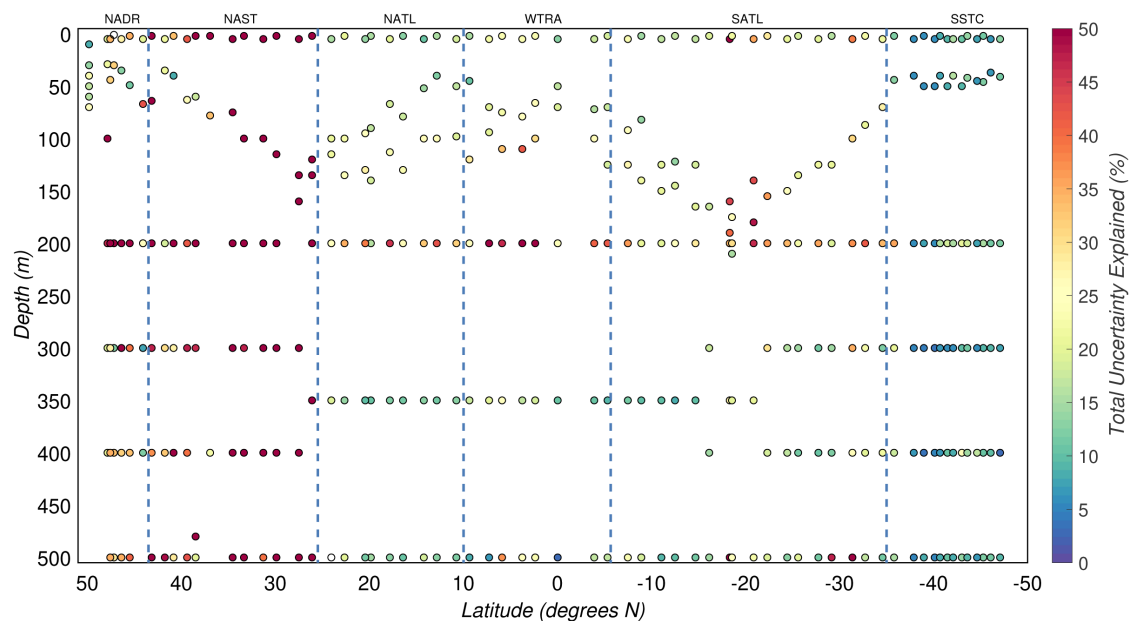


Figure 8. Portion of the total uncertainty of particulate organic carbon concentration that can be explained by the uncertainty budget. Borders of the sampled biogeographical provinces are marked by blue vertical lines.

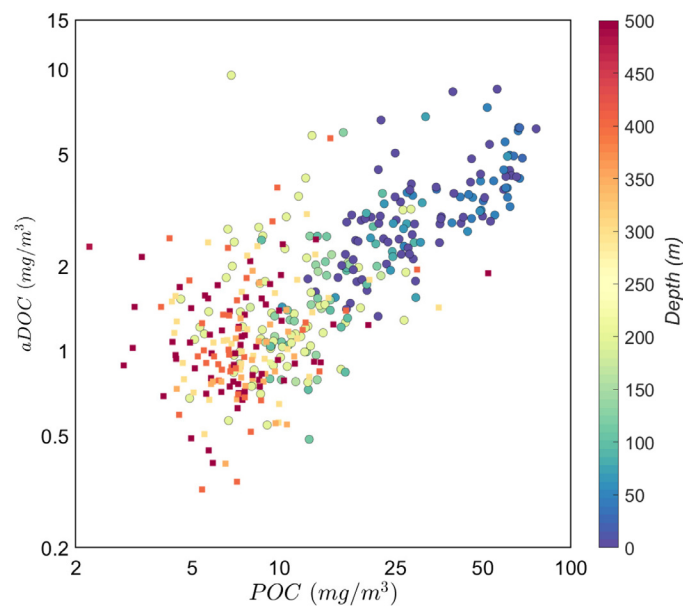


Figure 9. Correlation between aDOC and POC values. Colours refer to sample depths. Squares represent mesopelagic samples (MZ, $r = 0.38$). Circles represent samples in the productive zone (PZ, $r = 0.87$).

front in the California Current System using a high resolution digital camera system. They observed that the front had a different composition of particulate matter, and it was a zone of higher marine snow particles where the volume of all size fractions of suspended particulate matter, especially organic aggregates, increased several times in comparison with the surrounding seawater. Therefore, despite mixing samples in carboys before dispensing them to the filtration bottles, some of our duplicate filters might have captured

different types of particles, i.e. rare large aggregates or (invisible) zooplankton might have appeared only on one of the two duplicate filters.

Furthermore, [Wangersky \(1974\)](#); [Wangersky \(1976\)](#) found that a water mass in the open ocean had a homogeneous background of POC concentration, upon which occasional small patches with up to five times POC concentration were superimposed. He reported uncertainty of a single POC estimate derived from replicate 5-litre samples to be equal to $2.5(\pm 1) \text{ mg/m}^3$.

Our median total uncertainty for the mesopelagic region of $3(\pm 1) \text{ mg/m}^3$ is comparable to these findings, suggesting that samples might have been drawn from water masses of similarly spatially variable POC concentrations.

Further experiments are required to better understand the natural heterogeneity and small-scale patchiness of seawater using Niskin bottles. Since the collection of intact and flocculent marine snow particles using bottle samplers is highly challenging and time-consuming ([Bochdanský et al., 2016](#)), the oceanographic community would benefit from analysing the natural heterogeneity of suspended particles and the effect of patchiness on estimates of POC concentration and their uncertainties using optical observations from instruments such as the Underwater Vision Profiler or holographic cameras which have the advantage of displaying details of the *in-situ* vertical distribution of particles across a range of sizes ([Bochdanský et al., 2016](#); [Ohman et al., 2012](#)).

3.6.2 Contamination during filtration. The filtration system employed during this study was an open-funnel filtration set. This set up might have increased the risk of contamination by exposing the samples to particles rich in carbon (e.g., dust, ashes, ship's engine exhaust), during sampling from the Niskin bottles and during filtration in the laboratory ([Gardner et al., 2003](#)); (IOCCG POC Sampling and Measurement Protocols).

We consider that mainly uPOC filters would have been contaminated in such a way because they were exposed to the laboratory atmosphere for longer than the underlying aDOC filters. Assuming that the uPOC filters were contaminated during filtration, their duplicate filters might have received a similar amount of contamination. Thus, atmospheric contamination of uPOC filters would result in greater differences between uPOC duplicates compared to the differences between less contaminated aDOC duplicates. Indeed, the median of the absolute differences between duplicate uPOC concentrations was $2(\pm 2) \text{ mg/m}^3$, which is higher than the median of the absolute difference between duplicate aDOC concentrations of $0.2(\pm 0.4) \text{ mg/m}^3$. This result, however, is also consistent with our previous finding that the differences between duplicate concentrations depend on POC concentrations (see [Figure 2](#)). As a consequence, we do not have enough grounds to state that differences between duplicate uPOC concentrations are higher than the differences between duplicate aDOC concentrations because of contamination of uPOC filters.

The longer a filter is exposed to the laboratory atmosphere, the more contamination it should receive. The duration of filter exposure to the laboratory atmosphere depends on the volume of seawater filtered through the filter. To further investigate contamination of uPOC filters, we analysed how differences between duplicate uPOC concentrations depended on differences between volumes of seawater filtered through two duplicate filters and found no significant correlation.

Thus, there was no evidence to suggest that the time of exposure of filters to the atmosphere was the cause of disagreement between duplicate estimates. However, analysing duplicates to determine this source of contamination might not be sufficient as duplicate estimates could have been affected by other biases or contaminants that mask a single source of contamination. Consequently, with the available data, we cannot quantify this source of contamination and its uncertainty.

For future experiments, especially when using an open-funnel setup, filtering Milli-Q water through additional blank GF/F filters may be used to quantify contamination from the laboratory atmosphere. Even better, to minimise contamination from airborne particles, it would be advisable to filter samples under a laminar flow hood or by employing a closed filtration system ([Cetinić et al., 2012](#)).

3.6.3 Storage of samples. Freezing and storing of sample filters might have also introduced some contamination. Published values suggest that the average mass of unused GF/F filters from a cruise may range from $3(\pm 10) \mu\text{g}$ to $10(\pm 5) \mu\text{g}$ ([Cetinić et al., 2012](#); [Menzel, 1966](#); [Stramski et al., 2008](#); [Wangersky, 1974](#)). The

difference between our non-acidified ($3 \pm 1 \mu\text{g}$) filter blanks and these published values might indicate that the contamination during filter storage could amount to between 0 and $7 \mu\text{g}$. However, assuming homogeneous contamination of uPOC and aDOC filters, we would expect that this contamination would be accounted for when aDOC is subtracted from uPOC (Equation 2). To quantify uncertainties due to filter storage, we recommend preserving multiple unused GF/F filter blanks along with the samples for post-cruise analysis.

3.6.4 Collection of samples. Uncertainties can also be introduced by different operators. In our case, samples were collected by two operators, whereby one operator systematically collected samples from pre-dawn casts, while the other from noon casts. Thus, we thought that analysing duplicates pairs collected during pre-dawn and noon casts separately might give us an insight into this source of uncertainty. Due to constraints in the water budget, pre-dawn duplicates were collected from quasirandom depths, while 25 out of 28 duplicate pairs collected during noon time represented deep waters ($\geq 400 \text{ m}$). For pre-dawn duplicates, the median POC concentration and the median absolute differences of duplicate POC concentrations were $9(\pm 9)$ and $1(\pm 2) \text{ mg/m}^3$, respectively, while for noon duplicates were $9(\pm 5)$ and $2(\pm 2) \text{ mg/m}^3$, respectively, suggesting that there was no statistical difference between these medians in the two groups of duplicates. If we take into consideration that the majority of noon duplicates were collected from deep waters ($\geq 400 \text{ m}$), pre-dawn duplicates seem to be slightly more precise than noon duplicates with medians of the relative differences of duplicates collected from deep waters of $19(\pm 10)\%$ and $19(\pm 31)\%$ for pre-dawn and noon duplicates, respectively. However, there is no evidence that samples collected by different operators are biased by the operators themselves rather than by varying composition of particles at different depths. Even though the higher uncertainties that we found in the mesopelagic zone might be partially explained by the varying precision of duplicates collected by the two operators from pre-dawn and noon casts, we cannot quantify the bias and the uncertainty introduced by each operator.

3.6.5 Uncertainty model. The uncertainty model we employed in this study (Equation 11) was based on the empirical relationship we observed between duplicate differences and POC concentrations (Figure 2). Admittedly, this model is likely an approximation of the experimental uncertainty of our POC measurements. To improve the model (e.g., by adding a constant offset to it), we would need to better understand the role that each source of uncertainty plays in the total uncertainty of POC concentrations and to reconcile experimental and theoretical estimates of the POC uncertainty. These additional steps would ultimately allow us to then understand the extent to which each source of uncertainty is either a multiplicative or an additive term to the total uncertainty as POC, as the POC concentration in the ocean varies. Further dedicated experiments and analyses would be needed to achieve this deeper understanding.

3.7 aDOC blanks

Even though the double-filter technique employed in this study significantly increases filtration times, this procedure allowed us to collect a sample blank (i.e., aDOC filter) for each uPOC filter. Therefore, it is interesting to investigate how the uncertainty in the final POC concentrations would vary if fewer or no aDOC blanks were collected.

Some researchers avoid collecting aDOC blanks, under the assumption that by maximising the filtered volumes of seawater, uncertainties related to aDOC could be minimised (Stramski *et al.*, 2008). Since we collected multiple types of blanks (i.e., aDOC, non-acidified, and acidified), we can test this assumption under different scenarios.

First, uPOC concentrations were higher than POC concentrations by about $13(\pm 7)\%$ in the productive zone and $19(\pm 11)\%$ in the mesopelagic zone. Second, by subtracting the median carbon mass of non-acidified filters (i.e., clean GF/F filter blank) from the uPOC carbon mass, we obtained uPOC concentrations (corrected from clean GF/F filter blank) that are greater than our POC concentrations by $9(\pm 6)\%$ in the productive zone and $12(\pm 9)\%$ in the mesopelagic zone. Finally, by subtracting the median carbon mass of the acidified filters from the uPOC carbon mass, the uPOC concentrations (corrected from acidified GF/F filter blank) are larger than our original POC concentrations by $8(\pm 6)\%$ in the productive zone and $10(\pm 8)\%$ in the mesopelagic zone. Thus, by not correcting for aDOC blanks, we would have introduced positive biases in POC concentrations of the order of 10–20%, even if we filtered up to 8 liters of water. It is important to realise that these results depend on the relative amount of adsorbed DOC and POC present on the filters and therefore on the volumes of water filtered and the POC concentration. One must be careful when extrapolating our conclusions to POC values determined from different sample volumes and different ocean regions.

To reduce filtration time, one could collect fewer aDOC blanks (e.g., only one deep sample per station). To quantify the potential uncertainty introduced by this method, we corrected our uPOC estimates using a single value of aDOC blank, which was determined from the median of the aDOC blanks from deep (≥ 200 m) stations. The resulting POC concentrations were $3(\pm 4)\%$ and $0(\pm 6)\%$ higher than the original concentrations in the productive and the mesopelagic zones, respectively. Thus, by using fewer aDOC blanks, one could significantly reduce the bias generated when not using an aDOC blank. Overall, the above exercises can guide quantitatively how many (if at all) aDOC blanks to collect, based on the level of uncertainty that one is willing to accept.

Finally, an alternative method to decrease filtration times could be to collect aDOC samples by filtering smaller amounts of water, by collecting aDOC samples separately from the uPOC ones. For example, based on an analysis of various coastal and open-ocean samples [Novak *et al.* \(2018\)](#) suggested that GF/F filters are saturated with DOC after about 0.6 liters of sample water have been filtered. Even by adopting a conservative approach and doubling this suggested DOC saturation volume one could significantly decrease filtration times at sea, while ensuring that POC values are corrected for the adsorbed DOC.

3.8 The need for a POC reference material

The accuracy of oceanographic chemical analyses is typically assessed by measuring consensus or certified reference materials (CRM). Unfortunately, at present no such CRM has been selected by the oceanographic community for POC analyses ([IOCCG POC Sampling and Measurement Protocols](#)). CRMs exist that potentially might be used to represent organic matter in the ocean, e.g., NIST Buffalo River Sediment RM 8704 (National Institute of Standards and Technology), but their precise bio-organic elemental composition has not been determined, which prevents one from assessing how representative they are of open-ocean pelagic particulate matter ([National Research Council, 2002](#)). In addition, these CRMs are mainly comprised of marine sediments, rich in aluminosilicates and quartz, but with no pelagic opal and carbonate matrices, thus misrepresenting the complex matrix associated with open-ocean pelagic POC samples and potentially introducing artefacts in the accuracy assessment ([National Research Council, 2002](#)). The Committee on Reference Materials for Ocean Science of the US National Research Council recommended that a consensus or certified reference material for POC representative of open-ocean particulate matter could be obtained by mixing cultures from a diatom, a dinoflagellate, and a coccolithophore ([National Research Council, 2002](#)). Unfortunately, as of today, no CRM for POC analyses has been developed and, as a consequence, POC analyses are difficult to compare over time, among groups, and when different analytical protocols are used. Future work to improve POC determinations should focus on agreeing upon and producing a certified or consensus reference material. Finally, intercomparison exercises are also needed to minimise uncertainties arising from all sample collection and processing steps before the CHN analysis. Dedicated funding and an international effort are needed to achieve these two crucial objectives.

4 Conclusions

In this study, we modified the standard protocol used to determine POC concentration ([Knap *et al.*, 1996](#); [Martin *et al.*, 1993](#)) by: sampling different volumes of water in accordance to the expected in-situ POC concentrations; adopting different types of blanks, i.e., aDOC filter blanks and acidified and non-acidified GF/F filter blanks to quantify the dissolved organic carbon and any contamination (biases); and collecting and analysing duplicate samples. This methodology enabled us to quantify experimental uncertainties of our POC concentrations. Moreover, the various filters used as blanks allowed us to discuss several sources of uncertainty that contributed to the discrepancies between the duplicate estimates.

We found that the total experimental uncertainty of our POC estimates varied with depth and with POC concentration and was $\sim 14\%$ and $\sim 35\%$ in the productive and in the mesopelagic zones, respectively. However, the quantification of all the different sources of uncertainty associated with POC concentrations was impossible with our measurements, and we could explain only $\sim 21\%$ of the total experimental POC uncertainty we observed. Further work is required to identify the unexplained portion of the uncertainty.

Nevertheless, we moved one step forward in understanding uncertainties of bottle-derived POC estimates. The analysis of duplicates and of the various blanks presented in this paper improved our understanding of the limitations of the methods and of some of the stages of sample collection and processing that contributed to the variability of our results. Further experiments would be required to fully understand the uncertainty budget of POC estimates. This understanding would allow us to concentrate our effort on those parts of the

methodology that are more prone to introduce uncertainties and to develop a better-informed protocol to foster comparability of POC estimates across different studies.

Data availability

Underlying data

Published Data Library (PDL), British Oceanographic Data Centre (BODC): AMT24 (JR20140922/JR303) Particulate organic carbon (POC) measurements from CTD bottles. <http://doi.org/10/fzw5> (Dall'Olmo *et al.*, 2021).

This project contains the following underlying data:

- README.txt. (contains important information that is commonly required to understand the following files or spreadsheets deposited in CSV format.)
- Standards.csv. (Contains information regarding standards used to calibrate the CHN analyser, see README.TXT)
- Capsules.csv. (Contains information regarding empty tin capsules used to estimate uncertainties related to the sample handling in the lab, see README.TXT)
- NonAcidifiedFilters.csv. (Contains information regarding non-acidified filter blanks used to estimate uncertainties related to the sample handling in the lab, see README.TXT)
- AcidifiedFilters.csv. (Contains information regarding acidified filter blanks used to estimate uncertainties related to the sample handling in the lab, see README.TXT)
- aDOCFilters.csv. (Contains information regarding aDOC filters used to determine POC concentrations, see README.TXT)
- uPOCFilters.csv. (Contains information regarding uPOC filters used to determine POC concentrations, see README.TXT)
- DuplicateaDOC.csv. (Contains information regarding duplicate aDOC filters used to estimate total experimental uncertainties, see README.TXT)
- DuplicateuPOC.csv. (Contains information regarding duplicate uPOC filters used to estimate total experimental uncertainties, see README.TXT)
- POC.csv. (Contains overall information regarding POC concentrations, including nominal depth, amount of seawater filtered for each filter, geographic coordinates, date and time of collection, POC mass and POC concentration of each sample, see README.TXT)

This dataset is available under the terms of the [UK Open Government Licence version 1.0](#) for public sector information. This licence governs access to and use of Open Data supplied by the Natural Environment Research Council (NERC).

Software availability

- Source code available from: <https://github.com/pstrubinger/Uncertainties-of-particulate-organic-carbon-concentrations-in-the-mesopelagic-zone-of-the-Atlantic-o/tree/v1.0.0>
- Archived source code at time of publication: <https://doi.org/10.5281/zenodo.4630974> (pstrubinger, 2021)
- License: [GNU General Public License, version 3 \(GPL-3.0\)](#)

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References

- Abdel-Moati AR: **Adsorption of dissolved organic carbon (DOC) on glass fibre filters during particulate organic carbon (POC) determination.** *Water Res.* 1990; **24**(6): 763–764.
[Publisher Full Text](#)
- Altman DG: **Statistics with confidence: confidence intervals and statistical guidelines.** London: BMJ Books, 2nd edition, 2000.
[Reference Source](#)
- Analytical Methods Committee AMCTB No. 92: **The edge of reason: reporting and inference near the detection limit.** *Anal Methods.* 2020; **12**: 401–403.
[Publisher Full Text](#)
- Aumont O, van Hulten M, Roy-Barman M, et al.: **Variable reactivity of particulate organic matter in a global ocean biogeochemical model.** *Biogeosciences.* 2017; **14**(9): 2321–2341.
[Publisher Full Text](#)
- Balch WM, Bowler BC, Drapeau DT, et al.: **Biomining and the vertical flux of particulate organic carbon from the surface ocean.** *Geophys Res Lett.* 2010; **37**(22): L22605.
[Publisher Full Text](#)
- Banoub MW, Williams PJB: **Measurements of microbial activity and organic material in the western Mediterranean sea.** *Deep Sea Research and Oceanographic Abstracts.* 1972; **19**(6): 433–443.
[Publisher Full Text](#)
- Bishop JKB, Edmond JM: **A new large volume filtration system for the sampling of oceanic particulate matter.** *J Mar Res.* 1976; **34**(2): 181–198.
[Reference Source](#)
- Bishop JKB, Wood TJ: **Particulate matter chemistry and dynamics in the twilight zone at VERTIGO ALOHA and K2 sites.** *Deep-Sea Research Part I: Oceanographic Research Papers.* 2008; **55**(12): 1684–1706.
[Publisher Full Text](#)
- Bishop JKB, Wood TJ: **Year-round observations of carbon biomass and flux variability in the Southern Ocean.** *Global Biogeochem Cy.* 2009; **23**(2).
[Publisher Full Text](#)
- Bochdanský AB, Clouse MA, Herndl GJ: **Dragon kings of the deep sea: Marine particles deviate markedly from the common number-size spectrum.** *Sci Rep.* 2016; **6**: 22633.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Brewin RJW, Sathyendranath S, Platt T, et al.: **Sensing the ocean biological carbon pump from space: A review of capabilities, concepts, research gaps and future developments.** *Earth-Sci Rev.* 2021; **217**: 103604.
[Publisher Full Text](#)
- Carlson CA, Hansell DA, Peltzer ET, et al.: **Stocks and dynamics of dissolved and particulate organic matter in the Southern Ross Sea, Antarctica.** *Deep Sea Res 2 Top Stud Oceanogr.* 2000; **47**(15–16): 3201–3225.
[Publisher Full Text](#)
- Cetinić I, Perry MJ, Briggs NT, et al.: **Particulate organic carbon and inherent optical properties during 2008 North Atlantic bloom experiment.** *J Geophys Res Oceans.* 2012; **117**(C6): C06028.
[Publisher Full Text](#)
- Chaves J, Cetinić I, Dall'Olmo G, et al.: **Particulate organic carbon sampling and measurement protocols: Consensus towards future ocean color missions.** {IOCCG} {Ocean} {Optics and} {Biogeochemistry} {Protocols for} {Satellite} {Ocean} {Colour} {Sensor} {Validation}, {Volume 6.0. 2021.
[Publisher Full Text](#)
- Currie LA: **Nomenclature in evaluation of analytical methods including detection and quantification capabilities (IUPAC Recommendations 1995).** *Pure Appl Chem.* 1995; **67**(10): 1699–1723.
[Publisher Full Text](#)
- Dai M, Meng F, Tang T, et al.: **Excess total organic carbon in the intermediate water of the South China Sea and its export to the North Pacific.** *Geochim Geophys Geosyst.* 2009; **10**(12).
[Publisher Full Text](#)
- Dall'Olmo G, Ross JG, Boada RRJ, et al.: **Particulate organic carbon (poc) measurements from ctd bottle samples collected during the atlantic meridional transect cruise amt24 (jr20140922/jr303) between september and november 2014.** British Oceanographic Data Centre, National Oceanography Centre, NERC, UK, 2021.
<http://www.doi.org/10.5285/bd2d14a3-18c9-6a53-e053-6c86abc01c89>
- Feeley R: **Chemical characterization of the particulate matter in the near bottom nepheloid layer of the Gulf of Mexico.** *Ph.D. Thesis.* 1974.
[Reference Source](#)
- Gardner WD, Mishonov AV, Richardson MJ: **Global POC concentrations from in-situ and satellite data.** *Deep-Sea Research Part II: Topical Studies in Oceanography.* 2006; **53**(5–7): 718–740.
[Publisher Full Text](#)
- Gardner WD, Richardson MJ, Carlson CA, et al.: **Determining true particulate organic carbon: bottles, pumps and methodologies.** *Deep-Sea Research Part II: Topical Studies in Oceanography.* 2003; **50**(3–4): 655–674.
[Publisher Full Text](#)
- Gardner WD, Richardson MJ, Smith WO Jr: **Seasonal patterns of water column particulate organic carbon and fluxes in the Ross Sea, Antarctica.** *Deep-Sea Research Part II: Topical Studies in Oceanography.* 2000; **47**(15–16): 3423–3449.
[Publisher Full Text](#)
- Gardner WD, Walsh ID, Richardson MJ: **Biophysical forcing of particle production and distribution during a spring bloom in the North Atlantic.** *Deep Sea Research Part II: Topical Studies in Oceanography.* 1993; **40**(1–2): 171–195.
[Publisher Full Text](#)
- Gordon DD Jr, Sutcliffe WH Jr: **Filtration of seawater using silver filters for particulate nitrogen and carbon analysis.** *Limnol Oceanogr.* 1974; **19**(6): 989–993.
[Publisher Full Text](#)
- Graff JR, Westberry TK, Milligan AJ, et al.: **Analytical phytoplankton carbon measurements spanning diverse ecosystems.** *Deep Sea Res 1 Oceanogr Res Pap.* 2015; **102**: 16–25.
[Publisher Full Text](#)
- Hansell DA, Carlson CA: **Localized refractory dissolved organic carbon sinks in the deep ocean.** *Global Biogeochem Cy.* 2013; **27**(3): 705–710.
[Publisher Full Text](#)
- Hedges JJ: **Global biogeochemical cycles: progress and problems.** *Marine Chemistry.* 1992; **39**(1–3): 67–93.
[Publisher Full Text](#)
- Henson SA, Sanders R, Madsen E: **Global patterns in efficiency of particulate organic carbon export and transfer to the deep ocean.** *Global Biogeochem Cy.* 2012; **26**: 1028.
[Publisher Full Text](#)
- Hopkins J, Henson SA, Poulton AJ, et al.: **Regional characteristics of the temporal variability in the global particulate inorganic carbon inventory.** *Global Biogeochem Cy.* 2019; **33**(11): 1328–1338.
[Publisher Full Text](#)
- Hyslop NP, White WH: **Estimating precision using duplicate measurements.** *J Air Waste Manage Assoc.* 2009; **59**(9): 1032–1039.
[Publisher Full Text](#)
- JCGM: **Guide to the expression of uncertainty in measurement.** Technical report, BIMP, IEC IFCC, ISO UIPAC, IUPAC, OIML, Switzerland, 2008.
[Reference Source](#)
- Kharbush JJ, Close HG, Van Mooy BAS, et al.: **Particulate organic carbon deconstructed: Molecular and chemical composition of particulate organic carbon in the ocean.** *Front Marine Sci.* 2020; **7**: 518.
[Publisher Full Text](#)
- Kiko R, Biastoch A, Brandt P, et al.: **Biological and physical influences on marine snowfall at the equator.** *Nat Geosci.* 2017; **10**: 853–858.
[Publisher Full Text](#)
- King P, Kennedy H, Newton PP, et al.: **Analysis of total and organic carbon and total nitrogen in settling oceanic particles and a marine sediment: An interlaboratory comparison.** *Mar Chem.* 1998; **60**(3–4): 203–216.
[Publisher Full Text](#)
- Kinney PJ, Loder TC, Groves J: **Particulate and dissolved organic matter in the Amerasian basin of the Arctic oceans.** *Limnol Oceanogr.* 1971; **16**(1): 132–137.
[Publisher Full Text](#)
- Knap A, Michaels A, Close A, et al.: **Protocols for the Joint Global Ocean Flux Study (JGOFS) core measurements.** (reprint of the IOC Manuals and Guides no. 29, UNESCO 1994), JGOFS Report Nr. 19, 1996.
[Reference Source](#)
- Kwon EY, Primeau F, Sarmiento JL: **The impact of remineralization depth on the air-sea carbon balance.** *Nat Geosci.* 2009; **2**(9): 630–635.
[Publisher Full Text](#)

- Lam PJ, Doney SC, Bishop JKB: **The dynamic ocean biological pump: Insights from a global compilation of particulate organic carbon, CaCO_3 , and opal concentration profiles from the mesopelagic.** *Global Biogeochem Cy.* 2011; **25**(3).
[Publisher Full Text](#)
- Liu Z, Cochran JK, Lee C, *et al.*: **Further investigations on why POC concentrations differ in samples collected by niskin bottle and *in situ* pump.** *Deep-Sea Res Pt II.* 2009; **56**(18): 1558–1567.
[Publisher Full Text](#)
- Liu Z, Stewart G, Cochran JK, *et al.*: **Why do POC concentrations measured using niskin bottle collections sometimes differ from those using *in-situ* pumps?** *Deep-Sea Res Pt I.* 2005; **52**(7): 1324–1344.
[Publisher Full Text](#)
- Loder TC, Hood DW: **Distribution of organic carbon in a glacial estuary in Alaska.** *Limnol Oceanogr.* 1972; **17**(3): 349–355.
[Publisher Full Text](#)
- Longhurst AR: **Chapter 9 - The Atlantic Ocean.** In Alan R. Longhurst, editor, *Ecological Geography of the Sea (Second Edition)*. Academic Press, Burlington, second edition, 2007; 131–273.
[Publisher Full Text](#)
- Marra J, Langdon C, Knudson CA: **Primary production, water column changes, and the demise of a *Phaeocystis* bloom at the Marine Light-Mixed Layers site (59n, 21w) in the northeast Atlantic ocean.** *J Geophys Res.* 1995; **100**(C4): 6633–6643.
[Publisher Full Text](#)
- Martin JH, Kadar S, Leinen M, *et al.*: **U.S. JGOFS Equatorial Pacific Process Study, sampling and analytical protocols, determination of particulate organic carbon (POC) and nitrogen (PON) in seawater.** 1993.
[Reference Source](#)
- Menzel DW: **Bubbling of sea water and the production of organic particles: A re-evaluation.** *Deep Sea Res.* 1966; **13**(5): 963–966.
[Publisher Full Text](#)
- Menzel DW: **Particulate organic carbon in the deep sea.** *Deep-Sea Research and Oceanographic Abstracts.* 1967; **14**(2): 229–238.
[Publisher Full Text](#)
- Menzel DW, Goering JJ: **The distribution of organic detritus in the ocean.** *Limnol Oceanogr.* 1966; **11**(3): 333–337.
[Publisher Full Text](#)
- Menzel DW, Vaccaro RF: **The measurement of dissolved organic and particulate carbon in seawater.** *Limnol Oceanogr.* 1964; **9**(1): 138–142.
[Publisher Full Text](#)
- Mishonov AV, Gardner WD, Richardson MJ: **Remote sensing and surface POC concentration in the South Atlantic.** *Deep Sea Research Part II: Topical Studies in Oceanography.* 2003; **50**(22): 2997–3015.
[Publisher Full Text](#)
- Moran SB, Charette MA, Pike SM, *et al.*: **Differences in seawater particulate organic carbon concentration in samples collected using small- and large-volume methods: The importance of DOC adsorption to the filter blank.** *Mar Chem.* 1999; **67**(1–2): 33–42.
[Publisher Full Text](#)
- National Research Council: **Chemical reference materials: Setting the standards for ocean science.** 2002.
[Reference Source](#)
- Novak MG, Cetinić I, Chaves JE, *et al.*: **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.* 2018; **16**(6): 356–366.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ohman MD, Powell JR, Picheral M, *et al.*: **Mesozooplankton and particulate matter responses to a deep-water frontal system in the southern California Current System.** *J Plankton Res.* 2012; **34**(9): 815–827.
[Publisher Full Text](#)
- Parekh P, Dutkiewicz S, Follows MJ, *et al.*: **Atmospheric carbon dioxide in a less dusty world.** *Geophys Res Lett.* 2006; **33**(3): L03610.
[Publisher Full Text](#)
- Poulton AJ, Sanders R, Holligan PM, *et al.*: **Phytoplankton mineralization in the tropical and subtropical Atlantic ocean.** *Global Biogeochem Cycles.* 2006; **20**(4): GB4002.
[Publisher Full Text](#)
- pstrubinger: **pstrubinger/Uncertainties-of-particulate-organic-carbon-concentrations-in-the-mesopelagic-zone-of-the-Atlantic-o: First Release.** 2021.
<http://www.doi.org/10.5281/zenodo.4630974>
- Rasse R, Dall'Olmo G, Graff J, *et al.*: **Evaluating optical proxies of particulate organic carbon across the surface Atlantic ocean.** *Front Mar Sci.* 2017; **4**: 367.
[Publisher Full Text](#)
- Rawlings JO, Patula SG, Dickey DA: **Applied Regression Analysis: A Research Tool.** New York: Springer-Verlag, 2nd edition, 1998.
[Publisher Full Text](#)
- Sarmiento JL, Gruber N: **Ocean Biogeochemical Dynamics.** Woodstock: Princeton University Press, 2006.
[Reference Source](#)
- Sharp JH: **Improved analysis for “particulate” organic carbon and nitrogen from seawater.** *Limnology Oceanography.* 1974; **19**(6): 984–989.
[Publisher Full Text](#)
- Smith WO Jr, Nelson DM, DiTullio GR, *et al.*: **Temporal and spatial patterns in the Ross Sea: Phytoplankton biomass, elemental composition, productivity and growth rates.** *Journal of Geophysical Research C: Oceans.* 1996; **101**(C8): 18455–18465.
[Publisher Full Text](#)
- Stramska M, Cieszyńska A: **Ocean colour estimates of particulate organic carbon reservoirs in the global ocean – revisited.** *Int J Remote Sens.* 2015; **36**(14): 3675–3700.
[Publisher Full Text](#)
- Stramska M, Stramski D: **Variability of particulate organic carbon concentration in the north polar Atlantic based on ocean color observations with Sea-viewing Wide Field-of-view sensor (SeaWiFS).** *J Geophys Res Oceans.* 2005; **110**(C10): C10018.
[Publisher Full Text](#)
- Stramski D, Reynolds RA, Babin M, *et al.*: **Relationships between the surface concentration of particulate organic carbon and optical properties in the eastern south Pacific and eastern Atlantic oceans.** *Biogeosciences.* 2008; **5**(1): 171–201.
[Publisher Full Text](#)
- ThermoQuest: **FlashEA 1112 Elemental Analyzer Operating Manual.** ThermoQuest Italia S.p.A., Strada Rivoltana 20090 Rodano, Milan, Italy, 1999.
- Turnewitsch R, Springer BM, Kiriakoulakis K, *et al.*: **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.* 2007; **105**(3): 208–228.
[Publisher Full Text](#)
- Wangersky PJ: **Particulate organic carbon: Sampling variability.** *Limnol Oceanogr.* 1974; **19**(6): 980–984.
[Publisher Full Text](#)
- Wangersky PJ: **Particulate organic carbon in the Atlantic and Pacific oceans.** *Deep Sea Research and Oceanographic Abstracts.* 1976; **23**(5): 457–465.
[Publisher Full Text](#)
- Wheeler PA, Watkins JM, Hansing RL: **Nutrients, organic carbon and organic nitrogen in the upper water column of the Arctic ocean: implications for the sources of dissolved organic carbon.** *Deep Sea Research Part II: Topical Studies in Oceanography.* 1997; **44**(8): 1571–1592.
[Publisher Full Text](#)
- Zhou K, Maiti K, Dai M, *et al.*: **Does adsorption of dissolved organic carbon and thorium onto membrane filters affect the carbon to thorium ratios, a primary parameter in estimating export carbon flux?** *Mar Chem.* 2016; **184**: 1–10.
[Publisher Full Text](#)

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The authors have added in extra explanation and detail which is great to see. The paper provides data that will benefit the scientific community and presents uncertainties in a range of scenarios to allow others to adopt the necessary methods for the level of uncertainty they are willing to accept. It is good that the authors highlight the importance of correcting for adsorbed DOC whilst also highlighting the potential for contamination of POC on the aDOC lower filter. Their work suggests that having sufficient mesopelagic samples, where POC concentrations are lower to help estimate this contamination. They also present a useful investigation on the use of aDOC blanks, with an appreciation for what is practical at sea, having extra information on the possible bias if fewer aDOC blanks are taken.

Considering that POC concentrations can be very patchy, as discussed by the authors, I think it would be good to highlight this in the conclusions, as in some areas of the ocean in particular, this patchiness, and resultant difference between replicates will be far far larger than the methodological uncertainties here. Perhaps also in section 3.6.1 the authors could provide their recommendations/suggestions for how to take into account this patchiness, in terms of number of replicates etc. Or at least a mention that this needs to be considered when deciding on the sampling area and number and location of replicates to characterise a region etc.

I think the paper makes a contribution to the field and that the authors have explained the limitations of their works and uncertainties that they cannot quantify.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Jul 2022

Giorgio Dall'Olmo

We thank Dr. Belcher for the time invested in re-reviewing our manuscript. Below we present a point-by-point response to all her comments/suggestions. (Reviewer comments in italics)

The authors have added in extra explanation and detail which is great to see. The paper provides data that will benefit the scientific community and presents uncertainties in a range of scenarios to allow others to adopt the necessary methods for the level of uncertainty they are willing to accept. It is good that the authors highlight the importance of correcting for adsorbed DOC whilst also highlighting the potential for contamination of POC on the aDOC lower filter. Their work suggests that having sufficient mesopelagic samples, where POC concentrations are lower to help estimate this contamination. They also present a useful investigation on the use of aDOC blanks, with an appreciation for what is practical at sea, having extra information on the possible bias if fewer aDOC blanks are taken.

Considering that POC concentrations can be very patchy, as discussed by the authors, I think it would be good to highlight this in the conclusions, as in some areas of the ocean in particular, this patchiness, and resultant difference between replicates will be far far larger than the methodological uncertainties here.

RESPONSE: We have discussed particle patchiness at length in Section 3.6.1. Yet, we do not have evidence that leads us to believe that patchiness was the main source of the missing uncertainty that we have documented. Therefore, we do not feel it is justified to specifically mention this in the conclusions.

ACTION: None taken.

Perhaps also in section 3.6.1 the authors could provide their recommendations/suggestions for how to take into account this patchiness, in terms of number of replicates etc. Or at least a mention that this needs to be considered when deciding on the sampling area and number and location of replicates to characterise a region etc.

RESPONSE: It is very difficult to provide details of what exactly should be done in the future, because these details depend on the particle patchiness in each specific water mass.

ACTION: We have therefore only mentioned that this should be considered: "In future studies, when possible, these and other optical instruments should be used to quantify the patchiness of marine particles and its potential impacts on the uncertainty of the estimated POC concentrations. Such quantification could also inform the best strategies to collect discrete water samples in the presence of particle patchiness."

Competing Interests: No competing interests were disclosed.

<https://doi.org/10.21956/openreseurope.15193.r27737>

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Frederic Planchon 

UMR 6539 LEMAR, CNRS, IRD, Ifremer, University of Brest, Plouzané, France

The manuscript presents an interesting and detailed dataset on the occurrence of POC and associated uncertainties in the upper water column of the Atlantic Ocean. This subject is relevant since POC concentrations especially in the mesopelagic zone remain poorly documented at a global scale. In addition, and despite decades of POC measurements, large discrepancies remain between the different methodologies used in terms of sampling techniques, sample preparation, storage, analysis and data validation. The present work, which is a revised version based on the comments of two reviewers, is a further contribution.

The main objectives of the work are to quantify the uncertainties related to POC determination based on two independent approaches, the first one with duplicate samples and the second one with a detailed uncertainty budget taking into account three distinct contributions (volume, analytical determination, sample processing). These two estimates of the overall precision of the method are compared and discussed. The most important results of this study are that the determination of POC in the upper water column remains imprecise, especially in the mesopelagic zone (up to 35% error at 68% confidence interval) and adsorption of DOC represents a major source of bias that requires to be considered when POC is obtained from small volume seawater samples. The latter result is not surprising and has already been documented in a number of studies and should be better underlined in the abstract. The former result (precision deduced from duplicate samples) is far less documented and represents a valuable contribution that deserves publication. Regarding the methodology for estimating the precision, the duplicate sample approach is not common and probably suffer from a statistical significance as already mentioned by one reviewer. However, and as mentioned by the authors, this represents a valuable compromise.

As a general comment, the paper reads well and is relatively well illustrated. The material and method section describes in much detail the samples considered, the analytical determination and the statistical analysis applied to the data. However, some points need clarification and better organization. Results should not be included in this section as much as possible. For instance, Figure 2 is presented in section 2.4.1 and is not necessary for the total experimental uncertainty calculation since no correlation is assumed between duplicates and the total uncertainty is calculated essentially using the scaled relative difference. It would be more appropriate to present the lack of correlation in the result section to confirm that your calculation is appropriate. Another occurrence of a result presentation can be found p10 after equation 11. Table 2, which summarizes the different contributions, should also be moved to later in manuscript and not introduced in section 2.5. Similarly, the section 3.2 dealing with the detection limit should be moved to the method section, at least for the details of the calculation.

Regarding the detection limits, you choose to apply a 95% confidence interval, which makes sense and is commonly adopted in analytical chemistry. Why all other uncertainties are at 68% (1 sigma)?

Still on the L_d and L_c values and as it can be deduced from Fig 4. It appears that all procedural blanks are below the L_d , only field blanks (aDOC) and samples (uPOC filters) are above. Does it make sense to report a C quantity that is below the L_d ? It could even be worse if you considered the quantification limit.

Regarding the total experimental uncertainty (section 2.4.1), I do not understand why it is necessary to apply a propagation law for estimating the precision (Eq. 7, 8, 9). Eq 10 related to the scaled relative difference of duplicates, which is actually your estimator of the relative precision, does not imply such a calculation. Absolute precision can be easily deduced by considering the average POC concentration of each duplicate. Still on this parameter ($\sigma_{\Delta r}$) and as mentioned in Hyslop and White (2009), it could be worth checking that your scaled relative differences are normally distributed. In that studies, three different estimators of the precision have been used including the root mean square (RSM), the scaled mean absolute difference (MAD) and the percentiles approach. Why did you choose the percentile precision?

Another issue concerns the section 2.5.2 (uncertainty in mass predicted by the calibration equation), which is essentially the instrumental standard deviation. I do not understand why you include σ_{Mp} in this section, which refers to the correction of total POC mass with the corresponding DOC mass. This parameter is not related to the calibration equation and represent a post-analysis correction made possible with your sampling methodology. I think it could be interesting to consider separately these two components (instrument precision and precision on the corrected POC quantity by the DOC quantity). At this point it is not clear what the values reported in table 2 and associated with the calibration correspond to, instrument precision or precision on the corrected mass.

Still on the uncertainty budget in the section 2.5.3 (uncertainty due to sample handling during CHN analysis), the proposed calculation based on the three acidified filter blanks does not only correspond to contamination due to the acidification step but includes also the contribution from the filter. In Eq 17, I do not think it is necessary to introduce two distinct variables ($\sigma_{\eta 1}$ and 2) since the two are equal and are estimated from the three acidified filter blanks. It could be interesting to consider separately these two distinct sources of uncertainty. Impact of acid fuming by considering the difference between non-acidified and acidified filter and the filter alone.

Another remark on the impact of filter concerns their role in the analytical determination and especially on the sensitivity of the instrument. This is not taken into account in your dataset since your CHN standards were prepared without filters. Filters can affect the combustion process and as a consequence the sensitivity of the instrument (the slope of the calibration curve). Accordingly, it is highly recommended to tune the elemental analyzer with standards containing filters. In general, an increase in O_2 gas is necessary to prevent partial combustion of standards and samples. Also, adding a filter to the standards allows for better reproduction of the sample matrix and provides a means for quantifying filter blanks through the regression analysis (b parameter). This procedure is required in C isotope studies for both concentration and isotopic corrections when samples are loaded on a filter.

The result and discussion section is clearly written with abundant references to previous studies. I have only few concerns on this section. The first one is related to the description of the results. You present the median of your results with an associated uncertainty. Could you clarify how

these uncertainties have been obtained (number of values and calculation)? My guess is that it corresponds to the standard deviation of your data. Looking at these uncertainties, they appear to be highly variable indicating a high dispersion of uncertainty data. I think this dispersion could be better considered and at least mentioned, especially when you compare the productive zone with the mesopelagic zone. For instance in section 3.7 when you compare uPOC with POC concentrations, I do not think that the difference between sampling zones is statistically different.

Another point concerns the observed positive correlation between aDOC and POC concentrations (section 3.3 and figure 9). In addition to cells break and release of DOC, another hypothesis may concern the potential impact of small ($<0.7 \mu\text{m}$) particles associated with picophytoplankton (0.2-2 μm in size) and small heterotrophs (free bacteria, archaea, etc.). These organisms can be abundant in surface waters representing an important part of the biomass especially out of the bloom period.

Detailed comments:

Abstract L6-7: check sentences and change accuracy term with precision.

Section 2.1: clarify the number of samples taken at each station, were they taken at the same cast? You mention 392 uPOC samples. if you collected 6 samples at 67 stations, this makes a total of 402 samples.

Similarly, you mention duplicate samples were taken at each cast. You should have 67 duplicate samples and not 57. Please clarify

Page 5, last paragraph: if filter blanks were placed in the dessicator they are obviously acidified. I would change to: process as filter sample and subjected to acid fuming in the dessicator.

Page 6, third paragraph: correct your description of the sample analysis in the CHN. The samples are combusted in the oven (combustion chamber subjected to a dedicated temperature program), and then ashes fall in the ash trap, which progressively fills up. This is not a reaction tube. Then combustion gases are carried to the oxidation and reduction "tubes" (chambers) and then to the chromatographic column for separation and final detection.

P6, paragraph 4-5: Theoretically, all standards measured require to be included in the calibration. The first set called "calibration standards" are generally used to check the linearity. For state of the art analytical sequence, linearity is checked also at the end of the sequence. Also, standards showing poor recovery (due to weighting error, bad analytical run) can be removed according to z-score.

P6, paragraph 6: you often use the term robust throughout the manuscript (here for the fitting model), what does it mean? Does it differ from a classical linear fitting model?

P6, bottom page: the two equations are not numbered and the definition of M star is not given. Furthermore, there is a missing term in these two equations corresponding to the C mass associated to the filter. The difference between acidified and non-acidified filter blanks corresponds only to the C added by the acidification process.

P8, last paragraph: indicate the p value of the linear fits to explore the significance of the regression analysis.

P9, figure 2: y axis of figure 2b is unitless.

P10, section 2.5: an equation showing how you compute all uncertainty contributions would help the reader.

P10, Table 2: to be clearer, add a column with the corresponding variables associated with each line. If this table is moved to the result section, please include the sd for each median value and adjust to significant digit.

P14, last paragraph: indicate the p value of the linear fits and homogenize the significant digit.

P17, table 4: same remarks as for table 2.

P18, Figure 9: indicate the p value of the linear fits.

References

1. Hyslop NP, White WH: Estimating precision using duplicate measurements. *J Air Waste Manag Assoc.* 2009; **59** (9): 1032-9 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for developing the new method (or application) clearly explained?

Yes

Is the description of the method technically sound?

Partly

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Analytical chemistry, isotopic studies, marine biogeochemistry, biological carbon pump

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Jul 2022

Giorgio Dall'Olmo

We thank very much Dr Planchon for the time he has taken to review our work and for his thoughtful and constructive criticisms. We have addressed all his comments and have incorporated (where needed) changes in the new version of the manuscript.

REVIEWER'S COMMENTS: The manuscript presents an interesting and detailed dataset on the occurrence of POC and associated uncertainties in the upper water column of the Atlantic Ocean. This subject is relevant since POC concentrations especially in the mesopelagic zone remain poorly documented at a global scale. In addition, and despite decades of POC measurements, large discrepancies remain between the different methodologies used in terms of sampling techniques, sample preparation, storage, analysis and data validation. The present work, which is a revised version based on the comments of two reviewers, is a further contribution.

The main objectives of the work are to quantify the uncertainties related to POC determination based on two independent approaches, the first one with duplicate samples and the second one with a detailed uncertainty budget taking into account three distinct contributions (volume, analytical determination, sample processing). These two estimates of the overall precision of the method are compared and discussed. The most important results of this study are that the determination of POC in the upper water column remains imprecise, especially in the mesopelagic zone (up to 35% error at 68% confidence interval) and adsorption of DOC represents a major source of bias that requires to be considered when POC is obtained from small volume seawater samples. The latter result is not surprising and has already been documented in a number of studies and should be better underlined in the abstract. The former result (precision deduced from duplicate samples) is far less documented and represents a valuable contribution that deserves publication.

RESPONSE: We agree with the reviewer that the uncertainty of POC measurements is not well documented. However, we did not mean to report our results on the adsorption of DOC as a novel result. As the reviewer mentioned, this is a well-known result. What we think is novel about the aDOC section (3.7) is that, because we have a large number of aDOC samples, we could test the impact of i) having these measurements available to correct the uPOC concentrations; ii) having only a small subset of these aDOC samples and using the statistics of these samples to correct the uPOC samples from the entire cruise; or iii) not having them at all. We demonstrated that a good compromise could be to collect a limited number of deep aDOC samples. We believe that this analysis both confirms the importance of collecting aDOC measurements, and also provides a simple solution to minimise filtration times.

ACTION: We have modified the original text and added references to section 3.7 to better explain that the biases introduced by the adsorbed DOC are not new. Because the result is not new and we had not mentioned anything about the aDOC biases in the abstract, we did not think it was necessary to add any comment about this to the abstract.

Regarding the methodology for estimating the precision, the duplicate sample approach is not common and probably suffer from a statistical significance as already mentioned by one reviewer. However, and as mentioned by the authors, this represents a valuable compromise.

RESPONSE: We agree with the reviewer that the method is not common, but given the large

volumes of water that needs to be filtered, and as the reviewer recognises, it is a valuable compromise. As we have already discussed in one of our responses to the first round of reviews, statistical significance was achieved by combining the results of all duplicates and by considering their relative differences. In other words, we did not use single duplicate pairs by themselves, but we analysed the statistics of their population. Of course, this technique only allowed us to derive an "across-cruise" estimate of the uncertainty based on the duplicates.

ACTION: We added text to section 2.1 to explain why we collected only one duplicate per cast. In the same section, we have also explained that the whole duplicate population is used to derive "overall-cruise estimates of the relative POC experimental uncertainty".

As a general comment, the paper reads well and is relatively well illustrated. The material and method section describes in much detail the samples considered, the analytical determination and the statistical analysis applied to the data. However, some points need clarification and better organization. Results should not be included in this section as much as possible. For instance, Figure 2 is presented in section 2.4.1 and is not necessary for the total experimental uncertainty calculation since no correlation is assumed between duplicates and the total uncertainty is calculated essentially using the scaled relative difference. It would be more appropriate to present the lack of correlation in the result section to confirm that your calculation is appropriate.

RESPONSE: We respectfully disagree. Figure 2 is necessary to demonstrate a crucial part of our method, namely that we needed to work with relative rather than absolute duplicate differences, because the magnitude of the duplicate differences depended on the POC concentration.

ACTION: We have modified the text in section 2.4.1 to make this point more clear.

Another occurrence of a result presentation can be found p10 after equation 11.

RESPONSE: We agree.

ACTION: We have removed the text after eq 10 presenting the results of the percent uncertainties in POC.

Table 2, which summarizes the different contributions, should also be moved to later in manuscript and not introduced in section 2.5.

RESPONSE: We agree.

ACTION: We have moved the presentation of the old Table 2 (now Table 3 in the revised version) to the result section.

Similarly, the section 3.2 dealing with the detection limit should be moved to the method section, at least for the details of the calculation.

RESPONSE: We agree.

ACTION: We have now added a new subsection (2.5) to the methods to describe how the detection limit was estimated.

Regarding the detection limits, you choose to apply a 95% confidence interval, which makes sense and is commonly adopted in analytical chemistry. Why all other uncertainties are at 68% (1 sigma)?

RESPONSE: All other uncertainties are reported at the 1-sigma level to conform to the well-

known concept of "standard deviation". It should be easy for a reader to multiply these uncertainties by ~2 to obtain estimates of the 95% confidence intervals.

ACTION: None taken.

Still on the L_d and L_c values and as it can be deduced from Fig 4. It appears that all procedural blanks are below the L_d , only field blanks (aDOC) and samples (uPOC filters) are above. Does it make sense to report a C quantity that is below the L_d ? It could even be worse if you considered the quantification limit.

RESPONSE: We understand the reviewer's concern. Yet, we still believe it is useful to report results from all the measurements we have conducted, so that our results can be compared to those from other studies.

ACTION: None taken.

Regarding the total experimental uncertainty (section 2.4.1), I do not understand why it is necessary to apply a propagation law for estimating the precision (Eq. 7, 8, 9). Eq 10 related to the scaled relative difference of duplicates, which is actually your estimator of the relative precision, does not imply such a calculation. Absolute precision can be easily deduced by considering the average POC concentration of each duplicate.

RESPONSE: We thank very much the reviewer for spotting this mistake in our methods.

ACTION: We have now revised sect 2.4.1 and removed the old equations 7-9.

Accordingly, we have revised all quantitative results and figure related to the POC uncertainty. This mistake resulted in just small changes in our quantitative results and did not change our results qualitatively.

Still on this parameter ($\sigma_{\Delta r}$) and as mentioned in Hyslop and White (2009), it could be worth checking that your scaled relative differences are normally distributed. In that studies, three different estimators of the precision have been used including the root mean square (RSM), the scaled mean absolute difference (MAD) and the percentiles approach. Why did you choose the percentile precision?

RESPONSE: It is not a requirement that the scaled relative differences are normally distributed. If they are, the different metrics proposed in Hyslop and White 2009 will deliver approximately equivalent results. In our case, the distributions of the scaled relative differences are affected by outliers. The influence of these outliers on our metrics needs to be minimised, because we want to derive an estimate of the uncertainty for all measurements collected during the cruise. The percentile approach is the least sensitive to outliers (Hyslop and White, 2009). This is why we have selected the percentile range as a metrics (eq. 7 in the revised text).

ACTION: To explain the above argument in the manuscript, we have modified the text related to equation 7 in the revised text.

Another issue concerns the section 2.5.2 (uncertainty in mass predicted by the calibration equation), which is essentially the instrumental standard deviation. I do not understand why you include σ_{Mp} in this section, which refers to the correction of total POC mass with the corresponding DOC mass. This parameter is not related to the calibration equation and represent a post-analysis correction made possible with your sampling methodology. I think it could be interesting to consider separately these two components (instrument precision and precision on the corrected POC quantity by the DOC quantity). At this point it is not clear what the values

reported in table 2 and associated with the calibration correspond to, instrument precision or precision on the corrected mass.

RESPONSE: Our objective in this section was to propagate the uncertainty of the calibration equation to the POC concentration. We agree with the reviewer that the original text was not clear enough. But we have already dedicated one section to the impact of how the aDOC correction can affect the resulting POC, therefore we have decided to focus this section on the impact of the uncertainties in the calibration equation to the final POC concentration.

ACTION: We have modified the text in this section ("Uncertainty in POC concentration due to uncertainties in the calibration equation ") to clarify that we want to estimate the impact of the calibration equation on the POC concentration. We have also clarified this in Tables 3 and 4 of the revised manuscript.

Still on the uncertainty budget in the section 2.5.3 (uncertainty due to sample handling during CHN analysis), the proposed calculation based on the three acidified filter blanks does not only correspond to contamination due to the acidification step but includes also the contribution from the filter. In Eq 17, I do not think it is necessary to introduce two distinct variables (σ_1 and 2) since the two are equal and are estimated from the three acidified filter blanks. It could be interesting to consider separately these two distinct sources of uncertainty. Impact of acid fuming by considering the difference between non-acidified and acidified filter and the filter alone.

RESPONSE: We agree with the reviewer: the variability of the carbon mass of the acidified filters depends both on the uncertainties in measuring carbon on the acidified and on the clean filters. However, given that this source of uncertainty contributes a very small fraction of the final uncertainty, we have decided not to separate these two sources of uncertainty.

ACTION: We have now modified this section ("Uncertainty due to laboratory contamination") to clarify that it deals with the uncertainty due to filter contamination during laboratory analyses.

Another remark on the impact of filter concerns their role in the analytical determination and especially on the sensitivity of the instrument. *This is not taken into account in your dataset since your CHN standards were prepared without filters. Filters can affect the combustion process and as a consequence the sensitivity of the instrument (the slope of the calibration curve). Accordingly, it is highly recommended to tune the elemental analyzer with standards containing filters. In general, an increase in O₂ gas is necessary to prevent partial combustion of standards and samples. Also, adding a filter to the standards allows for better reproduction of the sample matrix and provides a means for quantifying filter blanks through the regression analysis (b parameter). This procedure is required in C isotope studies for both concentration and isotopic corrections when samples are loaded on a filter.*

RESPONSE: We thank the reviewer for this interesting remark. We were not aware of this methodological aspect, nor does it appear in the most updated protocols for POC determination. However, we have thoroughly searched the literature and have not managed to find a reference that describes this methodological detail.

ACTION: We have added a sentence to the method section to mention this additional aspect and cited a "personal communication" by Dr Planchon. "Finally, we note that, in carbon-isotope analyses filters are expected to affect the combustion process and thus the sensitivity of the analyser (Planchon, personal communication, 2022). As a consequence, to reduce uncertainties and improve sensitivity, future studies should consider investigating

this effect on POC determinations."

The result and discussion section is clearly written with abundant references to previous studies. I have only few concerns on this section. The first one is related to the description of the results. You present the median of your results with an associated uncertainty. Could you clarify how these uncertainties have been obtained (number of values and calculation)? My guess is that it corresponds to the standard deviation of your data.

RESPONSE: The reported uncertainties are robust standard deviations computed as described by eq. 9 (after substituting Δ_r with the appropriate variable for which the uncertainty is being estimated). The number of samples for the productive and mesopelagic zones are reported in Table 2 of the revised manuscript.

ACTION: In the revised manuscript, we have added a new subsection (2.6) to the methods to explain how uncertainties associated with medians were computed. We have also mentioned at the start of the Results section that the number of samples for each zone is reported in Table 2.

Looking at these uncertainties, they appear to be highly variable indicating a high dispersion of uncertainty data. I think this dispersion could be better considered and at least mentioned, especially when you compare the productive zone with the mesopelagic zone. For instance in section 3.7 when you compare uPOC with POC concentrations, I do not think that the difference between sampling zones is statistically different.

RESPONSE: Our intention in Sect 3.7 was not to state that the comparison between uPOC and POC was different between the productive and mesopelagic zones. What we wanted to do was to show that uPOC was higher than POC and therefore that the aDOC blank subtraction was important (as already well known). We presented separate results for productive and mesopelagic zones because throughout the manuscript the two zones are always described separately. In general, the uncertainties associated with the median values reported represent the spatial variability of the dataset in the two zones.

ACTION: In the revised manuscript (sect 3.1), we have added a sentence to clarify that these uncertainties represent the spatial variability of the dataset.

Another point concerns the observed positive correlation between aDOC and POC concentrations (section 3.3 and figure 9). In addition to cells break and release of DOC, another hypothesis may concern the potential impact of small (<0.7 μ m) particles associated with picophytoplankton (0.2-2 μ m in size) and small heterotrophs (free bacteria, archaea, etc.). These organisms can be abundant in surface waters representing an important part of the biomass especially out of the bloom period.

RESPONSE: We agree with the reviewer although these particles smaller than 0.7 μ m, if important, should also pass through the aDOC filter. We agree however that having two stacked filters increases the chances that a small particle is retained.

ACTION: In the revised manuscript we have added text (just above the beginning of section 3.4) to include the reviewer's suggestion as a potential alternative explanation of the aDOC-POC correlation.

Detailed comments:

Abstract L6-7: check sentences and change accuracy term with precision.

RESPONSE: We agree.

ACTION: Done.

Section 2.1: clarify the number of samples taken at each station, were they taken at the same cast? You mention 392 uPOC samples. if you collected 6 samples at 67 stations, this makes a total of 402 samples.

RESPONSE: We agree that the numbers did not added up correctly, because we had not mentioned exceptions (e.g., lost samples).

ACTION: We have now added information that should allow the reader to verify the number of samples (sect 2.1).

Similarly, you mention duplicate samples were taken at each cast. You should have 67 duplicate samples and not 57. Please clarify

RESPONSE: We agree that the numbers did not added up correctly, because we had not mentioned exceptions (e.g., lost samples).

ACTION: We have now added information (sect 2.1) that should allow the reader to verify the number of duplicates.

Page 5, last paragraph: if filter blanks were placed in the dessicator they are obviously acidified. I would change to: process as filter sample and subjected to acid fuming in the dessicator.

RESPONSE: We agree.

ACTION: Done.

Page 6, third paragraph: correct your description of the sample analysis in the CHN. The samples are combusted in the oven (combustion chamber subjected to a dedicated temperature program), and then ashes fall in the ash trap, which progressively fills up. This is not a reaction tube. Then combustion gases are carried to the oxidation and reduction "tubes" (chambers) and then to the chromatographic column for separation and final detection.

RESPONSE: Thank you.

ACTION: We have now corrected the sentence.

P6, paragraph 4-5: Theoretically, all standards measured require to be included in the calibration. The first set called "calibration standards" are generally used to check the linearity. For state of the art analytical sequence, linearity is checked also at the end of the sequence. Also, standards showing poor recovery (due to weighting error, bad analytical run) can be removed according to z-score.

RESPONSE: We thank the reviewer for this clarification. At the time of the analysis we were not aware that linearity should be also checked at the end of the analytical sequence. The calibration sequence we used was defined based on the manufacturer recommendation. Furthermore, even the most recent protocols for POC determination do not specify this is a requirement. Nevertheless, we agree with the reviewer about this potential additional check that can be performed on the analytical sequence. We also thank the reviewer for the suggestion to use the z-score metrics to flag outliers in the calibration process. As discussed in the text, the instrument instability was detected as an increased noise (but not a bias) in most of the stability standards, rather than in few of them. That is why we decided to not remove outliers, but to use all standards to generate a calibration equation.

ACTION: In the revised text (4th paragraph in sect 2.3) we have added the following sentence: "To better characterise the instrument's behaviour during the analysis, future

work should also use standards of with different weights at the end of the CHN run."

P6, paragraph 6: you often use the term robust throughout the manuscript (here for the fitting model), what does it mean? Does it differ from a classical linear fitting model?

RESPONSE: Yes it does. This is a fitting algorithm ("iteratively reweighted least-squares") that reduces the influence of outliers on the estimate regression parameters (i.e., slope and intercept)

ACTION: In the revised text we have clarified this in the paragraph before eq 1.

P6, bottom page: the two equations are not numbered and the definition of M star is not given. Furthermore, there is a missing term in these two equations corresponding to the C mass associated to the filter. The difference between acidified and non-acidified filter blanks corresponds only to the C added by the acidification process.

RESPONSE: We agree.

ACTION: Corrected.

P8, last paragraph: indicate the p value of the linear fits to explore the significance of the regression analysis.

RESPONSE: We agree.

ACTION: Done.

P9, figure 2: y axis of figure 2b is unitless.

RESPONSE: Thanks.

ACTION: Corrected.

P10, section 2.5: an equation showing how you compute all uncertainty contributions would help the reader.

RESPONSE: We agree.

ACTION: In the revised text we have now added a new equation (11) to describe how the uncertainty contributions were computed.

P10, Table 2: to be clearer, add a column with the corresponding variables associated with each line. If this table is moved to the result section, please include the sd for each median value and adjust to significant digit.

RESPONSE: We agree.

ACTION: Done.

P14, last paragraph: indicate the p value of the linear fits and homogenize the significant digit.

RESPONSE: We agree.

ACTION: Done.

P17, table 4: same remarks as for table 2.

RESPONSE: We agree.

ACTION: Done.

P18, Figure 9: indicate the p value of the linear fits.

RESPONSE: We agree.

ACTION: Done.

Competing Interests: No competing interests were disclosed.

Version 1

Reviewer Report 17 May 2021

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Anna Belcher

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The manuscript aims to develop a method to quantify the experimental uncertainties of POC concentrations, and hopes to contribute to developing a uniform protocol. Quantifying POC in the ocean is important and thus ensuring that a reliable and standardised method is adopted amongst researchers is important. Ensuring appropriate blanks are taken and that the necessary corrections are made is key, and the corrections made need to be transparent in each study, and ideally consistent amongst the research community.

The manuscript is clearly written and the authors have presented a clear explanation of the uncertainties that they have investigated. It is good to see each stage of the sampling to measurement process investigated, however there are some gaps in this which limit the uncertainty assessment. Particularly, certified reference materials should have been used to assess the accuracy of the CHN analysis. Additionally, it is a shame that a filtration blank was not taken – as the authors mention themselves, using Mili-Q water and an additional blank.

It is good to see the consideration of filtration time when assessing the use of double stacked filters, as this may well be a factor for many studies. Although the level of acceptable uncertainty may be different for different studies, it would be good for a 'gold standard' method to be identified by the authors in terms of giving their recommendation.

The distinction between DOC and POC is an interesting one, particularly when thinking about DOC contained within particles. A second filter is used in the study to estimate the concentration of DOC that is adsorbed onto the filter, which can be used to correct for DOC. However, as stated in section 3.3, fragmentation of particles into smaller particles could contaminate the aDOC filters. Additionally, particles will also contain DOC trapped within the particle matrix, some of which may be released during filtration due to damage of particles and DOC leakage. The loss due to DOC

leakage with the break up of particles on the filter is difficult to quantify, but poses an interesting question as to what we want to measure when we take a sample for POC. Gaining a measure of the 'true' POC in the water column is useful – i.e. correcting for DOC contamination, but if we want to think about the total carbon that can be transported by particles, then we should consider the trapped DOC in the particles. In a detailed uncertainty assessment such as this study, it would be worth mentioning/discussing this as a point to consider when we make POC measurements.

Although, the authors provide a useful assessment of uncertainty with clear and careful calculations, there are a few aspects of the work that make the uncertainty assessment incomplete. There is merit in the work undertaken and useful information for the scientific community, but some of these missing aspects need to be acknowledged and discussed further, and the limitations of the presented uncertainty values made clear. The manuscript therefore needs revisions before it can be considered to be indexed.

Specific comments:

Introduction, second paragraph, end of first sentence, 'coverage of POC', perhaps clearer to add 'POC estimates'? If this is what you mean?

It would be useful in the introduction to give more information on the IOCCG and JGOFS protocols in terms of what is missing from these, and how this paper will address these.

Section 2.3 – The authors say that constant adjustments to the CHN analyser were made. What are these, if these is important for accuracy then needs to be stated for reproducibility.

Section 3.3 – if aDOC and POC concentrations are spatially correlated as the authors suggest, it would be good to see the aDOC and corresponding POC value plotted against one another. Figure 5 and figure 3 are hard to compare for this purpose. It would be good to do this for mesopelagic and surface values separately, as this would also allow to separate out any depth driven correlation.

Section 3.7 – Please make clearer what you mean by 'original POC concentrations' as this is vague. Not all variables are defined in table 1.

Table 3: Add units.

Figure 4: should be 'black' not 'blank'.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Partly

Are sufficient details provided to allow replication of the method development and its use by others?

Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions about the method and its performance adequately supported by the

findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biological carbon pump, ocean biogeochemistry.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Aug 2021

Giorgio Dall'Olmo

Reviewer comment: General comments. The manuscript aims to develop a method to quantify the experimental uncertainties of POC concentrations, and hopes to contribute to developing a uniform protocol. Quantifying POC in the ocean is important and thus ensuring that a reliable and standardised method is adopted amongst researchers is important. Ensuring appropriate blanks are taken and that the necessary corrections are made is key, and the corrections made need to be transparent in each study, and ideally consistent amongst the research community. The manuscript is clearly written and the authors have presented a clear explanation of the uncertainties that they have investigated. It is good to see each stage of the sampling to measurement process investigated, however there are some gaps in this which limit the uncertainty assessment. Particularly, certified reference materials should have been used to assess the accuracy of the CHN analysis. Additionally, it is a shame that a filtration blank was not taken – as the authors mention themselves, using Mili-Q water and an additional blank.

Response: We thank very much the reviewer for the time they have spent in commenting our manuscript. We agree that a certified reference material (CRM) is needed for POC measurements, but as of now, no such CRM has been agreed by the community (see also responses to reviewer 1). We also agree that adding procedural filters would be helpful and that is why we have for example introduced blank filters to quantify the contamination that could occur during the acidification step. However, before calculating the uncertainty budget, we were not aware of the potential *critical* role of sources of uncertainties different from the CHN calibration and the DOC adsorption on filters. As such, we had not planned for testing contamination from, for example, the atmosphere of the ship's laboratory. Our hope is that by presenting our uncertainty budget and the disagreement between experimental and modelled uncertainties and by listing potential sources of uncertainty, in future studies such uncertainties will be addressed, quantified, and minimised.

Action: None taken.

Reviewer comment: It is good to see the consideration of filtration time when assessing the use of double stacked filters, as this may well be a factor for many studies. Although the level of acceptable uncertainty may be different for different studies, it would be good for a 'gold standard' method to be identified by the authors in terms of giving their recommendation.

Response: We do not think it is up to us to define a "gold standard" for the community to

follow. If at all, the definition of “gold standard” should be a community effort. We believe the standards to follow depend on the level of accuracy that is required to answer a specific question using data from a specific ocean region. What we have done is to quantify the additional uncertainties introduced by not collecting an aDOC sample for every uPOC sample (section 3.7). We expect these findings to guide future studies in potentially reducing filtration times.

Action: None taken.

Reviewer comment: The distinction between DOC and POC is an interesting one, particularly when thinking about DOC contained within particles. A second filter is used in the study to estimate the concentration of DOC that is adsorbed onto the filter, which can be used to correct for DOC. However, as stated in section 3.3, fragmentation of particles into smaller particles could contaminate the aDOC filters. Additionally, particles will also contain DOC trapped within the particle matrix, some of which may be released during filtration due to damage of particles and DOC leakage. The loss due to DOC leakage with the break up of particles on the filter is difficult to quantify, but poses an interesting question as to what we want to measure when we take a sample for POC. Gaining a measure of the ‘true’ POC in the water column is useful – i.e. correcting for DOC contamination, but if we want to think about the total carbon that can be transported by particles, then we should consider the trapped DOC in the particles. In a detailed uncertainty assessment such as this study, it would be worth mentioning/discussing this as a point to consider when we make POC measurements.

Response: We agree that particles (e.g., cells) contain DOC. Yet, this DOC contained in particles is operationally defined as part of the POC (unless particle breaks and this DOC is released). The concentration of this particle-related DOC is expected to be significantly smaller than the ambient DOC. To determine the extent to which this particle-related DOC should be a major avenue of future research, dedicated experiments should be devised and conducted to quantify how much DOC is contained in cells.

Action: We added a sentence to make the reader aware that broken cells release their inner DOC in the environment.

Reviewer comment: Although, the authors provide a useful assessment of uncertainty with clear and careful calculations, there are a few aspects of the work that make the uncertainty assessment incomplete. There is merit in the work undertaken and useful information for the scientific community, but some of these missing aspects need to be acknowledged and discussed further, and the limitations of the presented uncertainty values made clear. The manuscript therefore needs revisions before it can be considered to be indexed.

Response: We thank again the reviewer for their suggestions.

Action: We have addressed all questions/comments (see below).

Reviewer comment: Specific comments. Introduction, second paragraph, end of first sentence, ‘coverage of POC’, perhaps clearer to add ‘POC estimates’? If this is what you mean? It would be useful in the introduction to give more information on the IOCCG and JGOFS protocols in terms of what is missing from these, and how this paper will address these.

Response: We agree.

Action: We have replaced “coverage” with “measurements”. We have added a paragraph at

the beginning of Section 2 in which we introduce the two protocols. However, since we have also focused the manuscript more on quantifying the POC uncertainties and their sources, we have not presented a detailed description of these protocols.

Reviewer comment: Section 2.3 – The authors say that constant adjustments to the CHN analyser were made. What are these, if these is important for accuracy then needs to be stated for reproducibility.

Response: This was clarified just after the words “constant adjustments”, i.e., “to sample delay, which represents the time that it takes the CHN analyser to combust each sample, to deliver CO₂ released from the sample to the detector, and to run the analysis.”

Action: We have now written “sample delay” in quotes so that the reader is aware that the next part of the sentence explains what the adjustments were referring to. We hope this help making the text clearer.

Reviewer comment: Section 3.3 – if aDOC and POC concentrations are spatially correlated as the authors suggest, it would be good to see the aDOC and corresponding POC value plotted against one another. Figure 5 and figure 3 are hard to compare for this purpose. It would be good to do this for mesopelagic and surface values separately, as this would also allow to separate out any depth driven correlation.

Response: We agree.

Action: We have now added a new figure plotting aDOC vs POC and added related text with the correlation coefficients for the mesopelagic and productive zones.

Reviewer comment: Section 3.7 – Please make clearer what you mean by ‘original POC concentrations’ as this is vague.

Response: Thank you for pointing out this issue.

Action: We have now removed “original”, which should make the text clearer.

Reviewer comment: Not all variables are defined in table 1. Table 1 is a non-exhaustive list, please include the rest of variable. Table 3: Add units.

Response: Thank you for pointing out these issues.

Action: We have now updated the Tables 1 and 3.

Reviewer comment: Figure 4: should be ‘black’ not ‘blank’.

Response: Thank you for pointing this out.

Action: Corrected.

Competing Interests: No competing interests were disclosed.

Reviewer Report 10 May 2021

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Eric Achterberg 

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The submitted manuscript deals with an uncertainty assessment for the measurement of POC in oceanic water columns. POC is relevant as its removal through sinking from the surface ocean provides a means of long-term sequestration of carbon in the deep ocean.

POC levels are low in the ocean, and its measurement is influenced by a range of factors which affect data quality. A range of papers have been published on this issue, and the submitted manuscript aims to make a further contribution.

The paper makes a nice assessment of the various uncertainties associated with oceanic POC measurements, and attempts to quantify the uncertainties. For a range of uncertainties, it was not possible to constrain them, as the experimental set-up was inappropriate.

The starting point for the work was duplicate sampling and analysis of a subset of POC samples on a research cruise. The difference between the duplicate samples formed the basis of the work in the manuscript. It seems that the overall uncertainty work was not the result of a carefully planned experiment prior to the cruise, but an afterthought following the cruise. Unfortunately this shows in the manuscript, and seriously impacts quality.

In case a thorough uncertainty assessment was to be planned, then instead of duplicate samples, the authors would have sampled 5 samples, allowing thorough statistical assessment. The sampling equipment, and sample processing equipment (incl containers) would have been assessed for contamination risk/level. Filtration instrument blanks would have been collected (with and without clean MQ water filtration), and also filtering would have been conducted under more controlled conditions on-board ship.

The analytical analysis is lacking a certified reference material measurements, which hampers accuracy assessment.

Overall, the manuscript provides a detailed assessment of various causes for uncertainty, but the unfortunate experimental set up precludes meaningful conclusions to be drawn from the work. A dedicated experiment will need to be conducted to assess POC measurement uncertainty, with careful planning prior to the experiment which will allow for sound statistical data analysis. I cannot support indexing of this manuscript.

Specific comments

Intro: first line. Please be very careful in the distinction between POC and OM. POM is composed of POC, POC is not composed of POM. Rephrase sentence.

.....extensive coverage of POC? Coverage of what aspect of POC? Explain.

...methods for determining POC? Unclear what you are referring to. The CHN analysis is well established. Which methods are you referring to? I assume it is the sampling, and sample processing and filter blank assessment rather than the laboratory analysis?

Accuracy assessment for POC. This can be achieved for the laboratory analysis using certified reference materials, but not for the overall process of sampling, sampling processing and lab analysis. Intercomparisons etc. can be conducted for data validation purposes.

The intro would benefit from a better context of the topic. In 2 sentences, place POC in the context of DIC and DOC, and PIC. POC is of course the C fraction occurring at the lowest concentration. The authors should also mention the particle sizes that they are referring to when discussing POC (rather than DOC).

Data and methods.

I suggest that the authors only use the term accuracy when referring to the analysis of a certified reference material.

Section 2.1 Provide clear details on sampling approach. Including make of bottles and type of rosette frame.

Analysis of duplicate samples does not provide information on accuracy. It provides information on replication.

Section 2.2. Were procedural filters analysed (filters placed in filtration set-up at sea, with no seawater passed over them)?

Why is it important to reduce humidity in the desiccator.

Provide details on acetanilide.

Page 6...Therefore, the true mass... Please avoid the term true, as you cannot prove this. You mean 'blank corrected', I assume.

2.5.2. It is worrying that there is a weighing error introduced into the overall method assessment. This undermines all the conducted work.

There are certified reference materials (soil material), which can be analysed as part of the calibration, and also a regular check sample during the analyses. This will provide the researcher with more confidence in the measurement quality, which currently is unconstrained. Hence, the word accuracy and accurate should not appear in the manuscript.

Results & discussion. I do not understand why the authors say the unit mg/m^3 . I think using a unit with carbon in moles, and then L (or m^3) or kg, would be more appropriate.

Fig. 3 Colour differences between samples is hard to decipher.

P 15, bottom sentence: this statement does not allow for a comparison with the quantitative uncertainty approach conducted by the authors. Please quantify the potential consequence of the large particles.

Is the rationale for developing the new method (or application) clearly explained?

Partly

Is the description of the method technically sound?

Partly

Are sufficient details provided to allow replication of the method development and its use by others?

No

If any results are presented, are all the source data underlying the results available to

ensure full reproducibility?

Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Chemical oceanography, marine chemistry, carbon export, carbon cycle.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 27 Aug 2021

Giorgio Dall'Olmo

Reviewer comment: General comments: The submitted manuscript deals with an uncertainty assessment for the measurement of POC in oceanic water columns. POC is relevant as its removal through sinking from the surface ocean provides a means of long-term sequestration of carbon in the deep ocean. POC levels are low in the ocean, and its measurement is influenced by a range of factors which effect data quality. A range of papers have been published on this issue, and the submitted manuscript aims to make a further contribution.

Response: We thank the reviewer for the time they invested in providing comments to our draft manuscript. These comments have allowed us to improve the presentation of our work in the revised version.

Reviewer comment: The paper makes a nice assessment of the various uncertainties associated with oceanic POC measurements, and attempts to quantify the uncertainties. For a range of uncertainties, it was not possible to constrain them, as the experimental set-up was inappropriate. The starting point for the work was duplicate sampling and analysis of a subset of POC samples on a research cruise. The difference between the duplicate samples formed the basis of the work in the manuscript. It seems that the overall uncertainty work was not the result of a carefully planned experiment prior to the cruise, but an afterthought following the cruise. Unfortunately this shows in the manuscript, and seriously impacts quality. In case a thorough uncertainty assessment was to be planned, then instead of duplicate samples, the authors would have sampled 5 samples, allowing thorough statistical assessment. The sampling equipment, and sample processing equipment (incl containers) would have been assessed for contamination risk/level. Filtration instrument blanks would have been collected (with and without clean MQ water filtration), and also filtering would have been conducted under more controlled conditions on-board ship.

Response: The "uncertainty work" we have presented in the manuscript can be divided into two separate sections. First, we have presented an empirical method to estimate the uncertainty of POC estimates based on duplicate samples: to the best of our knowledge the

technique we presented has not been used for POC before, so we believe it is worth publishing on its own. Second, we have modelled the uncertainty of our POC measurements, based on a limited set of input sources of uncertainty. Finally, by comparing the empirical and modelled uncertainties, we showed that the latter accounted for a relatively small fraction of the empirical uncertainties, strongly suggesting that we did not include some important sources of uncertainties in our modelled uncertainty. The first part of our uncertainty work, was planned before the cruise. That is why we collected duplicate measurements at all stations, as well as aDOC blanks for each sample. The reviewer states that, had we planned this work in advance, we would have collected five (it is not clear why exactly five) replicate samples, rather than the duplicates we collected. We agree that larger number of replicates could have improved our estimates of the uncertainties, for example by allowing us to determine an uncertainty for each specific measurement. This reviewer, however, does not seem to be taking into consideration the large water volumes that we did sample (up to 8 litres, another pre-cruise planned activity to minimise uncertainties in the low-signal waters of the mesopelagic) and the constraints imposed on how much water was available from each cast for each scientist during the field campaign. Put simply, there would not have been enough water for the rest of the science party if we had collected five replicates at each station. Instead, we opted for a practical compromise and collected duplicates at different depths and used the statistics of the relative differences between the duplicates to quantify the overall uncertainty of our POC measurements during the entire field campaign. These duplicate uncertainties are very useful in putting all the measured values into context. The second part of our “uncertainty work”, the uncertainty budget, instead was an exercise that we decided to undertake after the cruise to better understand how POC measurements can be improved. We agree that this exercise has not provided definitive answers, but it has nevertheless clearly indicated that the sources of uncertainty we initially assumed to be more important (i.e., the calibration of the CHN analyser, aDOC correction) were NOT the dominant sources of uncertainty in the measurements. We have therefore discussed several potential sources of uncertainty that should be investigated in the future. Despite the limitations of this section, we still believe it is worth publishing as it can change attitudes on the dominant sources of uncertainty and therefore guide future studies. Finally, we would like to note that in 2014 (when we collected the samples described in this manuscript) the POC protocols (Knapp *et al.*, 1996) did not describe all the potential sources of uncertainties affecting POC measurements that we have presented in our manuscript. As a consequence, our thinking was guided by previous POC measurements we had collected and on the literature we were familiar with. Only recently has a new version of the POC protocol appeared (still in draft form) and we are part of this new effort to improve the quality of POC determinations (https://ioccg.org/wp-content/uploads/2019/11/poc_ioccg_protocol_2019_public_draft-18nov-2019.pdf). In other words, had we known all the major potential sources of uncertainty, we would have collected additional measurements to quantify their contributions to the total uncertainty. To conclude, while analytical protocols for other chemical species (e.g., macro- and micro-nutrients) may be well established, the situation is different for POC. Clear protocols are not yet well established, intercomparisons between laboratories are not routinely conducted, and there is no certified reference material for POC. As a consequence, POC measurements are still relatively uncertain, especially in the mesopelagic, where POC is extremely low.

Action: We clarified in the introduction the two different parts of the “uncertainty work”. We also improved the method section explaining what protocols were available at the time of

sampling and what has appeared later.

Reviewer comment: The analytical analysis is lacking a certified reference material measurements, which hampers accuracy assessment.

Response: We agree with the reviewer that a certified reference material (CRM) would be desirable to assess the accuracy of our measurements. However, as of today, no such reference material is available. As the reviewer suggest, there exist some CRMs that might be used, but these are all based on sediment samples, with very different matrices from open-ocean pelagic particulate matter (National Research Council. "Chemical Reference Materials: Setting the Standards for Ocean Science". The National Academies Press, Washington, DC, 2002. ISBN 978-0-309-08500-7. doi: 10.17226/10476.

<https://www.nap.edu/catalog/10476/chemical-reference-materials-setting-the-standards-for-ocean-science>.) In addition, not all bio-organic elements of these CRMs have been quantified, making it difficult to assess how similar they are to open-ocean pelagic sediments. Thus, these existing materials are not recognised by the POC community as reference materials for POC and are not routinely used to assess the accuracy of POC measurements (https://ioccg.org/wp-content/uploads/2019/11/poc_ioccg_protocol_2019_public_draft-18nov-2019.pdf).

Action: In the revised version of the manuscript, we have added a new section discussing the need for CRMs for POC analyses.

Reviewer comment: Overall, the manuscript provides a detailed assessment of various causes for uncertainty, but the unfortunate experimental set up precludes meaningful conclusions to be drawn from the work. A dedicated experiment will need to be conducted to assess POC measurement uncertainty, with careful planning prior to the experiment which will allow for sound statistical data analysis. I cannot support indexing of this manuscript.

Response: We agree with the reviewer that more work is needed to identify all sources of uncertainty for POC and we have listed several potential ones in our manuscript. However, we disagree that the work we have conducted and described in this manuscript is not worth publishing. First, we have presented a dataset of POC measurements in the mesopelagic (0-500m) along an Atlantic Meridional Transect. By itself such a dataset is very useful, for example to assess model output. Second, we have presented a detailed method to quantify experimentally the total uncertainty associated with these POC measurements based on duplicate samples. This is important because it will allow other researchers to similarly quantify and report their uncertainties, which is key to assess and compare datasets, especially in the mesopelagic where signals are very low. Third, we have modelled the total POC uncertainty based on what, at the time of the analysis, were thought to be the largest sources to the uncertainty in POC. Results from this modelling analysis demonstrated that the assumed sources of uncertainty were able to explain only a fraction of the total experimental uncertainty. We have thus listed potential additional sources of uncertainty that should be minimised and quantified in the future. Unfortunately, the reviewer only focused on this last point and did not seem to consider the other parts of our work.

Action: We have revised the manuscript to clarify the different contributions of our work.

Reviewer comment: First line. Please be very careful in the distinction between POC and OM. POM is composed of POC, POC is not composed of POM. Rephrase sentence.

Response: We agree with the reviewer.

Action: We have removed the term "POM" from the manuscript.

Reviewer comment:extensive coverage of POC? Coverage of what aspect of POC? Explain.

Response: We agree with the reviewer.

Action: We have replaced "coverage" with "measurements".

Reviewer comment: ...methods for determining POC? Unclear what you are referring to. The CHN analysis is well established. Which methods are you referring to? I assume it is the sampling, and sample processing and filter blank assessment rather than the laboratory analysis?

Response: Here we are referring to all the steps needed to determine POC, including sampling, sample processing and acidification, filter blank assessment, as well as CHN analyses. While the CHN analyses are well established, we still do not know how much of the total POC uncertainty they contribute.

Action: We have clarified this in the revised version of the manuscript.

Reviewer comment: Accuracy assessment for POC. This can be achieved for the laboratory analysis using certified reference materials, but not for the overall process of sampling, sampling processing and lab analysis. Intercomparisons etc. can be conducted for data validation purposes.

Response: No CRMs are available for POC. We agree that intercomparison exercises are needed to quantify and minimise uncertainties due to sample collection and processing before the CHN analysis in the laboratory.

Action: We have added a paragraph that specifically discusses the above.

Reviewer comment: The intro would benefit from a better context of the topic. In 2 sentences, place POC in the context of DIC and DOC, and PIC. POC is of course the C fraction occurring at the lowest concentration. The authors should also mention the particle sizes that they are referring to when discussing POC (rather than DOC).

Response: We agree.

Action: We have now added a part of the introduction that defines POC and places it in the context of the other carbon pools.

Reviewer comment: I suggest that the authors only use the term accuracy when a referring to the analysis of a certified reference material.

Response: We agree.

Action: Where relevant, we have replaced "accuracy" with "uncertainty".

Reviewer comment: Section 2.1 Provide clear details on sampling approach. Including make of bottles and type of rosette frame.

Response: We agree.

Action: We have added more details including make of bottles and type of rosette frame.

Reviewer comment: Analysis of duplicate samples does not provide information on accuracy. It provides information on replication.

Response: We agree.

Action: We have replaced “accuracy” with “uncertainty”, where relevant.

Reviewer comment: Section 2.2. Were procedural filters analysed (filters placed in filtration set-up at sea, with no seawater passed over them)?

Response: No procedural filters were used. This is something that we realised is needed, when we computed the uncertainty budget. No such procedural filters were recommended in the Knaps 1996 POC protocols.

Action: None taken.

Reviewer comment: Why is it important to reduce humidity in the desiccator.

Response: We have some experimental evidence that silica gel in the desiccator reduces the contamination during the acidification step. However, we do not fully understand what processes controls this. This is why we also introduced additional blanks to quantify the contamination.

Action: We have removed this sentence.

Reviewer comment: Provide details on acetanilide.

Response: Agreed.

Action: We have added the manufacturer and model number of the acetanilide used.

Reviewer comment: Page 6...Therefore, the true mass... Please avoid the term true, as you cannot prove this. You mean ‘blank corrected’, I assume.

Response: Agreed.

Action: Done.

Reviewer comment: Section 2.5.2. It is worrying that there is a weighing error introduced into the overall method assessment. This undermines all the conducted work.

Response: We disagree with the reviewer. As stated in the manuscript, “[...] there was no bias in the less accurate standards - they resulted in unbiased calibration coefficients, even though the random uncertainties around these coefficients were higher.” We also disagree that having less accurate, but unbiased, standards in two out of 16 CHN runs “undermines all the conducted work”.

Action: None taken.

Reviewer comment: There are certified reference materials (soil material), which can be analysed as part of the calibration, and also a regular check sample during the analyses. This will provide the researcher with more confidence in the measurement quality, which currently is unconstrained. Hence, the word accuracy and accurate should not appear in the manuscript.

Response: As stated in a previous response, there are no community agreed certified reference materials for POC. Therefore, the accuracy of POC measurements cannot be quantified at present.

Action: Where relevant, we have removed the words “accuracy” and “accurate” from the revised version.

Reviewer comment: Results & discussion. I do not understand why the authors say the unit

mg/m³. I think using a unit with carbon in moles, and then L (or m³) or kg, would be more appropriate.

Response: We disagree. The community dedicated to measuring POC concentrations has not selected official units for POC (e.g., see IOCCG POC protocol draft: https://ioccg.org/wp-content/uploads/2019/11/poc_ioccg_protocol_2019_public_draft-18nov-2019.pdf). Thus, both grams and moles are commonly used for POC. It is easy to convert from POC in grams/m³ to POC mol/m³ by dividing the mass of C by the atomic weight of carbon: $\text{mol_POC} = \text{g_POC}/12.0107$.

Action: None taken.

Reviewer comment: Fig. 3 Colour differences between samples is hard to decipher.

Response: We agree with the reviewer that coloured plots are sometimes hard to interpret quantitatively. However, the purpose of Fig. 3 is to present the general patterns in POC during ATM24 (i.e., higher POC at the start and end of the transect vs. the oligotrophic gyres and higher POC near the surface than at depth). We believe these general patterns are clear in Fig. 3. The dataset was also made publicly available so that the reader can inspect any specific point they may be interested in.

Action: None taken.

Reviewer comment: P 15, bottom sentence: this statement does not allow for a comparison with the quantitative uncertainty approach conducted by the authors. Please quantify the potential consequence of the large particles.

Response: The sentence the reviewer refers to is part of a longer paragraph that might have been broken up in the typesetting process.

Action: We have now ensured that the paragraph is all connected.

Competing Interests: No competing interests were disclosed.