

General comments

This study examines the influence of filter blanks, pore sizes, and storage conditions on fluorescent dissolved organic matter (FDOM) measurements in seawater. Given the importance of FDOM as a tracer in oceanographic research, ensuring the accuracy and consistency of its measurements across different sampling protocols is critical. The manuscript presents clear objectives and a thorough discussion of the findings.

The study's primary objective is to refine methodologies for more reliable FDOM measurements. To achieve this, the researchers evaluated uncertainties associated with various sample preparation techniques. Using parallel factor analysis (PARAFAC), they identified three key FDOM components: the terrestrial humic-like peak (C peak), the marine humic-like peak (M peak), and the protein-like peak (T peak).

The main findings of the study are as follows:

1. Effect of blanks: Systematic blank values were observed when samples were filtered through pre-combusted glass fiber filters (0.7 μm pore size) and membrane filters (0.2 μm pore size) without pre-cleaning. This indicates that the filtration process can introduce contamination that affects FDOM measurements.
2. Effect of pre-washing: The blank values became negligible when the filters were pre-washed with 5 mL of 0.1 M HCl or 20 mL of distilled water, suggesting that proper pre-treatment of filters is essential for accurate measurements.
3. Effect of storage: FDOM_H concentrations remained consistent for 21 days when stored in a refrigerator or freezer. However, the T peak concentrations showed significant decreases within five days, indicating that protein-like components are more susceptible to degradation.

I recognize the effort put into this study and the importance of the findings. However, I disagree with one recommendation made in the manuscript that can potentially have important impacts. In the discussion, the authors state:

the filtration procedure is not necessary for measuring FDOM concentrations in open ocean waters

This statement seems counterintuitive, as filtration is a standard practice in oceanographic research to remove particulate matter and ensure sample homogeneity.

- First, you never know what you might find in a sample and it might be too late when you figure out that you should have filtered it.
- Second, based on Figure 3, filtering appears to have a significant effect on the T component. From the data provided in the appendix, I extracted the T peak concentrations for different storage conditions and measurement days (0, 5, and 21 days). The boxplot below illustrates

that the T peak concentrations for unfiltered samples can be significantly higher than those for filtered samples. Therefore, I would advise caution in recommending against filtering samples.

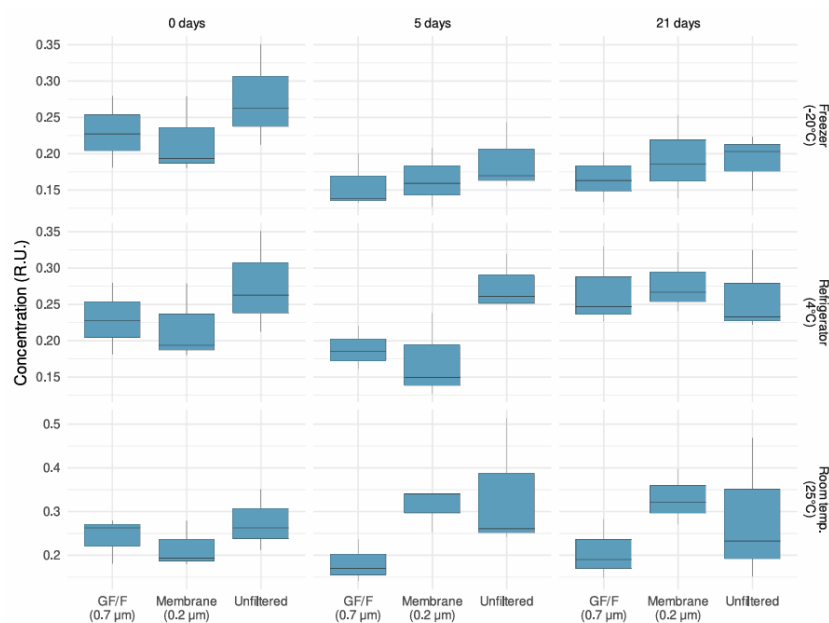


Figure 1 Boxplot of T peak concentrations for different storage conditions and measurement days.

I also wonder if there are many researchers that do not filter their samples. I would suggest discussing this point further and providing more context on the implications of not filtering samples in oceanographic research.

→ We sincerely appreciate your insightful comments and will carefully incorporate them into the revised manuscript. Regarding the filtration process for FDOM measurements, we will tone down the description to account for the potential influence of contamination.

Specific comments

Abstract

- Line 13: *the samples identified three..*: the samples cannot identify, the researchers or the statistics identified.

→ This sentence has been rephrased as follow:

“Three primary components were identified from these samples using parallel factor analyses: terrestrial humic-like peak (C peak), marine humic-like peak (M peak), and protein-like peak (T peak).”

- Line 14: what is a *high procedural blank*? This should be rephrased for clarity. Maybe something like *high blank values*.

→ This sentence has been revised to clarify the meaning, as follow:
“Relatively high blank values were obtained ...”

- Line 18: Define the H in $FDOM_H$. I guess this means humic-like, but it should be spelled out the first time.

→ All abbreviations are defined upon their first time in the manuscript.

- Line 21: It is said that significant bacterial degradation occurs after five days, but later in the same sentence it is said three days. Please clarify.

→ The phrase “three days” was changed to “five days”.

Introduction

- Line 33: I suggest removing the last sentence.

→ removed

Methods

- Authors used 1 cm quartz cuvettes for the measurements. It seems a bit unusual to use so small pathlength cuvettes for samples from the open ocean. Could you provide more details on this choice?

→ The path length of quartz cuvettes depended on the FDOM concentrations. In marine environments, a 1 cm path length quartz cuvette was used, which is a standard and widely adopted practice in FDOM measurements (Murphy et al., 2013).

Reference:

Murphy, K. R., Stedmon, C. A., Graeber, D., & Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. *PARAFAC. Analytical methods*, 5(23), 6557-6566.”

- Line 59: It is specified that the deepest samples were measured at 500 meters, but in Fig. 4, we can see 2300 meters. Please clarify.

→ Sampling was conducted at surface (3 m), 300 m, 2300 m, and this correction has been incorporated into the revised manuscript.

- Line 86: Maybe rephrase the sentence to be precise that the components were compared to the components found in the OpenFluor database.

→ revised to clarify as follow:

“The fluorescence spectra results were compared with the OpenFluor spectra database (Murphy et al., 2014), and statistical matches were found at a confidence level of > 95%, with 28 matches for the C component, 37 for the M component, and 45 for the T component.”

- Line 95: The term the filter blank is often used. I would suggest replacing it with something like the fluorescence of the C/M/T peaks measured on the filtrate, or something similar.
 → The term “filter blank” was changed to “fluorescence of the C/M/T peaks measured on the filtrate” in the revised manuscript.

Results

- The authors report some statistical results in the text, but they do not specify which tests were used.
 → In the revised version, we have added a brief explanation in this section where the p-value is first introduced (line 118), clarifying that p values higher than 0.05 are considered not statistically significant.
- Figure 3:
 - Why is there a second y-axis on the right side of the plot? It is confusing and unnecessary. Both unfiltered and filtered samples should be on the same y-axis (they already are on the same scale).
 - Add the legend for the circle and triangle markers in the legend.
 - Add a tag/annotation on each subplot to identify them in the text.
 → The y-axis corresponding to the unfiltered samples has been removed, and the data are presented together with the filtered samples, as per reviewer’s suggestion. Annotations from (a) to (i) will be added to distinguish each subplot, and a description for each subplot will be provided in the figure caption.

Discussion

- Line 133: I suggest replacing *in the course of water filtration* with *by the filtration process*.
 → the phrase “in the course of water filtration and sample storage” was changed to “in the course of water filtration with by the filtration process”.
- Line 157: I think you meant Fig. 4.
 → corrected
- Line 158: The authors say *However, at room temperature*, we observed *significant changes in FDOM_H concentrations after five days*. However, I do not see many changes in the figure for peaks C and M. Do you mean the T peak?
 → FDOM_H concentrations after five days showed significantly differences compared to those within five days, particularly for the M peak in the open ocean and the C and M peaks in the coastal ocean (Fig. 5). In the revised manuscript, we have incorporated this information to

improve the clarity.

Conclusions

In the conclusions and elsewhere, the authors suggest that the decision to filter or not should depend on the research question—specifically, whether the T peak is of interest. Could the authors clarify what types of research questions would not require consideration of the T peak? Providing a few examples would be valuable.

→ As suggested, we have clarified in the revised manuscript the types of research questions that may not require consideration of the T peak.

For example:

- 1) In open ocean observations where sample storages typically exceeds one weeks before analysis, degradation of the T peak over time can lead to misinterpretation.
- 2) In costal studies focused on identifying the sources of humic substances, rather than investigating biological processes.

Other comments

- The samples come from different areas with varying salinity. Can this influence fluorescence intensity?

→ In general, FDOM components show various intensity range depending on different sampling stations (these can be either coastal vs. open ocean, surface layer vs. benthic condition), but the salinity effect is not clear. For example, in the open ocean, the T peak exhibited high intensity in the biologically productive surface layer and decreased sharply with depth. In addition, FDOM_H showed low intensity at the surface and gradually increased with depth.

- Please identify each panel in the figures and cite them properly in the text when discussing them.

→ corrected