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Technical note: Measurements of fluorescent dissolved organic matter (FDOM) in seawater (Filter blanks, pore sizes, and storage)

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Abstract.

Fluorescent dissolved organic matter (FDOM) provides crucial information regarding the sources and characteristics of DOM in oceans. However, the FDOM measurements vary depending on filter blanks, pore sizes, and sample storage. To develop more reliable methods for FDOM measurements, in this study, we examined the uncertainties associated with different preparation methods for seawater samples. These samples identified three primary components using parallel factor analyses: terrestrial humic-like peak (C peak), marine humic-like peak (M peak), and protein-like peak (T peak). Relatively high procedural blank values were obtained from samples filtered through the pre-combusted glass fiber filter (0.7 μm pore size) and membrane filter (0.2 μm pore size) without pre-cleaning. However, the blank values became negligible when the filter was pre-washed with 5 mL of 0.1 M HCl or 20 mL of distilled water. The effects of different filter pore sizes were not observed for the C and M peak concentrations ($FDOM_H$), but relatively low T peak values were observed for filtered samples (0.7 or 0.2 μm) relative to unfiltered samples. For all samples, $FDOM_H$ showed consistent results for 21 days (8% ± 3%) when stored in a refrigerator or a freezer. However, T peak concentration decreased rapidly in both filtered (15%–50%) and unfiltered samples (10%–40%) after five days, indicating considerable bacterial degradation of protein-like components within three days. Therefore, our results suggest that reliable $FDOM_H$ values can be obtained either unfiltered and filtered samples stored in either a refrigerator or freezer for three weeks, but careful sample filtration, storage, blank controls are necessary for T peak measurements.

https://doi.org/10.5194/egusphere-2025-501
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1. Introduction

Fluorescent dissolved organic matter (FDOM), which emits fluorescent light after absorbing energy, is ubiquitous in the ocean and provides important information on the origins and behavior of the DOM in the ocean (Nelson and Siegel, 2013; Stedmon and Nelson, 2015). FDOM also has been used as a tracer for water circulation in the ocean (Galletti et al., 2019; Margolin et al., 2018), for estimating DOM turnover times in the global ocean (Catalá et al., 2015), and for calculating the fractions of different water masses in the ocean (Kim et al., 2020; Wang et al., 2022). FDOM can influence the optical properties of the water column. Siegel et al. (2002) suggested that FDOM can regulate the absorption of blue light (> 50%) in the global ocean. The absorption of UV by FDOM can control photosynthesis and the growth of marine microorganisms (Arrigo and Brown, 1996; Del Vecchio and Blough, 2002). Thus, FDOM is important to understand the biogeochemistry and optical properties of the ocean.

Over the last few decades, the measurements of FDOM in the ocean have been extensively conducted. Accordingly, various sampling and laboratory protocols have been developed in different laboratories without intercalibrations in sampling, storage, and measurements. In general, freezing and refrigeration have been used to store samples. The freezing of seawater samples after filtering with a pre-combusted (4 h, 450°C) glass fiber filter (GF/F, Whatman, 0.7 μm pore-size) is one of the widely used methods to preserve the FDOM concentration when the measurements are delayed (> one month) (Conmy et al., 2009; Yamashita et al., 2021). However, during the freezing and thawing process, FDOM concentration can vary up to ± 50% due to aggregation and disaggregation, especially when high levels of humic substances are present (Murphy et al., 2013; Spencer et al., 2007). Moreover, sample treatment with acid or formalin to avoid biological activity can also change FDOM concentrations and cause a shift to variable results (Spencer et al., 2007). Thus, FDOM sampling was commonly performed by filtering the water sample (~ 40 mL) with the pre-combusted (4 h, 450°C) GF/F and storing it in a pre-combusted (4 h, 450°C) amber vial without any treatment. Then, the FDOM samples generally were kept in a refrigerator (4°C) before the measurement (Coble et al., 1998; Stedmon et al., 2003). However, the uncertainties from these various sampling and storage methods for different DOM compositions have not been carefully evaluated yet. Therefore, in this study, we measured FDOM concentrations in open- and coastal-ocean samples under different conditions (i.e., filtration sizes and storage strategies) to obtain reliable methods for FDOM measurements.

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

2. Methods

2.1. Procedural blank

We used pre-combusted GF/F and membrane filters (Whatman, 0.2 μm pore size) to examine the filter blanks of FDOM measurements. The materials of GF/F and membrane filter are composed of borosilicate and mixed cellulose ester, respectively. The filter blank was tested with a sequential filtration process adding up to 100 mL of distilled water and 0.1 M HCl, respectively. The measurement of FDOM was conducted by collecting 5 mL water samples at the volume of 5, 10, 15, 20, 30, 50, and 100 mL during the sequential filtration process.

2.2. Sampling and preparation

Sampling was conducted in the East Sea (Japan Sea) (Station EC1; 37.33°N, 131.45°E) in April 2019 and the Jinhae Bay (JH; 35.04°N, 128.62°E) in August 2019. The water samples (3 m, 300 m, and 500 m depths) of Station EC1 were collected using a Niskin sampler onboard a ship (*R/V* Ieordo). The samples from the JH were collected at three sites (JH1, JH2, and JH3). The surface water samples (~0.5 m) from the JH were collected using a pump system onboard a ship. All water samples were stored in pre-cleaned 20 L polypropylene bottles and transported to the land-based laboratory without any treatment.

In the laboratory, the unfiltered and filtered (0.7 or 0.2 μm) water samples (~ 40 mL each) were stored in the pre-combusted amber vials at different temperatures (–20℃, 4℃, or 25℃), respectively. All the samples were triplicated. Thus, each sample was divided into 27 amber vial samples. The initial measurement was conducted within two day after seawater sampling. The measurement interval to examine the effect of storage time was 1, 3, 5, 7, 14, and 21 days, respectively, from the initial measurement.

2.3. Analytical protocols

FDOM fluorescence intensity was determined by a spectrophotometer (Aqualog, Horiba, USA). We used 10 mm path-length quartz cuvettes, which went through the signal-to-noise test. The measurement of ultra-pure water (milli-Q water, < 18.2 Ω) was performed at the start of FDOM analysis, and the result was considered as a blank value. The excitation-emission matrices (EEMs) were performed over the ranges of 240-700 nm in a 3 nm interval for excitation and 250-500 nm in a 5 nm

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

interval for emission. The integration time of EEMs was 5 seconds. The parallel factor analysis (PARAFAC) model was utilized through Solo software (Eigenvector Inc., USA) to identify and characterize key fluorescent components (Han et al., 2022). Before applying the model, corrections for the inner-filter effect (IFE) were conducted to reduce distortions in fluorescence measurements (Kothawala et al., 2013). To ensure the reliability of the extracted components, the model was assessed through split-half validation and random initialization, confirming its robustness (Bro, 1997; Stedmon and Bro, 2008; Zepp et al., 2004). Since fluorescence intensity is highly instrument-dependent, the intensity of FDOM was normalized by the Raman peak area using ultra-pure water to convert to Raman Unit (R.U.). The R.U. value represents the integrated area of the water Raman peak at an excitation wavelength of 350 nm (Lawaetz and Stedmon, 2009).

To compare the intensity of all samples, the PARAFAC model was applied to a single data set of the EEM data, including filter blank samples (distilled water and 0.1 M HCl) as well as open- and coastal-ocean samples, while considering filter pore sizes, storage time, and temperature for the seawater samples. Terrestrial humic-like peak (C peak, Ex/Em = 375/457 nm), marine humic-like peak (M peak, Ex/Em = 315/391 nm), and protein-like peak (T peak, Ex/Em = 270/313 nm) were identified by the PARAFAC model (Coble, 1996; Coble et al., 1998; Coble, 2007). The fluorescence spectra results were also compared with the OpenFluor spectra database (Murphy et al., 2014), yielding statistical matches with 28, 37, and 45 previous studies, respectively, at a confidence level of > 95%. The EEM contours and loading results from the PARAFAC model are presented in Figure 1.

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

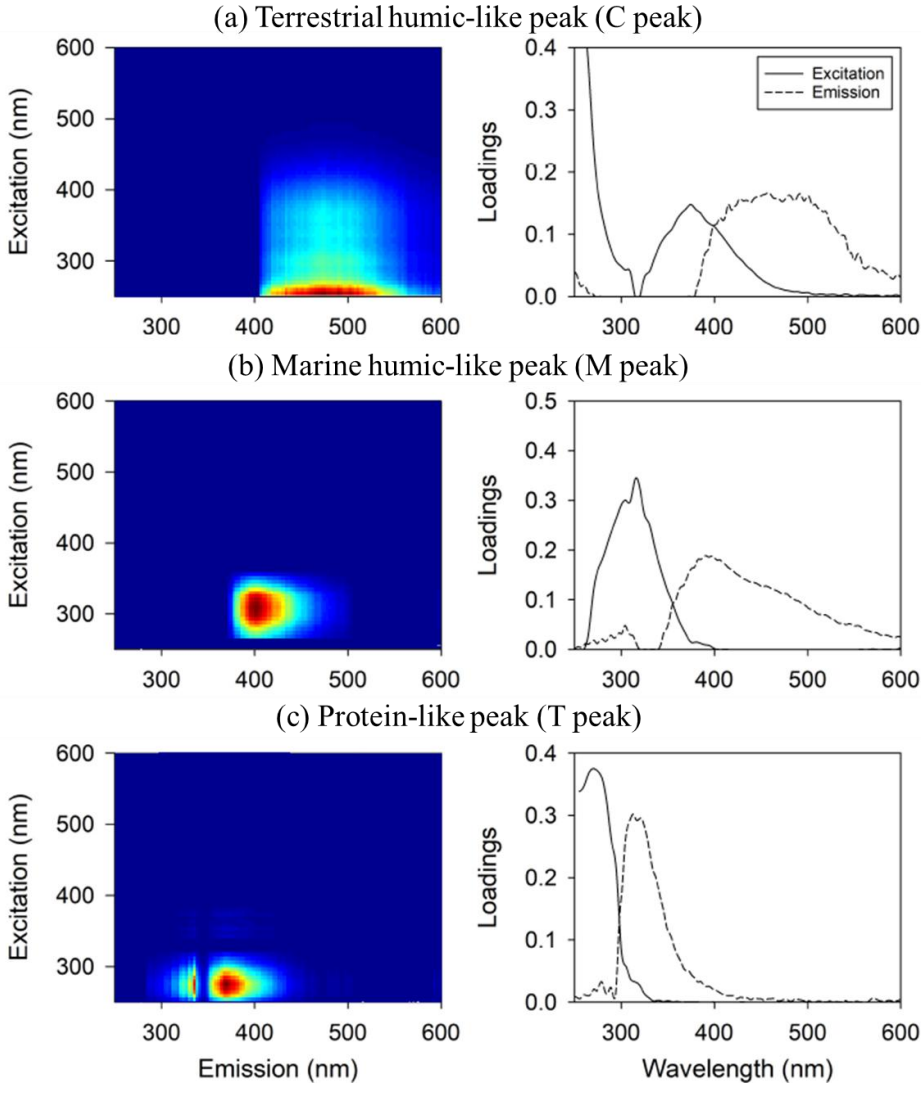

Figure 1: EEM contours and loading results from the PARAFAC model include: (a) terrestrial humic-like peak, (b) marine humic-like peak, and (c) protein-like peak.

3. Results

3.1. Filter blanks

During GF/F filtration, the filter blanks of the C and T peaks were negligible for both washing with distilled water and acid-treated filters (Fig. 2). Unlike the C and T peaks, a significant concentration of the M peak (up to 0.15 R.U.) was

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

observed in the GF/F filter before it was washed with 20 mL of distilled water, corresponding to up to 60% of the M peak in the coastal ocean (Station JH) samples. The acid-washed filter showed negligible filter blanks for the M peak.

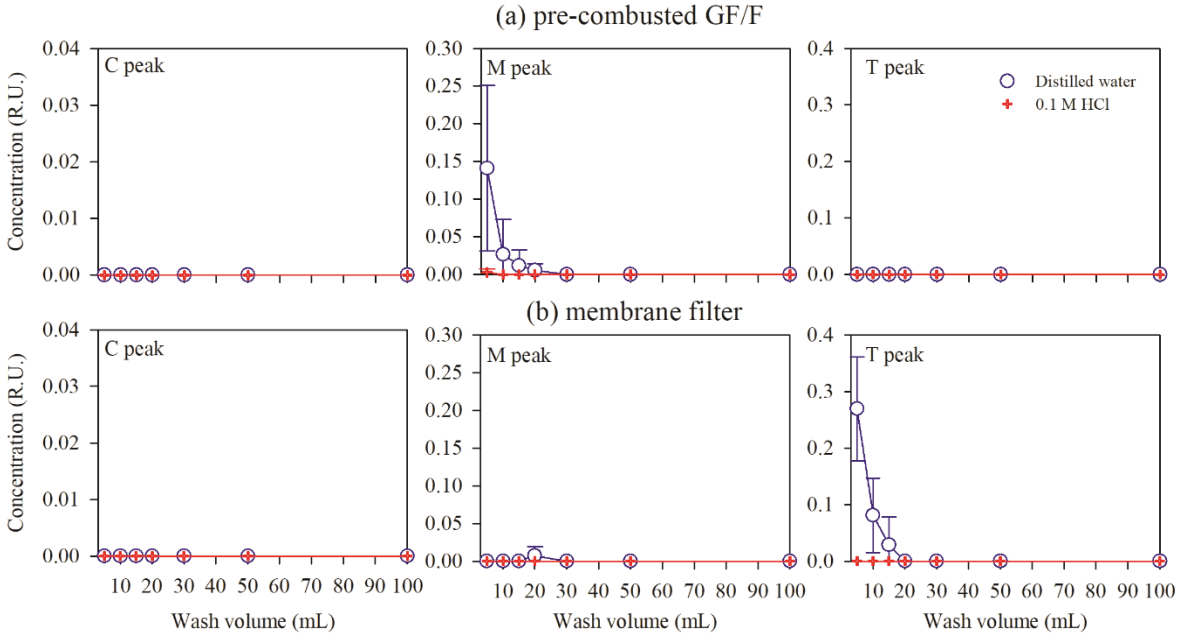

Figure 2: Filter blanks of FDOM concentrations for (a) pre-combusted GF/F and (b) membrane filters washing with distilled water or 0.1 M HCl.

During filtration with the membrane filter, humic-like peaks ($FDOM_H$; C and M peaks) showed negligible filter blank in both washing with distilled and acid-treated filters (Fig. 2). However, the T peak showed high concentrations from the filter which is washed with distilled water (up to 0.27 R.U.). The blank of T peak was almost 95% in the open ocean (Station EC1) samples. The acid-washed filter contained negligible filter blank for the T peak.

3.2. Filter pore sizes

The concentrations of $FDOM_H$ for unfiltered samples were similar to those for the filtered samples (0.7 μm) in the open ocean (97% ± 1%, $p > 0.05$) and coastal ocean (96% ± 4%, $p > 0.05$) (Fig. 3). The concentrations of T peak in unfiltered samples from the open ocean showed a slight difference with those in filtered samples (0.7 μm) (83% ± 9%, $p = 0.05$). Unlike the open ocean, the concentrations of T peak in unfiltered samples from the coastal ocean were 48% – 79% higher than those observed in filtered samples (0.7 μm or 0.2 μm), and this difference was statistically significant ($p < 0.05$).

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

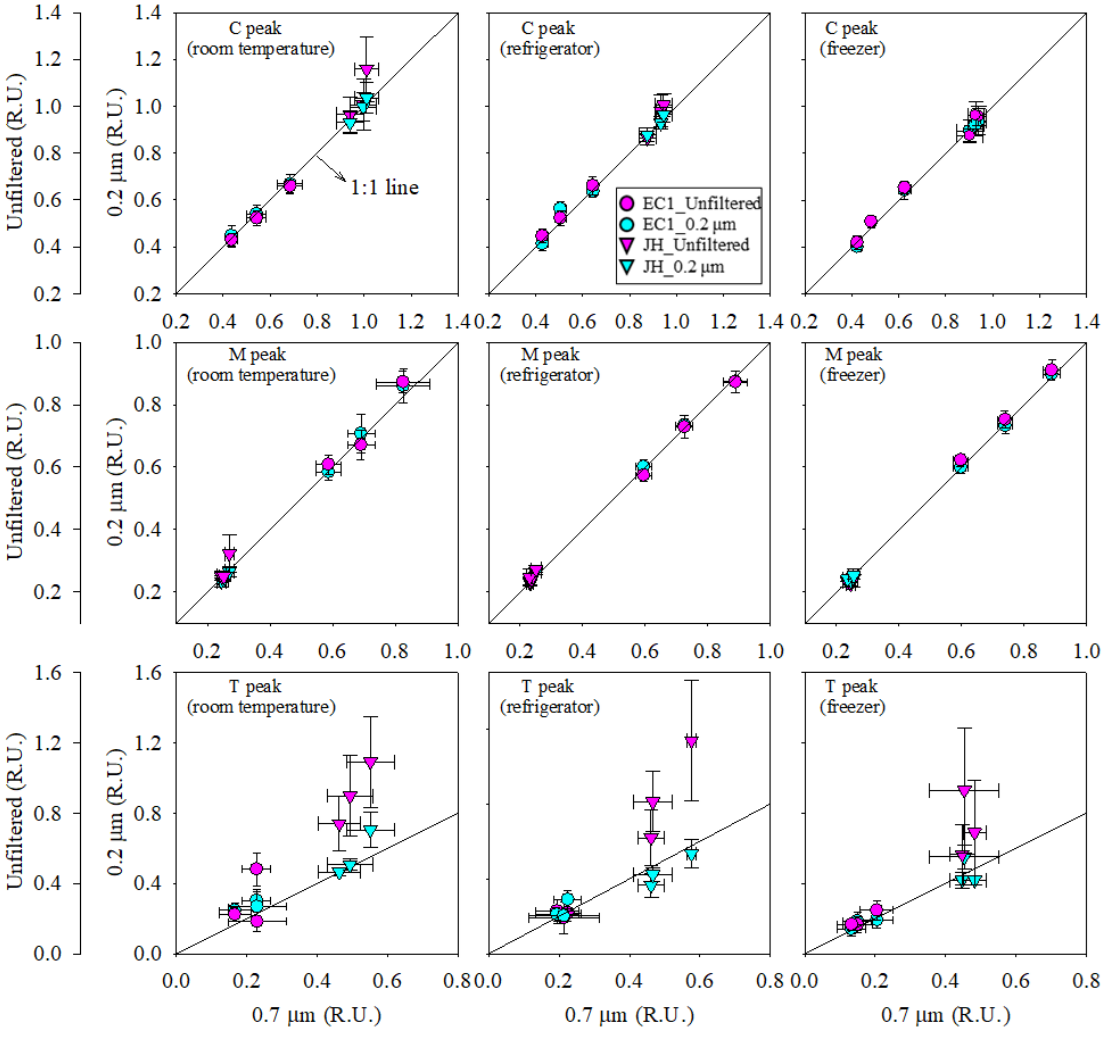

Figure 3: FDOM concentrations in samples obtained from the open ocean and the coastal ocean depending on the filter pore-sizes. Circles and triangles indicate the samples from the East Sea and Jinhae Bay, respectively. The solid line indicates the 1:1 line.

3.3. Storage strategies

The concentrations of $FDOM_H$ for unfiltered and filtered samples (0.7 and 0.2 μm) from the open- and coastal-ocean stored in the refrigerator (4℃) and freezer (–20℃) showed no clear differences between the initial and after 21 days measurements (8% ± 3%, $p > 0.05$) (Fig. 4). At room temperature, $FDOM_H$ concentrations in the unfiltered or filtered samples from the open- and costal-ocean also showed no clear differences within five days (7% ± 2%, $p > 0.05$) (Fig. 5).

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

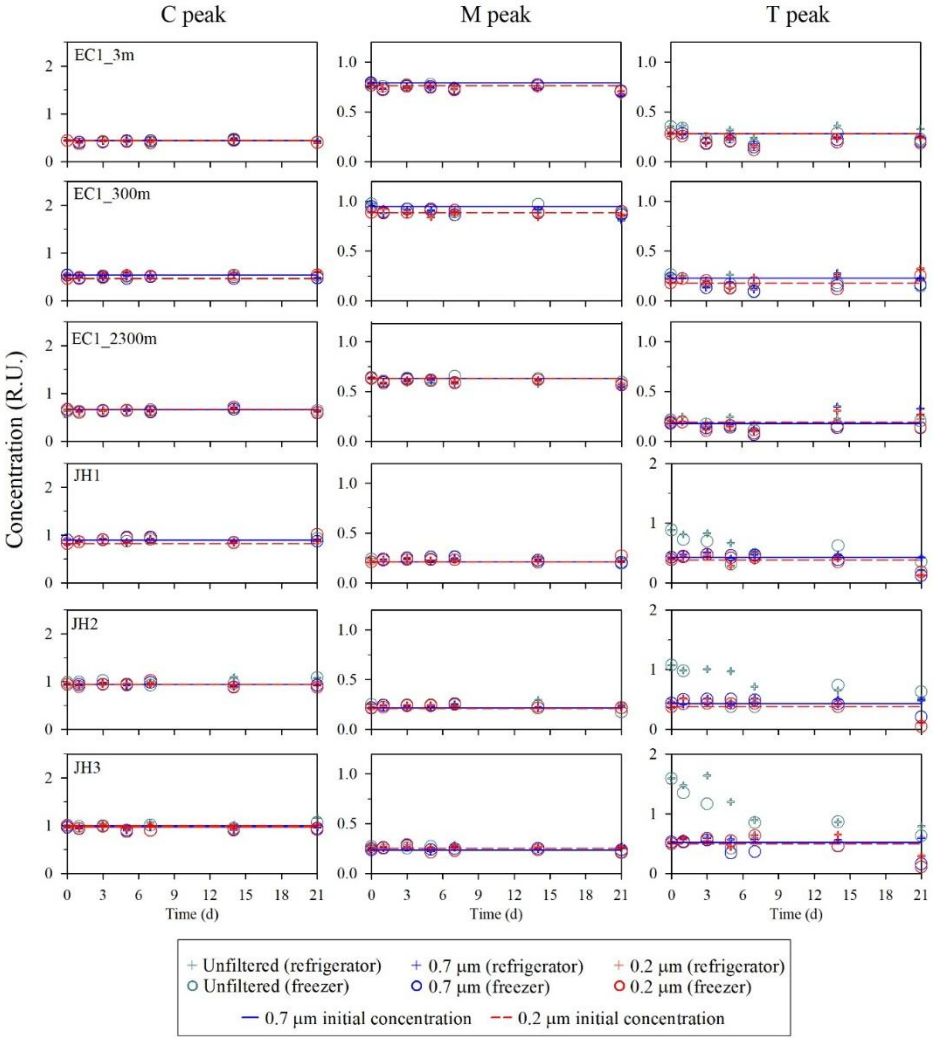

Figure 4: Changes in FDOM concentrations after stored in the refrigerator or freezer for samples obtained from the open ocean and the coastal ocean. Circles and crosses represent the refrigerator and freezer, respectively. Solid and dashed lines denote the initial concentration of FDOM after filtration with 0.7 µm pore size (blue) and 0.2 µm pore size (red) filters, respectively.

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

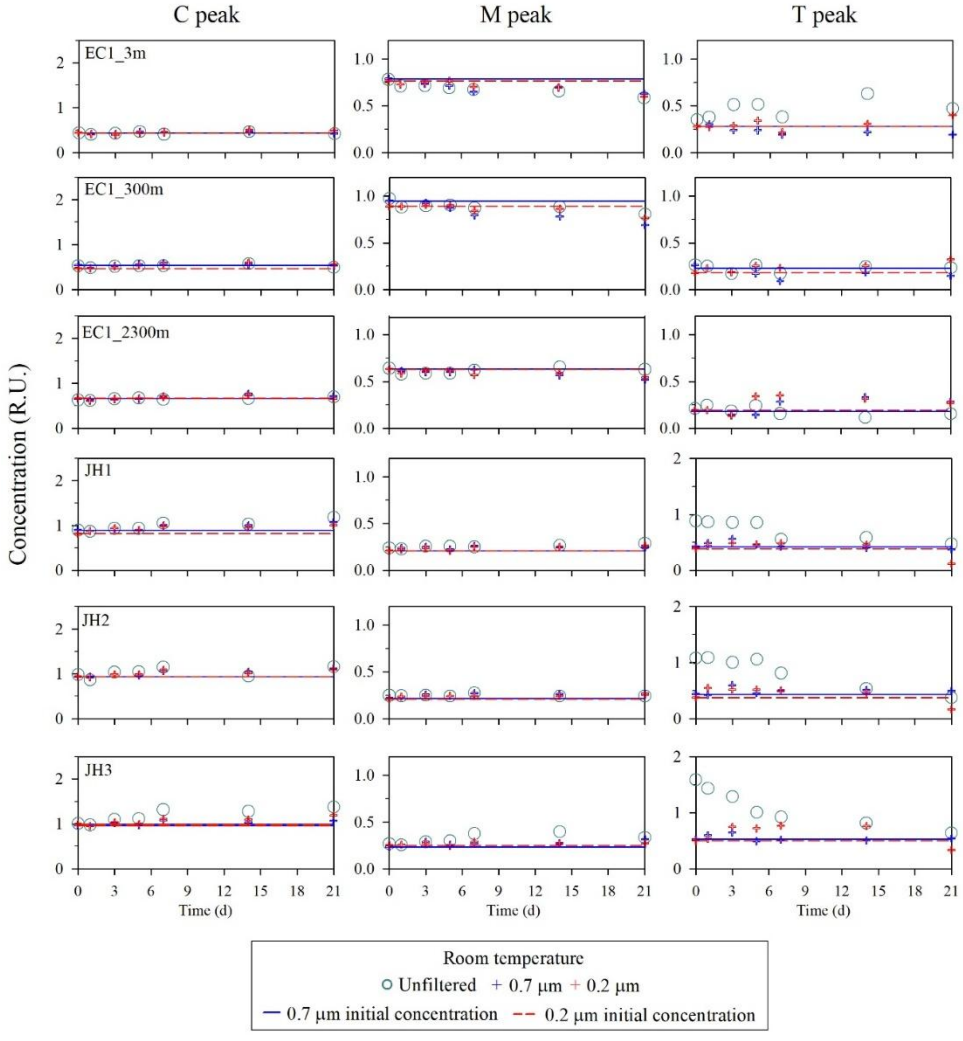

Figure 5: Changes in FDOM concentrations after stored at room temperature for samples in the open ocean and the coastal ocean. Circles and crosses represent the unfiltered and filtered samples, respectively. Solid and dashed lines denote the initial concentration of FDOM after filtering by 0.7 μm pore size (blue) and 0.2 μm pore size (red) filters, respectively.

However, regardless of the open- or coastal-ocean, the concentrations of T peak in unfiltered or filtered samples showed a variation after five days compared with the initial value (24% ± 5%, $p < 0.05$). The difference of T peak increased more significantly after 21 days in unfiltered (42% ± 3%, $p < 0.05$) and filtered samples (43% ± 15%, $p < 0.05$).

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

4. Discussion

The accuracy of FDOM measurements can be affected largely in the course of water filtration and sample storage. For water filtration, pre-combusted GF/F has been widely used for FDOM sampling due to its advantages in low DOM backgrounds after ignition, high flow rate, and large capacity. However, we observed high contamination of M peak even after washing with the distilled water. This seems to be produced by filter fiber particles from the ashed filter. This result suggests that GF/F should be washed with 20 mL of distilled water or 5 mL of 0.1 M HCl before the sample filtration for FDOM measurements. Although GF/F has such advantages, the large filter pore size (0.7 μm) can allow the passage of microorganisms or colloids (Tanoue, 1992), which could mislead the measurement due to the biological activity.

To prevent the effects of biological activity, the membrane filter with a 0.2 μm pore size has been often used for FDOM sampling (Rochelle-Newall and Fisher, 2002). However, we observed high blank values of the membrane filter for the T peak without pre-washing using 20 mL of distilled water or 5 mL of 0.1 M HCl. Our results from the blank test for different filters display that filter blank may introduce uncertainties in the measurement of FDOM in seawater. This is particularly noticeable for the M peak in open ocean samples and the T peak in coastal ocean samples. Therefore, careful pre-washing, including ashing processes, is necessary to prevent any contamination from filtration.

The concentrations of all FDOM components in the open ocean showed no significant difference between unfiltered and filtered samples (0.7 and 0.2 μm). This result suggests that the presence of particles in the sample did not significantly affect the fluorescent concentration in the open ocean. Thus, the filtration procedure is not necessary for measuring FDOM concentrations in open ocean waters. The measurement without filtration for open ocean waters is also advantageous in the fact that the contamination from filters can be avoided. However, in the coastal ocean, there are significant differences between unfiltered and filtered samples (0.7 and 0.2 μm) for the concentration of T peak, although no difference was observed for $FDOM_H$ concentrations. The high concentrations of T peak in unfiltered samples could be due to fresh protein-like organic component, which has relatively large particle sizes (Lin and Guo, 2020). These results imply that $FDOM_H$ measurements from any ocean environment can be performed without filtration. However, for T peak measurements, it is important to consider the size cutoff, especially for coastal water samples.

https://doi.org/10.5194/egusphere-2025-501
Preprint. Discussion started: 25 February 2025

For the sample storage, $FDOM_H$ in the open- and coastal-ocean waters can be preserved up to 21 days when stored in a refrigerator and freezer, regardless of whether samples were filtered or unfiltered (Fig. 5). However, at room temperature, we observed significant changes in $FDOM_H$ concentrations after five days. Thus, storage of $FDOM_H$ samples at room temperature for more than five days is not recommended in any sampling conditions. Unlike $FDOM_H$, the T peak showed significant changes within five days for any type of storage and filtration. These changes in T peak were presumably associated with rapid production and/or biodegradation of protein-like DOM. Thus, for the accurate measurement of the T peak, which is biologically labile, immediate measurements (< two days after samplings) is required.

5. Conclusions

We investigated the effects of filter blanks, filter pore sizes, and storage strategies for the measurement of FDOM using seawater samples from the open- and coastal-ocean. We observed high blank values of FDOM occurring from the filter without any pre-washing and ashing procedures. The $FDOM_H$ concentrations were not affected by filter pore sizes for both in the open- and coastal-ocean samples. However, filter pore sizes affected the T peak concentrations significantly, showing higher concentrations from 48% to 79% (unfiltered) in the coastal ocean samples than in the filtered samples. The concentration of $FDOM_H$ in seawater samples can be preserved for up to 21 days in a refrigerator or freezer, regardless of whether the samples are filtered or unfiltered. However, the concentration of T peak, even in filtered samples, rapidly decreased within five days regardless of storage temperature. Overall, if only $FDOM_H$ data are required, unfiltered samples can be stored in the refrigerator and measured within 21 days. However, for T peak measurements, filtrated samples should be immediately measured after sampling (< two days after sampling).

Data availability

The original contributions presented in the study are included in Supplementary Material, further inquiries can be directed to the corresponding author/s.

https://doi.org/10.5194/egusphere-2025-501
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Author Contribution Statements

JS and GK contributed to the conceptualization of the study. JS and HH performed sampling, experiments, and analyses. JS, IK, and GK were involved in the data interpretation and writing of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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