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RESEARCH PAPER

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UV-vis absorbance and chromophoric dissolved organic matter (CDOM) characterization of Sto. Tomas Cove surface water

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Abstract

Near Sto. Tomas Cove, multiple aquafarms were operational along the coastal area, specifically referred as the Techno Demo farms, which are monitored by the Bureau of Fisheries and Aquatic Resources via monthly water sampling. These Techno Demo farms are found in the coastal waters of Sto. Tomas and Rosario, La union. However, the dissolved organic matter (DOM) impact of these aquafarms in the coastal water are still unknown. Thus, in this study, to understand the DOM contribution of these aquafarms, physicochemical analysis and UV-Vis absorbance measurements were performed to derive spectral optical properties. The absorption spectra measurements were used to calculate the absorption coefficient, spectral slope coefficient and the specific UV absorbance which estimate the chromophoric DOM, the composition and structure of DOM, and the aromaticity, hydrophobicity, and molecular weight of DOM, respectively. Both physicochemical and spectral absorbance analyses were compared to understand the characteristics of dissolved organic matter found. The result was able to distinguish the difference between the two locations based on their spectral properties supported by their physicochemical properties. The phosphate and the TSS level were found related to the Napierian absorption coefficient at 254nm and 355nm, respectively. The study concluded that the aquafarms significantly have higher absorption coefficients due to high CDOM concentration than their respective control no take zones, indicating possible source mariculture pollution.

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Introduction

Over the last few decades, rapid development in global aquaculture industries is rampant because of the exponentially growing population in Asia and the Pacific (FAO, 2018). However, the accelerated development of the aquaculture industry brought threats to the coastal environment because of the disposal of organic waste and inorganic nutrients. If these aquaculture farms become improperly managed, continuous unregulated operation of these aquaculture farms may trigger severe pollution (Qin et al., 2005; Strain and Hargrave, 2005). Aquaculture pollution is contributed by various factors such as direct excretion, by-products of microbial metabolic activity, and excessive feeding aquafarms which resulted to highly enrich organic and inorganic substances in effluent (Brinker et al., 2005; Green et al., 2002; Sindilariu, 2007). These have direct negative impacts including eutrophication, water quality deterioration, and red tide downstream of the discharge outlet (Rosa et al., 2013). Aquaculturedriven eutrophication and environmental problems becomes a major concern because of its increasing frequency globally, on which a sustainable aquaculture operation is desired (Zhang et al., 2019).

In the coastal area of Lingayen Gulf, the aquaculture industry in the area had shown a microcosm in degradation and depletion of resources because of poor planning and mismanagement of resource utilization (Silvestre et al., 1989). In addition, various economic activities in the area had put a considerable stress on the resource system which includes the following triggers and/or issues: overexploitation, destructive fishing methods, siltation and sedimentation, and potential pollution from aquaculture runoff. Given the increasing population and intensifying economic activities, potential water pollution clearly exists. Despite the apparent problems and concern, the information necessary for proper management resources remains inadequate. Therefore, the area has been an appropriate site for an intensive study of coastal resource management plans.

The Department of Agriculture and the Bureau of Fisheries and Aquatic Resources stationed satellite

offices throughout the region to study the proper management of coastal resources. One of these satellite offices is in Casantaan, Sto. Tomas, La Union on which the regional mariculture technology demonstration center is found. The center conducts monthly monitoring of the general water parameters including dissolved oxygen (DO), temperature, salinity, pH, ammonia, nitrate, total solid suspended (TSS), and transparency (NEDA 1992). Such measures provide a glimpse of the water quality in the coastal area to safeguard public health and to assure the continuance of various coastal-dependent economic activities.

Dissolved organic matter (DOM) composition in water resources can provide important information about the pollutants which can pose risks to human health and to the ecosystem. Dissolved organic matter has optically sensitive fractions, which are characterized by light absorption and fluorescence, chromophoric-DOM (CDOM) termed as and fluorescent-DOM (FDOM), respectively. The spectral properties of CDOM and FDOM have been used to determine quantitative (e.g., the concentration of organic matter and pollutants) and qualitative (e.g., origins and molecular weight) simultaneously (Stedmon and Nelson 2015). Using the ultraviolet (UV) absorption of the organic solutes using a UV/Vis spectrum, the amount of organic carbon in the dissolved organic matter can be estimated (Jiang et al., 2020). In addition, the degree of humification and the aromatic constituents of the water samples can also be estimated using the UV-Vis absorbance values (Xu-jing et al., 2011; Santos et al., 2016). Colored dissolved organic matter (CDOM) parameters are known to be affected by aromaticity and molecular weights (Santos et al., 2016). Thus, CDOM and FDOM can be used to characterize DOM and large spatial scales of water quality monitoring. In addition, these optical analysis techniques are advantageous in terms of time (<30 mins) and cost.

Traditionally, the water quality of aquaculture is evaluated through laboratory analysis of the following parameters: chemical oxygen demand, biological chemical demand, and total organic carbon. The physicochemical analysis is a traditional and standard way to evaluate water quality. However, recent techniques such as ultra-high resolution mass spectroscopy, and Fourier-transform ion cyclotron resonance mass spectroscopy has enabled investigation of the molecular composition of organic pollutants (Kamjunke *et al.*, 2017). Yet, these chemical analysis techniques are costly and timeconsuming. Cadondon *et al.* (2021) suggest inclusion of the absorbance and fluorescence measurements to evaluate the water quality accurately.

In this study, UV- Vis absorbance measurements of the surface water of the Techno Demo Farm and control sites monitored by the Bureau of Fisheries and Aquatic Resources were carried out to estimate CDOM in the coastal-based aquaculture areas to understand the DOM behavior and origins from the aquafarms. The study focused on the spectral properties of CDOM in tracing anthropogenic DOM with carbon and nitrogen contents. The approach was based on the hypothesis that optical analysis can be applied to distinguish between the anthropogenic and natural origins of DOM (Kim *et al.*, 2021). The results of this study indicate that optical analysis has the potential as an efficient monitoring method for anthropogenic organic pollutants from aquafarms worldwide.

Materials and methods

Chromophoric dissolved organic matter (CDOM) largely contributes to the optical properties and the biological activity of marine waters.

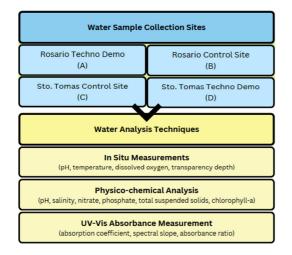


Fig. 1. Methodology flowchart.

CDOM characterization has been studied extensively and has been traditionally measured using UV-Vis spectroscopy for absorbance. The fig. below shows the procedural flowchart done in the study.

Collection of Surface Water Samples and In Situ Measurements

The sample collection proceeded similarly with the sampling site of BFAR monthly monitoring in the marine protected area. The four sampling sites were as follows: (A) Techno Demo, Rosario, La Union, (B) Rosario, La Union Control, (C) Sto. Tomas, La Union Control, and (D) Techno Demo, Sto. Tomas, La Union sites (shown in Fig. 3). The GPS location of the sampling sites were summarized below:

Table 1. GPS location of each sampling sites.

Coc	le Site	Latitude (°N)	Longitude (°E)
A	Techno Demo, Rosario, La Union	16.21995	120.39657
В	No Take Zone (Control Site) Rosario, La Union	16.22730	120.39042
С	No Take Zone (Control Site) Sto. Tomas, La Union	16.23682	120.38447
D	Techno Demo, Sto. Tomas, La Union	16.24269	120.38476

Sterilized polyethylene (PET) bottles were filled directly from the surface water of each site. A simple and standard grab water sampling method was performed during sample collection. Two liters of surface water sample was collected in each site -one liter for physico-chemical laboratory analysis, and one liter for UV-Vis absorbance measurement. The water samples were stored in a cooler with initial temperature of 10°C. The water collection was conducted from 8:30 am to 9:00 am of April 12, 2023 on a low-tide. In situ measurements such as temperature, dissolved oxygen, and pH were measured using YSI Pro 1020 (Xylem Analytics, Bellngham + Stanley, Inc.).

Physicochemical Analysis of the Surface Water Samples

After the surface water samples were collected, a liter of water sample for each site were submitted to the

BFAR NIFTDC, Bonuan, Dagupan City, Pangasinan for physico-chemical analysis. The water samples were submitted to the laboratory within the four (4) hours window for testing. The water sample were filtered using Whatman 47mm. The test parameters include the following: water pH, salinity, phosphate, nitrite, total suspended solids, and chlorophyll-a.

UV-Vis Absorbance Measurement of the Surface Water Samples

Some of the absorbance measurements were conducted in the Environmental Monitoring Unit, BFAR-NIFTDC, Bonuan, Dagupan City. The UV-Vis spectrophotometer in the facility is a Spectro UV-Vis Dual Beam with 8 Auto Cell reading (UVS-2700, Labomed, Inc.).

The cuvette used was a 10mm glass/ quartz cuvette. Absorbance at various wavelength (i.e., 240nm, 254nm, 260nm, 280nm, 310nm, 275nm, 295nm, 350nm, 400nm, 355nm) was measured for each sample. Six (6) readings were measured for each sample. Distilled water was the control cell for the UV-Vis spectroscopy.

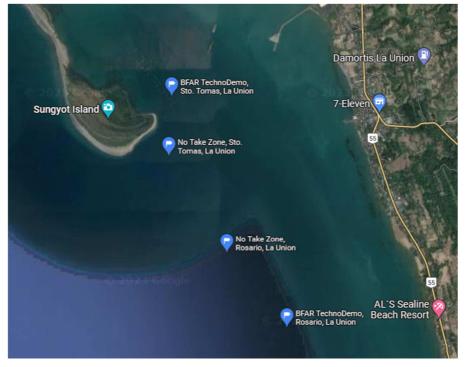


Fig. 2. Map showing the location of the four sampling sites.

In addition, absorbance measurements of the water samples were scanned through the wavelength range of 350nm to 700nm using the Spectronic 200 (Thermo Scientific, Inc.) and a 10 mm glass/quartz cuvette. Increments of 10nm were the data points for the absorbance measurement scan.

Results and discussion

In Situ Measurements of the Surface Water

Upon surface water collection, in situ measurements such as temperature, pH, dissolved oxygen (DO) and transparency depth were measured (~1.0m depth) as shown in the Table 2.

Table 2. In situ measurements of each sampling sites.

Code	pН	Temperature (°C)	DO (mg/L)	Transparency depth (m)
A (TD-RLU)	8.56	28.7	9.4	3.0
B (CS-RLU)	8.58	28.7	8.9	3.8
C (CS-STLU)	8.60	28.8	9.7	3.0
D (TD-STLU)	8.64	28.6	8.9	3.0

Physico-chemical Analysis of the Surface Water Samples

The water samples collected were transported immediately to the BFAR NIFTDC Environmental Monitoring Unit, Bonuan, Dagupan City, Pangasinan. Before any test were conducted, the water samples were filtered through a 0.45 μ m filter. The summary of the physico-chemical analysis was presented in the Table 3. The samples collected were from the BFAR Techno Demo at Rosario, La Union (TD-RLU) and Sto. Tomas, La Union (TD-STLU), and from

their no take zone for both towns (*i.e.*, CS-RLU & CS-STLU). From the physico-chemical analysis report, it is evident that the surface water collected from Rosario, La Union has lesser total suspended solids than the surface water collected from Sto. Tomas, La Union.

Table 3. Physico-chemical analysis report from BFAR-NIFTDC.

Code	pН	Salinity (ppt)	Nitrite (mg/L)	Phosphate (mg/L)	TSS (mg/L)	chlorophyll-a (µg/L)
A (TD-RLU)	8.11	33	0.017	0.020	36.80	0.001
B (CS-RLU)	8.12	33	0.023	0.018	35.80	0.002
C (CS-STLU)	8.10	33	0.015	0.039	41.20	0.001
D (TD-STLU)	8.08	33	0.019	0.027	42.20	0.002

UV-Vis Absorbance Measurement of the Surface Water Samples

From the UV-Vis absorbance measurements, the spectral properties of the CDOM present in the water samples were calculated using absorption coefficient, spectral slope, absorbance ratio, and specific UV absorbance. The CDOM concentration, DOM composition and molecular weight, humification degree of DOM, DOC/ TOC concentration, aromaticity, and hydrophobicity were estimated.

Estimation of the concentration of CDOM using intensity of a lambda at specific wavelength

Some of the filtered water samples were prepared for the absorbance measurement using an eight (8) Auto Cell Spectro UV-Vis Dual Beam (UVS-2700, Labomed, Inc.) spectrophotometer at BFAR NIFTDC, Bonuan, Dagupan City. Based on Zhang *et al.* (2022), the chromophoric dissolved organic matter (CDOM) is represented by the absorption coefficient at 355nm, α (355nm). The absorption coefficient is calculated using Equation 1.

The average α_{355} for the marine end members of the four sampling sites are shown in the fig. 4 below. The salinity for all sites was high with a value of 33. The CDOM abundance of the four sampling sites was higher at Sto. Tomas, La Union ($\alpha_{355} = 7.139 \text{ m}^{-1}$, 7.408 m⁻¹) than Rosario, La Union ($\alpha_{355} = 5.450 \text{ m}^{-1}$, 5.719 m⁻¹). The absorption coefficient at 355nm for

the no take zone and Techno Demo farm were comparatively little variance. No take zones had higher absorption coefficients than their respective techno demo farms.

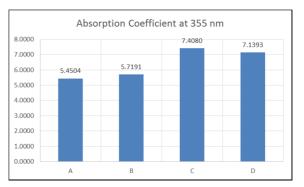


Fig. 3. Estimated CDOM concentration per site using the absoption coefficient at 355nm wavelength.

Shape of the Absorption Spectrum

The absorbance of each sample was measured from 350nm to 600nm using the Spectronic 200 (Thermo Scientific, Inc.). The data interval was increments of 10nm. The measured absorbance was then used to compute for the equivalent absorption coefficient. The calculated absorption coefficient was then plotted to analyze the shape of the absorption spectrum which can estimate the changes in the CDOM composition in each sample.

The fig. 5 below shows the absorption spectrum shape from the four sites and control which is the distilled water. The absorption spectra obtained from the four sampling sites indicate that the ascending order of the sampling sites based on their absorption spectra were as follows: (1) Techno Demo Rosario (A), (2) No Take Zone Sto. Tomas (B), (3) No Take Zone Rosario (C), and (4) Techno Demo Sto. Tomas (D).

Based from the work of Helms *et al.* (2008), two general trends were found. One is that the lower salinity upriver samples contained more high molecular weight (HMW) chromophoric dissolved organic matter (CDOM) relative to low-MW (LMW) CDOM than the high-salinity bay sample. Secondly, the percentage of CDOM in the LMW fraction in all three sampling sites increased upon light exposure, whereas in the HMW fraction, it decreased. This implicates that a shift from HMW to LMW CDOM is observed due to increased light exposure and salinity.

From these initial results and work of Helms *et al.* (2008), this result implies that the salinity and light exposure is responsible for the absorption spectra of the samples. Looking into the physico-chemical analysis report, the salinity of all sampling sites was 33 ppt. Thus, the contribution of salinity in the absorption spectra is negligible in this case, rather the amount of the chlorophyll-a can be associated to the absorption spectra. Sampling sites A and C had 0.001µg/L of chlorophyll-a, while sites B and D had 0.002µg/L.

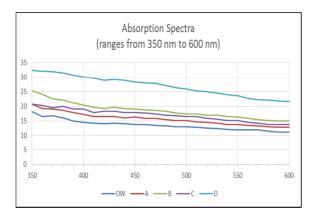


Fig. 4. Absorption spectra obtained from four sampling sites: (A) Techno Demo Rosario, (B) No Take Zone Rosario, (C) No Take Zone Sto. Tomas, (D) Techno Demo Sto. Tomas.

Estimation of DOC Values using Absorbance Properties of Water

According to various studies (Laudon *et al.*, 2004; Waterloo *et al.*, 2006), different wavelengths can be used to estimate DOC concentration from the spectral absorbances. Laudon *et al.* (2004) found that the absorbance from 254nm could explain 78% to 97% of TOC concentration in seven streams. Meanwhile, Waterloo *et al.* (2006) used absorbance at 255 and 350nm as proxies for estimating DOC with calibration using a TOC analyser.

Calculating the absorption coefficient at 254nm wavelength, the estimated DOC concentration is shown below. It can be inferred that the DOC levels at sampling site A and B do have the same value and quite lesser than the estimated DOC level at sampling site D and C.

The absorbance ratio of 350nm and 254nm estimates the proxies of DOC per site (Cadondon *et al.*, 2021), as shown in the fig. below. This implies that the DOC concentrations Dissolved organic carbons (DOCs) are known to play a primary role in the UV absorbance in aquatic ecosystems. In addition, the study of Heymann *et al.* (2017) relates the DOC concentration to the promotion of phosphate in leachate. Phosphate concentrations in excess of 0.024mg/L are related to eutrophication which happens in water bodies with low levels of nutrient contamination.

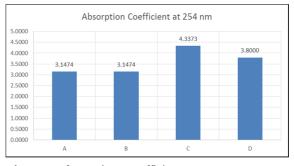


Fig. 5. Absorption coefficient at 254nm as an estimation of the DOC values.

From the physicochemical analysis, the phosphate concentration levels of the four sampling sites were as follows: (A) 0.020mg/L; (B) 0.018mg/L; (C) 0.039mg/L; and (D) 0.027mg/L. Indirectly, the DOC

concentration estimation using the 254nm absorption coefficient and the phosphate concentration levels were proportional. Therefore, eutrophication can be observed in sampling site C and D, which were at the Sto. Tomas waters, indicating low level of nutrient contamination.

Estimation of Color Intensity, Molecular Weight, Aromaticity using Carbon-Specific Absorption or Specific Absorption Coefficient

DOM Composition and Molecular Weight of the Surface Water Samples

According to Helms et al. (2008), Fichot et al. (2012), and Xido et al. (2013), the dissolved organic matter (DOM) composition and molecular weight can be determined by the spectral slope $(S_{275-295})$ at 275nm (as reference wavelength) and 295nm. It may indicate the DOC-normalized yield of dissolved lignin present in an ocean-influenced by rivers -estimating the DOC fraction of terrigenous origin (Fichot & Benner, 2011; Fichot & Benner, 2012; Helms et al., 2008, Opsahl & Benner, 1997). It may also indicate the photobleaching activity and the microbial alteration of DOC in estuarine and coastal environments (Helms et al., 2008; Ficher & Benner, 2014). The calculated absorption coefficient at 275nm and 295nm were shown in Table 4, as well as the calculated spectral slope $(S_{275-295})$.

Table 4. Calculated absorption coefficients andspectral slope at 275nm and 295nm wavelengths.

Code	$\alpha(275nm)$ (m ⁻¹)	α(295 <i>nm</i>) (m ⁻¹)	<i>S</i> _{275–295} (nm ⁻¹)
A (TD-RLU)	2.533	1.842	0.016
B (CS-RLU)	2.533	1.727	0.019
C (CS-STLU)	3.570	2.994	0.009
D (TD-STLU)	2.956	2.226	0.014

The spectral slopes for the control site at Rosario, La Union and Sto. Tomas, La Union were 0.019 and 0.009, respectively. This implies that the samples from the no take zone of Sto. Tomas, La Union have higher CDOM concentration than the samples from the no take zone of Rosario, La Union as reflected in the absorption coefficients at 275nm and 295nm wavelengths.

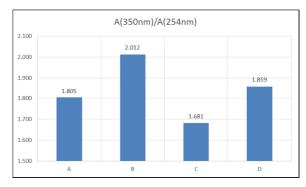


Fig. 6. Absorbance ratio of 350nm and 254nm as an estimation of the DOC values.

Alternatively, the molecular weight of the dissolved organic matter (DOM) can be estimated given as the ratio of absorbance at 250nm and 365nm, or simply referred as E_2/E_3 . This ratio indicates the molecular size of the CDOM. When E_2/E_3 ratio decreases, it implies that the CDOM of macromolecules gets larger in size. This happens because CDOM tends to absorb longer wavelengths more easily (Dorado *et al.*, 2003).

In this study, instead of the absorbance measurement of 250nm and 365nm to calculate the E_2/E_3 ratio, the absorbance measurement of 254nm and 355nm were used. The molecular size of the CDOM can still be estimated with these values, as shown in Table 5.

Table 5. Absorbance measurement at 254nm and 355nm and the computed E_2/E_3 ratio.

Code	A(254nm)	A(355nm)	E_2/E_3
A (TD-RLU)	0.014	0.024	0.577
B (CS-RLU)	0.014	0.025	0.550
C (CS-STLU)	0.019	0.032	0.585
D (TD-STLU)	0.017	0.031	0.532

Humification Degree of Dissolved Organic Matter Present

Another characteristic information that can be inferred from absorbance measurement is the humification degree of the DOM which is determined by the spectral slope ($S_{350-400}$) at 350 and 400nm (Hansen *et al.*, 2016). However, Loiselle *et al.* (2009) reported that the spectral slopes at 370nm have similar peak positions to the reference humic and fulvic acids.

The calculated absorption coefficient at 350nm and 400nm were shown in Table 6, as well as the calculated spectral slope ($S_{350-400}$).

Table 6. Calculated absorption coefficients andspectral slope at 350nm and 400nm wavelengths.

Code	$\alpha(350nm)$ (m ⁻¹)	$\alpha(400nm)$ (m ⁻¹)	$S_{350-400}$ (nm ⁻¹)
A (TD-RLU)	5.681	5.374	0.0011
B (CS-RLU)	6.333	6.640	-0.0009
C (CS-STLU)	7.293	6.333	0.0028
D (TD-STLU)	7.063	6.909	0.0004

In connection, the humification degree of the DOM molecule can be characterized by the ratio of absorbance at 350nm and 400nm, or simply referred as E_4/E_5 . The humification degree can be described either humic acid or fulvic acid.

The DOM is said to be mainly composed of humic acid when the E_4/E_5 is less than 3.5; otherwise, it is mainly fulvic acid (Artinger *et al.*, 2000). Humic acid is a mixture of acids that were formed due to the decay of both plants and animals. It may act as an effective agent in sequestering pollutants found in terrestrial and aquatic environments. It may form small solid pieces when a strong base extract is acidified. On the other hand, fulvic acid remain dissolved when a strong base is acidified. It is soluble at all pH levels and has a lower molecular weight due to its higher oxygen content. It is commonly found in soils, compost, marine sediments, and sewage. It is believed as a product of microbial metabolism.

Table 7. Absorbance measurement at 310nm and 400nm and the computed E_4/E_5 ratio.

Code	A(310nm)	A(400nm)	E_{4}/E_{5}
A (TD-RLU)	0.026	0.023	1.100
B (CS-RLU)	0.029	0.029	1.006
C (CS-STLU)	0.032	0.028	1.145
D (TD-STLU)	0.031	0.030	1.044

In calculating the E_4/E_5 ratio, the absorbance measurement of 300nm and 400nm were used

(Artinger *et al.*, 2000). However, absorbance measurement at 310nm is used rather than 300nm. Thus, the humification degree of DOM can be estimated with these values, as presented in Table 7.

As expected, the humification degree of the surface water samples were less than 3.5 which indicates that the DOM present were mainly composed of humic acid. There was comparatively large variability of the average S_R values for Techno Demo farms and the no take zones of Sto. Tomas and Rosario, La Union (as shown in the fig. below). The S_R values of the no take zones of Sto. Tomas and Rosario, La Union were 0.5714 μ m⁻¹ and -2.5714 μ m⁻¹, respectively. While the S_R values of the Techno Demo farms in Sto. Tomas and Rosario, La Union were (5.3333 μ m⁻¹ and 2.1429 μ m⁻¹) than their respective no take zone.

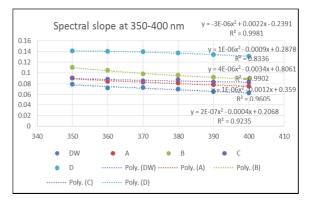


Fig. 7. Nonlinear regression plot of the absorbance from 350-400nm with its equation and r-squared value.

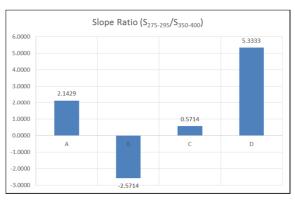


Fig. 8. Slope ratio of each sample.

The study found that the concentration levels of CDOM were higher for the techno demo farms than those of the no take zones.

This implies that the mariculture industry activities do contribute to the marine pollution in terms of the CDOM levels.

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