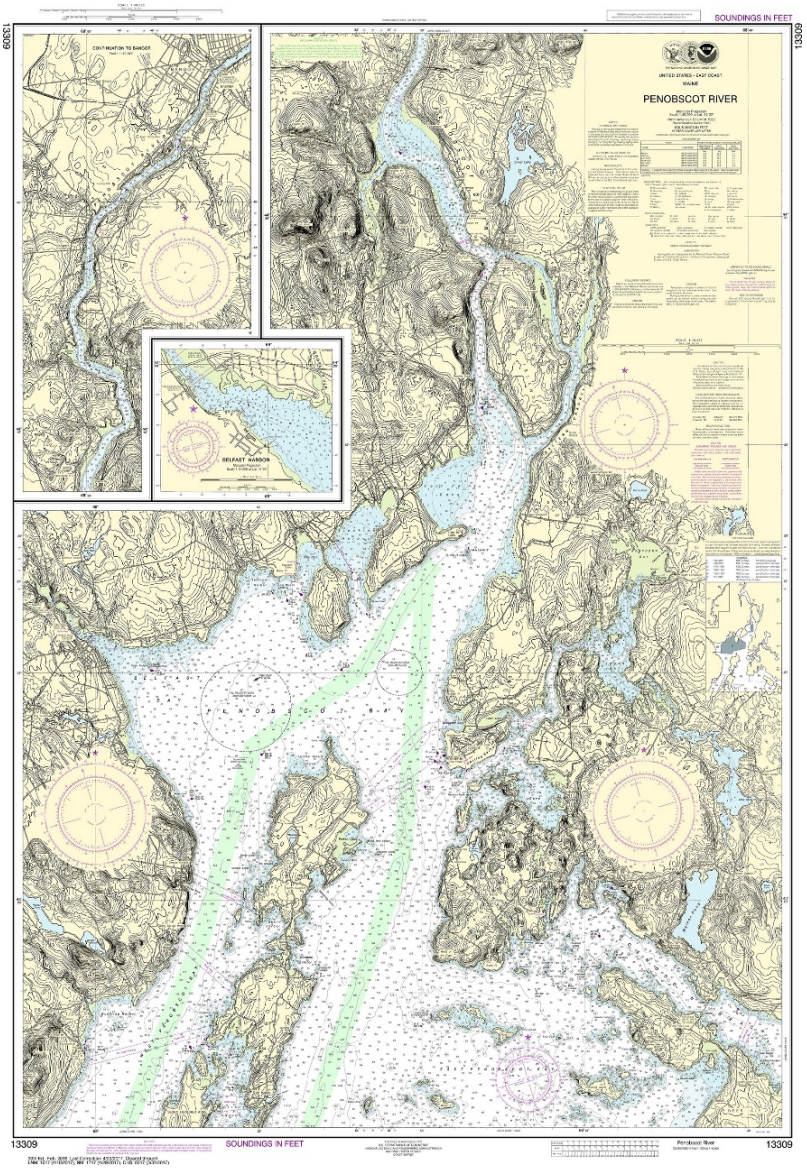

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Characterizing Dissolved Organic Matter in Penobscot Bay, ME, in Relation to Weather Events

Ellie Gellerson

Corning School of Ocean Studies, Maine Maritime Academy

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Abstract

Dissolved Organic Matter (DOM) is fundamental to marine ecosystems, as it forms the foundation of marine ecosystems. Due to DOM's vital role in ecosystem health and function, small changes in the DOM pool can result in much larger changes to overall ecosystem dynamics. With storm intensity and frequency expected to increase with progressing climate change, it is pertinent to understand how DOM is reacting to current weather events in order to make predictions about its behavior under future conditions. This study identified DOM components present in a tidal estuary system in Penobscot Bay, ME, through water sample collection along a three station salinity transect over a 5-week study period. Samples were analyzed through EEM-PARAFAC analysis, which determined three unique components present in the system: two fulvic acid like components, and one aromatic protein. The relative abundance of each of these components were then analyzed in relation to precipitation (mm), river discharge (m^3 s^{-1}) rates of the adjacent Penobscot River, and physical parameters that were collected at each station (temperature, salinity, dissolved oxygen, etc.). DOM component 2 (C2) was identified as a terrestrially sourced fulvic-acid and shown to increase in abundance following a major precipitation event. Components 1 and 3 (C1 and C3) were identified as a general fulvic acid and tryptophan like component, respectively, and require more investigation to understand the drivers of their distribution and abundance within this system. This study concluded that C2 of the DOM pool in Penobscot Bay, ME, is increasing in response to precipitation events, while the abundance of other components are controlled by other factors.

Introduction

Dissolved Organic Matter (DOM) is the most abundant form of carbon on Earth (e.g. Dupouy et al. 2020; Asmala et al. 2021). DOM is the fraction of organic matter suspended in water that can pass through a submicron (typically 0.77 μm) filter. This class of organic material holds a variety of constituents, varying from amino acids and vitamins to larger molecules like proteins and different carbohydrates, as well as less understood components such as humic materials and microbial byproducts (Repeta 2015). The DOM pool also holds approximately 97% of the ocean's carbon, is the largest sink for atmospheric CO_2, and has been identified as a major source of nutrients for biological activities (Parlanti et al. 2000; White and Roesler 2014; Dupouy et al. 2020). Therefore, analyzing the constituents of the DOM pool and their spatial-temporal variation can provide a more comprehensive understanding of ocean nutrient cycling, the global carbon cycle, and environmental responses to climate change (Benner 2002; Hedges 2002; Ridgewell and Arndt 2015). In marine systems, DOM is created as a byproduct of various biological processes and cell lysis whereas coastal zones have additional inputs from terrestrial runoff carrying organic material from soils and anthropogenic processes (Carlson 2002; Dupouy et al. 2020). Because of its importance in understanding many facets of ocean dynamics and global cycling, identifying DOM components and their influence in smaller systems can provide insight on the impact to the global ocean.

With increases in extreme weather events (such as hurricanes and cyclones), precipitation, and river influx expected with climate change, understanding the DOM pool's response to changing conditions is pertinent for predicting changes in carbon cycling and ecosystems (Rabalais 2009; Statham 2011; Seneviratne et al. 2012; White and Roesler 2014; Dupouy et al. 2020). With the Gulf of Maine (GoM) being a specific area of interest for climate

science, understanding how the DOM pool responds to current weather events from climate change may provide insight into the global oceans' future conditions (Dupouy et al. 2020; Asmala et al. 2021). With climate change impacts on the horizon, storm intensity and frequency are expected to increase because of higher contrasts in temperatures and changes in wind patterns, as well as changes in precipitation amount and intensity (Seneviratne et al. 2012). With these increases in the hydrologic cycle, terrestrial runoff will increase, and ultimately, river influx into coastal and estuarine regions will as well (Asmala et al. 2021). Since this runoff and freshwater influx will carry a variety of DOM, it is important to understand the current dynamics to make predictions about the fluctuations as a result of climate change (White and Roesler 2014; Huntington et al. 2016; Dupouy et al. 2020).

Recent research has shown that the DOM pool shifts in relation to precipitation and major storm events (Dupouy et al. 2020; Asmala et al. 2021). Coastal systems are most susceptible to shifts in the DOM pool because of their interactions with river inputs and terrestrial runoff – these inputs carry DOM that can enter the system intermittently, and in pulses following storm events that can persist in the environment (Huntington et al. 2016; Dupouy et al. 2020; Asmala et al. 2021). These pulses of DOM can carry large amounts of carbon and nutrients which fuel microbial growth that can then lead to eutrophication of waters and algal blooms (Fellman et al. 2008; White and Roesler 2014; Asmala et al. 2021). Following major storm events, dissolved organic carbon (DOC), a fraction of the DOM pool, increases significantly in estuarine systems, ranging from a 26% to 400% increase in estuarine streams depending on site location, intensity of the storm event, and time of year (Fellman et al. 2008). Another study observed a significant increase of terrestrial humic components following precipitation, as well as an increase in a protein-like component (Dupouy et al. 2020). The protein component was also found to increase

following higher wind levels during stormy weather. Dupouy et al. (2020) inferred that this was a result of increased biological activity following mixing of the water column and subsequent mixing of nutrients, through wind-induced mixing. Many of these changes can be traced back to the hydrologic cycle because hydrology is the primary transport method for DOM entering the water through coastal and estuarine ecosystems. Asmala et al. (2021) showed that following storm events, which increases groundwater runoff and river discharge rates, pulses of organic carbon delivered from terrestrial sources persist in coastal and estuarine environments for an average of 55 days. It is important that these pulses of DOM are accounted for when configuring carbon and nutrient budgets for coastal and global oceans.

One of the best ways to quantify presence and fluctuation of DOM in different ecosystems it through utilization of the optical properties of water samples (Blough and Vecchio 2002; Stedmon and Nelson 2015). The proportion of DOM that contributes to ocean color and has optical properties is called Chromophoric or Colored Dissolved Organic Matter (CDOM), and a smaller portion of the CDOM pool that fluoresces is referred to as Fluorescent Dissolved Organic Matter (FDOM) (Blough and Vecchio 2002; Stedmon and Nelson 2015). These chromophores and fluorophores, CDOM and FDOM molecules and compounds, can be used as optical markers to determine categories and sources of DOM components that are present in seawater and consequently shed light on biological and coastal mixing processes, along with general seawater optics (Parlanti et al. 2000; Stedmon and Nelson 2015). FDOM is of special importance when looking to identify DOM components because fluorophores excite at specific wavelengths of light and subsequently emit other characteristic wavelengths of light. This behavior of fluorophores results in a spectrum of fluorescence intensity under specific excitation and emission wavelengths that can be graphed in Excitation Emission Matrices (EEMs). These

matrices plot excitation, emission, and fluorescence intensity across a range of wavelengths in a 3D format; this generates peaks on 3D graphs that are unique to the DOM components present in the water, allowing them to be identified through spectrofluorometry (Fig.1) (Stedmon and Nelson 2015; Murphey et al. 2013).

The excitation and emission spectra of water samples, in conjunction with their fluorescence intensity, can be used to identify what DOM components are present in the sample, and their relative abundance (Murphey et al. 2013; Dupouy et al. 2020). The decomposition of the overall fluorescence of a water sample into its specific DOM components is done through Parallel Factor Analysis (PARAFAC). PARAFAC is an analysis technique for three way data that assumes the overall fluorescence of samples is the summation of several different fluorescence spectra of individual fluorophores (Bro, 1997; Murphey et al. 2013). This allows for the dominant sources of DOM to be identified based on their relative contribution to overall fluorescence. These FDOM analyses can be used as proxy measurements for changes in the overall DOM pool because of FDOM's closely tied relationship to the DOM pool (Murphey et al. 2013). This means that with environmental and time series data, such as river flux, precipitation, salinity, and chlorophyll concentrations, FDOM analyses can provide information on DOM fluctuations, changing composition, and drivers of DOM cycling in specific ecosystems (Parlanti et al. 2000; Yamashita et al. 2008; Dupouy et al. 2020). Typical components identified through FDOM analysis reveal protein-like and humic-like components and furthermore, humics can be traced to either a marine or terrestrial source (Stedmon and Nelson 2015). Understanding the origin and type of DOM components present provides insight into carbon and general nutrient cycling. Presence of terrestrial humic-like components, which have excitation and emission maximums between 250-400 nm and 375-550 nm, respectively, can indicate the

magnitude of the effect of terrestrial nutrient sources on an ecosystem. Whereas the presence of marine humic-like components indicates that the degradation of marine organisms is actively occurring (Parlanti et al. 2000; Yamashita et al. 2008). Protein-like components, which have excitation and emission maximums between 200-250 nm, and 200-372 nm, respectively, indicate that biological activity is present and active, as these protein-like components are generated as byproducts of many metabolites and organismal functions (Parlanti et al. 2000; Yamashita et al. 2008). Understanding the types of DOM that are dominant and fluctuating within systems provides insight into estuarine and watershed dynamics, carbon cycling, potential nutrient sources, and the biological activity in studied areas (Fellman et al. 2008; Dupouy et al. 2020; Asmala et al. 2021).

The GoM has been a specific area of research concerning changes in the DOM pool because of its demonstrated susceptibility to climate change, especially since it is warming at a faster rate than 99% of the world's oceans (Pershing et al. 2015). This warming can lead to fluctuations in precipitation amounts and intensity, as well as increases in storm intensity and frequency because of increased evaporation and temperature contrasts between currents and air masses (Seneviratne et al. 2012). A study examining the DOC export into the GoM found that changes observed over the last century had been related primarily to changes in the hydrologic cycle and showed little interaction with other variables such as warming temperature or biological activity (Huntington et al. 2016). White and Roesler (2014) examined DOM in four rivers draining into the GoM and found that the three terrestrial humic components identified increased following the spring snow melt. An identified protein-like component was likely sourced from a phytoplankton bloom because the component's fluorescence signal rose when the phytoplankton bloom reached its end and cell lysis began to occur (White and Roesler 2014).

Since the samples analyzed by White and Roesler (2014) were focused on areas of the Penobscot River with very low salinities, the DOM in Penobscot Bay may have slightly altered compositions and dynamics because of increased biological activity, higher salinities, and general differences between estuarine and marine environments.

This study will attempt to identify components and fluctuations of DOM in relation to weather events in Penobscot Bay, ME. To identify DOM components present, water samples were taken from three stations along a salinity transect from both the surface and 10m depth. They were then analyzed by a spectrofluorometer to generate excitation-emission matrices (EEMs) and use Parallel Factor Analysis (PARAFAC) to identify DOM components that were present in the samples. The seawater samples were collected weekly, along with corresponding hydrologic, oceanographic, and meteorological data to observe fluctuations in environmental conditions that may have caused changes in the DOM pool over the study of this period. The objectives of the study were to identify which DOM components are present in Penobscot Bay, as well as to begin to understand if and how weather events such as rain or drought, and changes in discharge rates from the Penobscot River, affect the DOM pool in this area.

Materials and Methods

This study investigated the identity and fluctuation of DOM components in surface waters in Penobscot Bay, ME, in relation to weather events. To identify DOM components, surface water samples were collected four times over a five week period at three stations in Penobscot Bay (Table 1; Fig. 2). A variety of *in situ* measurements and environmental conditions were measured and recorded at each station during sampling. These water samples were filtered through submicron filters (0.45 microns) and analyzed with a Hitachi-4500 fluorescence spectrophotometer for EEM-PARAFAC analysis to identify the DOM components and relative

abundance. These relative abundances were compared to weather data from NOAA buoy 44033, river discharge from a USGS water monitoring stations on the Penobscot River, and average precipitation for Penobscot Country, ME, for a correlation analysis of any fluctuations in DOM components attributed to the aforementioned conditions.

Water Sample Collection and Preparation

Sample collection bottles (60 ml amber borosilicate collection bottles) were cleaned according to USGS protocol for water sampling– all bottles were cleaned with a lab grade detergent, soaked in a 5% hydrochloric acid solution, and rinsed with methanol before being dried and wrapped in tinfoil for storage until sample collection (Groat 2004). Syringe filters were sterilized before shipment and did not need to be treated before sample filtration.

Samples were collected four times from three stations in Penobscot Bay over a five week period (Table 1; Fig. 2). Surface water samples were collected using a 5-gallon bucket with three seawater rinses prior to water collection at each station. At each station three 60 mL replicates of surface water were collected and filtered through a 0.45 micron sterile nylon syringe filter, and then stored in 60 mL glass borosilicate collection bottles that were covered with aluminum foil to prevent organic material from undergoing any photodegradation or photooxidation (Stedmon and Nelson 2015). After collection, samples were stored in the refrigerator in Rodgers Hall at MMA at approximately 4 ºC until spectral analysis was performed. The maximum amount of time a sample spent refrigerated prior to analysis was 37 days. Parlanti et al. (2000) analyzed similar water samples at one week and one year after collection and observed no measurable degradation of samples.

Environmental and Weather Data Collection

At each station information on environmental parameters was collected using different instrumentation. Windspeed was measured using a Sper Scientific mini environmental quality meter and dissolved oxygen and temperature were measured using a YSI Pro Optical Dissolved Oxygen meter. Salinity and atmospheric pressure were measured with a YSI Pro 30 salinometer. These data were recorded on data collection sheets while in the field and transferred to Excel files upon return.

Information on river flux ($ft^3\ s^{-1}$) and current speed ($m^2\ s^{-1}$) were collected from USGS water monitoring site 010345000 in West Enfield, ME, (USGS, waterservices.usgs.gov) and NOAA Buoy 44033 (NOAA, nbdc.noaa.gov) in Western Penobscot Bay, respectively. precipitation values, recorded in height (mm), were obtained from the NOAA Center for Environmental Information for all precipitation in Penobscot county, ME (NCEI, ncdc.noaa.gov). Precipitation data are reported by town, so all values for each town were averaged together to generate daily average precipitation for Penobscot county. River flux, current speed, and precipitation data were all collected for the 60 day period prior to the first cruise date through the last cruise date in according with findings from Asmala et al. (2021) that pulses of DOC persist in estuarine environments for 55 days following storm events. Each series of hydrologic data points were binned and averaged to generate mean daily values for each parameter. This information was recorded in a spreadsheet for correlative comparison to changes observed in the concentration and presence of DOM components.

Quinine Sulfate Standards

A quinine sulfate stock solution was created using quinine sulfate dihydrate $(C_{20}H_24N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O)$ and 0.5 M sulfuric acid (H_2SO_4) (Murphey et al. 2013; Tedetti et al. 2016). The stock solution was created using 120.7 mg of quinine sulfate dihydrate in 1 L of

0.5 M sulfuric acid for a concentration of 100 ppm. Quinine sulfate standards were used to convert the fluorescence of the EEMs to a standardized unit for fluorescence, called Quinine Sulfate Units (QSU), since this solution fluoresces similarly to DOM, where 1 ppb quinine sulfate = 1 QSU (Murphey et al. 2013). Six standards were made at concentrations of 1, 5, 10, 25, 50, and 100 parts per billion quinine sulfate to generate a regression of fluorescence intensity of the solution at the excitation/emission wavelengths 275/450 nm v. the concentration of quinine sulfate. This QSU calibration curve typically allows the relative concentrations of components identified by the PARAFAC analysis to be converted to a standardized fluorescence unit, and consequently interstudy comparison (Murphey et al. 2013; Tedetti et al. 2016). Due to software troubleshooting errors, the conversion from Raman Units (RU) to QSU was not able to be executed.

Spectrofluorometer and Spectrophotometer

Sample excitation and emission was measured in a Hitachi F-4500 fluorescence spectrophotometer at the Darling Marine Center (DMC) on October 4th, 2021, under the supervision of Dr. Meg Estapa. Water samples were transferred from Maine Maritime Academy to the DMC in an ice filled cooler, and all necessary supplies were transported as well. Samples were allowed to rest until they warmed to room temperature prior to analysis (Tedetti et al. 2016). Each sample was placed in a quartz fluorescence cuvette with a 10 mm pathlength and 3.5 ml volume; Millipore Milli-Q water was used as the blank for the instrument and subsequent samples. The excitation spectra were measured from 200 to 550 nm (wavelength; λ) in 5 nm increments and emission spectra from 280 nm to 600 nm at 5 nm increments. Instrument speed was set at 2400 nm min^{-1} with a 0.5 s slit width. Water Raman scans were collected at an excitation of 350 nm, which are used to normalize the fluorescence of the water samples to the

Raman peak. The Raman peak is a fixed area at excitation wavelength of 350 nm observed in pure water with high fluorescence intensities – normalizing to this area corrects for any remaining instrument bias not accounted for in the instrument correction files and allows for interstudy comparison (White and Roesler 2014; Tedetti et al. 2016). The absorbance of all samples was measured in a Thermo-scientific spectrophotometer (Genysys V-10) in 1 cm quartz cuvettes between 200 and 900 nm at 2 nm intervals. Data from all analyses were exported as .csv files for analysis in MATLAB.

EEM-PARAFAC Analysis

Excitation and emission data for samples, quinine sulfate standards, and Raman scans, as well as absorbance data, were entered into MATLAB R2020b (Mathworks) and analyzed using the dreem (version 0.6.13) package coupled with EEMlab. EEM-PARAFAC analyses followed the guidelines of Murphy et al. (2013) and Mico (2019), described briefly below. EEMlab is a user-friendly plug-in for MATLAB to make EEM-PARAFAC analysis simpler and more intuitive by focusing more on data analysis than the complex coding required when only using the dreem package. Figure 3 displays a general flow chart of the steps taken to perform EEM-PARAFAC. Instrument correction factors, which corrects the known error in fluorescence values from instrument variation and lamp intensity, were applied to the EEMs at the time of analysis. The EEM-PARAFAC analyses were performed in MATLAB using the EEMlab interface. After loading all files into EEMlab, the EEMs were corrected for inner filler effect using the absorbance scans taken on the spectrophotometer. This corrects the data for the amount of light the samples absorbed while they were being analyzed in the spectrofluorometer, allowing the fluorescence signals to be more accurate. The EEMs were then corrected for Rayleigh and Raman scatter so the EEMs show only the fluorescence of the DOM in the sample through

removing backscattering and specific signals unique to pure water. EEMlab's analyses tools know the specific wavelengths bands Raman and Raleigh scatter occur at allowing for the removal of both signals in the EEMs, so that fluorescence signals of pure water do not override the DOM signals (Mico 2019, Massicotte 2019).

Once the EEMs were corrected, PARAFAC was used to decompose the DOM signals into as many components as the model could accurately fit. Outliers identified through this process were removed from the data set after analysis at this phase so that the model could be optimized. Outliers are manually identified through scatter plots of maximum intensity (QSU) v. singlet state (a calculation performed by EEMlab) tagged with codes aligning with sample identities (Fig. 4). The model was validated with split-half analysis of the residuals, which separates the data set into three groups that are then split into subgroups, to make sure that the variances between all groups and sub-groups are homogenous and can be equally explained by the model. The excitation and emission peaks of components (e.g. protein-like, humic-like, fulvic-like) identified through PARAFAC were then compared to a plot of peak values in the dreem toolbox to identify the components general class (Fig. 5), and specific identities were made through comparison with literature values.

Comparison Between EEM-PARAFAC Model and Weather Conditions

Correlations between relative concentrations of PARAFAC components within water samples, relative to weather events, were compared. All statistical analyses were performed in R with the RStudio interface. A 2 way ANOVA test was performed to determine if there were significant differences between component concentrations among different stations and cruise dates. Independent variables were cruises and stations, and the dependent variable was component concentrations. A principal component analysis (PCA) was performed to analyze

relationships between concentration components and other environmental parameters, including precipitation, river influx, current speed, salinity, dissolved oxygen, and temperature. This allowed for a more comprehensive view of the interactions between environmental parameters and component concentrations, and to rank which parameters may have had the most influence over the presence and concentration of DOM components.

Uncertainty and Error

Uncertainty in this study could have come from measurements of environmental parameters and the locations from where environmental data is sourced. Each instrument for the environmental data collection while water sampling, the YSI dissolved oxygen probe, wind meter, and salinometer, have certain degrees of error that may take away from the accuracy of the information. The YSI Pro 30 salinometer has an accuracy of ± 1% of the salinity, or 0.1 ppt, whichever is greater. The YSI Pro Optical Dissolved Oxygen meter has an accuracy of ± 0.1 mg L^{-1} measuring dissolved oxygen, and ± 0.2 ºC accuracy for measuring temperature. Each cruise also occurred at different points in the tide schedule, which could have altered water conditions through mixing and other processes.

River flux and current data are not for the exact location that are being sampled; streamflow is approximately 90 km up the Penobscot River in West Enfield, ME, and current flow data is supplied approximately 35 km south for western Penobscot Bay off Camden, ME. This is something to take into consideration for error and uncertainty, because while these areas are influenced by one another and a part of the same overarching system, these variables are not direct measurements from the exact location that sampling is occurring.

Samples may have also experienced photooxidation and photodegradation. Depending on the light conditions when cruises are performed, surface samples could be experiencing different

levels of photooxidation, degradation, and microbial activity. This could cause degradation of the quality and quantity of the DOM in the samples. Although sample containers were blacked out to prevent degradation, some samples were stored in the refrigerator for a maximum of 37 days. Jaffe et al. (2008) showed that the spectral analyses results do not change significantly over a one month period after being filtered and refrigerated, but it is still possible that the DOM composition may change in this period and should be considered a potential source of variation.

When generating the PARAFAC model and PCA, both error and uncertainty could have major influences in my results. In PARAFAC, backscatter and inner filler effects are often identified through manual inspection of fluorescence graphs. If a backscatter wavelength was incorrectly identified or a range of wavelengths was left uncorrected that needed to be corrected, the model could generate results that are not accurate, even though the model may be validated. This analysis can add error and uncertainty to my project through the limitations of modeling software and computing power. While PARAFAC can provide a detailed look at DOM composition in certain areas, it is likely not capturing every individual DOM component present since some organic matter does not fluoresce and is thus unable to be detected by the techniques used in this study.

The nature of this study has limitations with the time constraint of the semester, materials, and complexity of DOM's relationship with the environment. DOM is constantly interacting with physical, chemical, and biological variables, making it difficult to encompass the full story of DOM in relation to weather events. There are minimal data on biological activity in the survey area, which could be a controlling factor for DOM instead of weather events. This would be an excellent path to progress this research in the future. While the data collected will be

adequate to draw conclusions and inferences from, it will not tell the entire story of what is controlling the fluctuations of the DOM pool.

Results

EEM-PARAFAC analysis identified three unique DOM components present in varying magnitudes across all stations and cruises. Components are labeled in order of their overall contribution to fluorescence, meaning that component 1 (C1) had the highest contribution to the overall fluorescence signal from the samples. Subsequently component 2 (C2) and component 3 (C3) contributed to overall sample fluorescence in smaller proportions. C1, C2, and C3 had fluorescence peaks were observed at excitation and emission wavelengths 220/595 nm, 230/440 nm and 225/280 nm (Fig. 6). Two of these components, C1 and C2, were identified as fulvic-acid like components, and C3 was identified as an aromatic protein (Fig. 7). While both C1 and C2 fall within the class of fulvic-acid like components, their unique excitation and emission peaks indicate that they are distinct DOM components.

The relative abundance of the three identified DOM components were observed to behave differently over the study period, instead of changing in the same temporal and spatial patterns. C1 was not found to change significantly (2 way ANOVA, $p > 0.05$, $n = 36$) between stations or cruise dates (Fig. 8), with the average fluorescence signal over the study period being 346 ± 68 RU for this component. While none of the changes were considered statistically significant, there was a notable change in the distribution of abundance of C1 between stations following the precipitation event observed during the study period. For cruises 2 and 3 the abundance of C1 appeared to be very similar in values between all stations; following the precipitation event during cruise 4, the lowest levels of C1 were detected at station 1, which then increased through stations 2 and 3 (Fig. 8).

C2 was found to change significantly between all stations over all cruise dates, with the exceptions of cruises 1 and 3 (2 way ANOVA, $p < 0.026$, $n = 36$; Fig. 9). The spatial distribution of C2's abundance was the same across cruises two, three, and four, with the highest levels of C2 detected at station one, which then decreased through stations two and three. Temporal variation of C2 was shown to respond to precipitation. The average fluorescence signal for C2 during cruise 1 was 61.2 ± 8.56 RU, followed by cruise 2's average fluorescence signal of 76.8 ± 18.1 RU. This increase is in line with a small precipitation event observed between cruises 1 and 2 (Fig. 10). Cruise 3 had the lowest abundance of C2, with an average fluorescence signal across stations being 47.3 ± 9.4 RU. The highest relative abundance of C2 for all stations over the study period was observed during cruise 4, which followed the major precipitation event observed during the study period. The average abundance of C2 across stations during cruise 4 was 118 ± 8.57 RU, showing a 151% increase in the abundance of this component following the rain event between cruises 3 and 4.

The aromatic protein component, C3, was found to only change significantly between cruises 2 and 3 (2 way ANOVA, $p = 0.036$, $n = 36$; Fig. 11), but its abundance did not change significantly between stations (2 way ANOVA, $p > 0.05$, $n = 36$). Cruise 2 had a higher average abundance of C3 with an average of 176 ± 44.6 RU. There was then a significant decrease in the abundance of this component, with cruise 3's average fluorescence signal being 98.5 ± 10.4 RU.

Data on physical parameters (temperature, salinity, dissolved oxygen) that were collected at each station while sampling over all cruises, were also observed to fluctuate. Salinity (PSU) was similar between cruise dates, with the lowest salinity values always occurring at station one, with an average of 24.4 PSU over the study period, and then increasing through stations 2 and 3 with averages of 26.3 and 26.8 PSU, respectively. Theses average salinities show the salinity

gradient of the study location where station 1 is closest to the mouth of the Penobscot River and station 3 is furthest into Penobscot Bay (Fig.2). Cruise 2 was observed to have the lowest salinities for all three stations over the entire study period, with an average salinity of 24.1 PSU for that cruise - the highest salinities occurred during cruise 3, with an average of 27.2 PSU.

Temperature (ºC) decreased consistently for stations two and three across the four cruises. Station two's temperature over the study period began at 19.8 ºC, decreasing to 18.9 ºC for cruise 2, with this trend continue through cruises 3 and 4 with respective temperatures of 17.1 ºC and 16.8 ºC. Station 3 had a larger total temperature change over the study period, beginning at 20.4 ºC, and subsequent temperatures of 19.2 ºC, 17.5 ºC, and 16.5 ºC, for a total change of 3.9 ºC. Station one, however, experienced an increase in temperature between cruises one and two, from 17.8 ºC to 19.4 ºC, and then decreased by 2.9 ºC between cruises 2 and 3, and a smaller decrease of 0.3 ºC between cruises 3 and 4 (Table 1).

Dissolved oxygen levels (mg L^{-1}) were observed to have a consistent trend between stations throughout all four cruises. Dissolved oxygen levels were always lowest at station one, with the average value for station one over the study period being 80.3 mg L^{-1}, and then increased through station two and three, with their respective averages over the study period being 94.3 mg L^{-1} and 96.5 mg L^{-1}. Peak dissolved oxygen content at station 1 was observed during different cruise dates than stations 2 and 3. Peak dissolved oxygen levels for station 1 occurred during cruise 2 (86 mg L^{-1}), whereas peak dissolved oxygen for stations two and three (96 and 100 mg L^{-1}) both occurred during cruise three (Table 1).

One major precipitation event was observed over the study period, with subsequent increases in river discharge (m^3 s^{-1}), and fluctuations in current speed (m^2 s^{-1}; Fig. 10). Peak precipitation (mm) for Penobscot County, ME, occurred on September 10th, 2021 (between

cruises three and four) with a rainfall of 52.09 mm. Smaller rain events were also observed between cruise dates, but none of similar magnitude. Average daily rainfall over the study period was calculated to be 4.11 ± 9.11 mm. Peak river discharge was delayed by approximately one day, with the peak discharge rate of 13,100 $m^3 s^{-1}$ compared to the average discharge rate over the entire study period of 4671 ± 2030 $m^3 s^{-1}$. The plot of river discharge closely mimics the precipitation plot because of their intimately tied relationship, where precipitation runs off of the land into the Penobscot River typically increasing discharge rates (Fig. 10). Current speed was observed to rise in the 5 days preceding the precipitation event from 0.13 $m^2 s^{-1}$ to a peak value the day after the precipitation event of 0.20 $m^2 s^{-1}$ (Fig. 10). Current speed then quickly decreased the following day to 0.14 $m^2 s^{-1}$.

The first two dimensions calculated by the Principal Component Analysis (PCA) were able to explain 62% of the variability in the overall dataset (Fig. 12). The data included in the PCA were all environmental parameters (river discharge, precipitation, current speed, atmospheric pressure, temperature, salinity, dissolved oxygen), the relative abundance of all three DOM components, and the station number. PCA results revealed that C1 is positively related to salinity, current speed, and station, and negatively related to the abundance of C2. Dissolved oxygen was strongly and negatively correlated to C2 abundance and were secondarily related to weather related parameters (atmospheric pressure, discharge, precipitation). Lastly, C3 was shown to be positively correlated with temperature, and negatively correlated with the previously listed weather parameters.

Discussion

The results of the EEM-PARAFAC analysis identified three unique DOM components present in Penobscot Bay, ME, two of which (C1 and C2) were independent fulvic acids, and the

third an aromatic protein (C3; Figure 2). Results showed that components C1 and C3 were resistant to change in response to weather events, whereas C2's abundance increased in response to a major precipitation event. C2's abundance was observed to be at its highest concentration for all stations over the study period following the precipitation event. This precipitation was significant, with a rainfall of 52.9 mm for Penobscot County, ME, where the average for this study was 4.11 ± 9.11 mm, making it more than 3 SD greater than the daily mean. This study can therefore conclude that certain portions of the DOM pool in Penobscot Bay, ME, are changing in response to precipitation and subsequent increases in river discharge, while other components are resistant to these environmental changes.

The first component detected by this study, C1, was identified as a fulvic acid with its fluorescence peak occurring at excitation/emission wavelength 295/595 nm. When compared to a variety of literature values a more specific identify of this component could not be determined, limiting the understanding of its behavior in this study. Murphey et al. (2008) described fulvic acids as having low excitation maximums with high emission maximums which supports the identification of this component. In general, fulvic acids are sourced from the degradation products of terrestrial plants and organisms, and thus mostly sourced from soils and marine sediments (Boggs et al. 1985). Because of their source material, fulvic acids are also most typically found in highest concentrations in environments subject to rain drainage, such as this studies location in a shallow coastal zone (Carder et al. 1989). This source and phenomenon would suggest that the concentration of C1 would change following precipitation events – but this was not observed since the changes in C1 over stations and cruise dates were not determined to be significant. Since the abundance of C1 remained stable, it would suggest that there is either some sort of regulation occurring of this DOM component within the system, or that marine

sediments are sourcing this component since it does not appear to be carried to Penobscot Bay, ME, through terrestrial runoff.

It is unclear whether this component is part of the labile or refractory portion of the DOM pool. Labile DOM is biologically available and thus processed and transformed quickly within environments, whereas refractory DOM persists in environments for large timescales. If this component is labile or semi-labile, a potential regulation of this component could occur through trace metals. Fulvic acids are known to bind to trace metals such as iron and remove both particles from the system. Lagler and van den Berg (2009) found that fulvic acids were responsible for the entirety of iron lignan concentrations in a shallow coastal zone, where the iron was traveling from land to sea since trace metals are mainly sourced terrestrially. Since this study area could also be classified as a shallow coastal zone, it could be inferred that fulvic acids are binding with iron to form iron lignans in this area. As iron and fulvic acids are entering the system through terrestrial runoff they may bind to one another and settle out of the system. This removal process may leave a background concentration of C1 that stays stable in response to weather events or fluctuates at levels too small for this study to detect. This hypothesis is supported by PCA results, which showed that the abundance of C1 was positively correlated with salinity. At lower salinities closer to the mouth of the Penobscot River, iron concentrations would be at its highest since it is being transported from land. The higher iron concentrations allow for more of C1 to be removed by binding to the iron and settling out of the system. As iron concentrations decrease along the salinity gradient there is then a surplus of this fulvic acid like component, explaining the positively correlated relationship between these two variables.

Another component, C2, was also identified as a fulvic acid like component, but it was determined to have a terrestrial origin by comparing its characteristic fluorescence peak with

literature values(Murphey et al. 2008). This identification is further supported by its behavior over the study period - this component distinctly increased in response to a precipitation event that was observed between cruises 3 and 4 (Fig. 10). All stations had their highest concentration of C2 (172, 116, 67.1 respectively) during cruise 4, following a distinct pattern of the highest abundance of C2 at station one, (closest to the Penobscot River), and then decreasing through station 3. This gradient of C2's abundance was observed across all cruise dates, further suggesting a terrestrial origin since the components abundance was always highest at the station closes to the mouth of the Penobscot River and land/sea margin. As precipitation from weather events occur, it is likely washing C2 from land into the Penobscot River – the precipitation runoff from this weather event increases river discharge rates and carries C2 out into Penobscot Bay. As it enters the bay its concentration is diluted, explaining the gradient of C2 observed across stations and subsequently, salinity. These results and observations show that C2 increases substantially in this system following precipitation events, showing that at least a portion of the DOM pool in Penobscot Bay, ME, changes in response to weather events.

Results from the PCA showed that C2 was also negatively correlated with dissolved oxygen (Fig. 12), which may indicate that this component also has a relationship with microbial communities. This PCA correlation suggests that as the abundance of C2 increases, dissolved oxygen decreases. This indicates that as C2 is entering the system, it is fueling growth that is utilizing oxygen – pointing towards fueling microbial communities that are contributing to remineralization. As C2 is utilized by these microbial communities and decreases in abundance, dissolved oxygen begins to increase again as photosynthesis begins to dominate the system.

Fulvic-acids as a class of DOM are all considered a type of tannin, which are compounds that increase the CDOM and turbidity of seawater (Boggs et al. 1985). This increase in tannins

can be a concern due to its potential to reduce photosynthesis by shallowing the photic zone and less light being able to enter the system. On the converse side, this increase in tannins could impede photooxidation and degradation of DOM, allowing DOM in systems with high tannin content to persist longer in the environment, potentially fueling microbial growth for longer periods of time. With increases in storm intensity and frequency with climate change, it is likely that Penobscot Bay will experience consistently higher loads of terrestrially sourced fulvic acids in pulses following precipitation events. The effects this could have remain unclear and needs further investigation. Decreased dissolved oxygen levels following precipitation events indicate the system shifting towards remineralization and oxygen utilization. These same pulses can also increase tannin levels, reducing photosynthesis and therefore oxygen production. These two factors point towards the concern of low dissolved oxygen content following precipitation events in Penobscot Bay. Further, if this fulvic acid also behaves in line with Lagler and van den Berg (2009) and binds to trace metals, C2 has the potential to deplete Penobscot Bay of trace metal concentrations further impeding photosynthesis.

The final component identified through EEM-PARAFAC was C3, which fell within the class of DOM considered aromatic proteins. The fluorescence peak was most similar to a peak identified by Tedetti et al. (2016) and White and Roesler (2014), which were both tryptophan-like compounds. Tedetti et al. (2016) identified their peak fluorescence at 225/295 nm and White and Roesler (2014) 225/285 nm, whereas this study's C3 had a fluorescence peak of 225/280. These differences in the peak emission wavelength could be a result of several factors, including differences in the sampled environment, as well as sample degradation. However, the conjunction of these studies results allows this study to say with confidence that this peak is a tryptophan-like compound.

C3 was only observed to change significantly between cruises 2 and 3, where the components abundance was observed to decrease. The environmental parameters that are controlling the abundance of this component remain unclear; the PCA showed a positive relationship with temperature, but a negative correlation with weather parameters (precipitation, river discharge, atmospheric pressure; Fig. 12). The positive correlation with temperature could indicate a seasonality of this component, as cruise 2 had the highest average temperature across all stations - the seasonality of the component could however be the result of an underlying factor such as biological activity. The negative relationship with weather parameters suggests that this component is observed at lower concentrations when precipitation and river discharge increase. This may indicate that this component is being removed from the system from the increased river discharge. While no relationship with salinity was observed through the PCA analysis, it is worthy to note the cruise 2 had the lowest salinities at all stations across cruise dates. This cruise was performed closest low tide compared to other cruises (< 2 hours) and may be an underlying variable worth of examination.

One other study has been performed examining the DOM in the Penobscot River through EEM-PARAFAC methods, which identified several different DOM components than this study. White and Roesler (2014) identified 5 unique DOM components, all of which had different peak fluorescence values then the three components of this study but two of which had similar identities. White and Roesler (2014) identified a component as a soil fulvic like material derived from agricultural catchments with a peak fluorescence value of 280/510 nm. While this is most similar to C1 of this study (peak 220/595) the differences in values make it very unlikely that it is the same component, despite both being identified as fulvic-acid like components. However, they did observe that the magnitude of fulvic material present in the Penobscot River was driven

by the mobilization of material on land, observing the highest values following the spring snowmelt and fall precipitation maximum, which is in line with the behavior observed for C2 in this study. This studies observation on the increase of C2 following precipitation events in conjunction with White and Roesler's (2014) observation on the driver of fulvic material in the Penobscot River, supports the conclusion that fulvic material in Penobscot Bay is largely driven by terrestrial runoff.

The tryptophan like component (C3) that was also identified by White and Roesler (2014), observed this component to be at its highest annual value in August. They found that this component had a small positive correlation with chlorophyll a concentration at all sampling sites along the Penobscot, with major peaks of this component occurring in line with summer phytoplankton blooms. Because of this it was hypothesized that this component was being sourced from the senescence of cells following blooms and subsequent cell death, as well as consumption by organisms at higher trophic levels. Since this study had no data on chlorophyll content, it was not possible to confirm the behavior of this component observed by White and Roesler (2014). Its positive correlation with temperature however, revealed through the PCA, may support their observation of seasonal increases of this component in line with phytoplankton blooms. More data on the temporal trends of this component at the stations surveyed for this study would be needed to understand the seasonality of this component more definitively.

The differences between White and Roesler (2014) and this study likely come from several confounding factors. White and Roesler (2014) analyzed over 1500 water samples collected from four different Maine rivers (including the Penobscot River) over a yearlong study period. These differences in sample size, study locations, and time of year likely all play confounding roles in the differing results, despite the interconnected ecosystems. Since Whie and

Roesler's (2014) samples were collected from different locations, but all used to generate the same EEM-PARAFAC model, DOM components that were present below detection levels in the Penobscot River may have been identified through higher concentrations in one of the other rivers. They also focused on the Penobscot River instead of Penobscot Bay or estuary -while it is likely that DOM components are being carried to Penobscot Bay through the river, DOM components could be utilized or undergo transformation before arriving there.

Uncertainties in this study are mostly sourced from the statistical power of PARAFAC and troubleshooting that occurred with generating a model to fit to the collected EEMs to identify DOM components. An initial limitation is the number of samples that were used to generate the model – this study collected 36 samples, but ideal studies use upwards of one hundred to generate a model with good resolution and to account for variation between samples. Original plans for this study included collecting the same number of samples but at depth, allowing for a model to be generated with 72 samples which likely would have increased the accuracy of the model. However, due to only being able to use the spectrofluorometer for one day, there was not enough time to also analyze the depth samples.

The spectrofluorometer also scanned samples at emission and excitation intervals of 5 nm, whereas 2 nm is more commonly executed to get a higher resolution of EEM results. This was done due to time limitations – measuring at 5 nm increments significantly reduced analysis time. Model iterations, or the number of times the computer runs different combinations of data to validate a model were done 10^3 times, which is adequate for the scope of this study. However, increased iterations and tuckers coefficients, (the statistical measure of confidence for PARAFAC), would allow for a more determinate model with more specific resolution.

Results for this PARAFAC model were also reported in Raman Units (RU) instead of the standard QSU. This was because of a trouble shooting error that occurred with EEMlab when attempting to convert the EEMs from RU to QSU, despite having the necessary conversion factor. There was no clear resolution to this issue within MATLAB, or instructions from the user manuals, so the results remained in RU. RU are a standardized unit of measurement, as normalizing the EEM data to the Raman peak of a Millipore water sample is a correction and normalization that is standard in EEM-PARAFAC analysis. This standard correction means that the results from this study are still appropriate for interstudy comparison.

Samples from each cruise were stored for different amounts of time since they were all analyzed on the same day. Although Parlanti et al. (2000) showed that there were no statistically significant differences between EEM signatures of water samples analyzed shortly after collection and one year, it is still possible that samples experienced some sort of degradation from photooxidation or microbial processing from bacteria that were able to pass through the filter. Ideally, if samples could not be collected at the same time they would be analyzed at the same interval between collection and analysis to allow for the most consistent results.

More data is needed on the larger scale temporal and spatial trends of the components identified by this study. Only one major precipitation event was observed within this study period; to definitively establish the trend of C2 entering Penobscot Bay in high amounts following precipitation events, more data is needed. More sampling should occur to see if the phenomenon observed over this study is being replicated after every precipitation event, only precipitation events of certain magnitudes, or if any other changes are regularly occurring that were not captured by this study. It would also be beneficial to see how long pulses of C2 persist in this environment in comparison with Asmala et al. (2021). C1 and C3 may also change

temporally, but only on seasonal or annual scales that were not able to be captured by this study. These changes may occur in response to spring snowmelt, deepening or shallowing of the thermocline, or other environmental fluctuations that typically occur over larger time scales. Understanding the fluctuations of these components in response to these conditions would be valuable in understanding the overall dynamics of DOM in the system.

This study can conclusively say that portions of the DOM pool in Penobscot Bay, ME, change in response to weather events. The increase in C2 following the observed precipitation events suggests that Penobscot Bay experiences increases in fulvic acids following storm events. As frequency and intensity of storm events increase with climate change, Penobscot Bay, ME, may continuously experience higher loads of fulvic acid to the system, which has potential to inhibit photosynthesis, increase remineralization, and potentially deplete the system of trace metals (Boggs et al. 1985; Seneviratne et al. 2012). While other DOM components, C1 and C3, are appearing to remain stable in response to precipitation events, it would be valuable to further explore the dynamics of DOM in Penobscot Bay with the goal of understanding the driving factors controlling the distribution and abundance of these two components. Understanding the affects and interactions these DOM components have with the system would increase understanding of nutrient and DOM cycling in this area, help generate more accurate carbon budgets, and provide a more wholistic view on the health of this ecosystem.

Future work based off this study could focus on building a machine learning framework from the dimensions identified through the PCA to predict DOM abundance in response to precipitation events. Supplementing the already collected environmental parameters with direct or satellite measurements of chlorophyll, data on remineralization rates and microbial activity, and tide levels at sampling times would all be beneficial variables to add to the machine learning

network. It may also be valuable to directly measure fulvic acid concentrations in Penobscot Bay so that the magnitude of their change following precipitation events can also be better understood. Overall, further long term monitoring should be implemented to achieve a more wholistic view of DOM in this system, as well as to confirm the recurrence of the trends initially identified by this study.

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I am very grateful for the financial support provided by Quahog Bay Conservancy that allowed for outside lab analysis and the purchase of my materials. I would like to give special thanks to Dr. Steven Baer, for pushing me to expand and deepen my project, skills, and aspirations. I appreciate your enthusiasm and passion for teaching, sharing knowledge, and inspiring curiosity. You have guided and inspired me towards a career path in research and academia. Thank you to Dr. Meg Estapa for her help with instrumentation and lab space to make my project possible. Extraordinary thanks to Pam Grindle for organizing and obtaining all my materials and lab space required for my project, and thank you to Finn Welch for taking me out on the vessels to collect my samples, as well as helping with sample collection and Niskin bottle technical difficulties. I am grateful for the many other people who helped me through this process including Dr. Carey Friedman, Dr. Alan Verde, Kaitlyn Reny, and my parents. Thank you all for continually encouraging me to explore my passions and curiosity. I would not be where I am today without the amazing group of people who have walked this journey with me.

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Figures and Tables

Table 1. Cruise logistic information (latitude, longitude, dates) as well as environmental data collected during sample collection in Penobscot Bay, ME across all four cruise dates.

Cruise 1 (08-25-2021)			
Parameter	Station 1 *(44'28.06 N, 68'48.32 W)*	Station 2 *(44'21.80 N, 68'51.14 W)*	Station 3 *(44'18.34 N, 68'51.91 W)*
Temperature (C^{o})	17.8	19.8	20.4
Salinity (PSU)	25.8	26.5	26.9
Dissolved Oxygen (mg L^{-1})	82	95	97
Atm. Pressure (mm Hg)	760.7	760.7	760.7
Cruise 2 (09-01-2021)			
Temperature (C^{o})	19.4	18.9	19.2
Salinity (PSU)	21.7	25.0	25.7
Dissolved Oxygen (mg L^{-1})	86	96	98
Atm. Pressure (mm Hg)	755.3	755.1	755.3
Cruise 3 (09-08-2021)			
Temperature (C^{o})	16.5	17.1	17.5
Salinity (PSU)	26.4	27.6	27.6
Dissolved Oxygen (mg L^{-1})	80	96	100
Atm. Pressure (mm Hg)	757.1	757.1	757.2
Cruise 4 (09-22-2021)			
Temperature (C^{o})	16.2	16.8	16.5
Salinity (PSU)	23.5	25.9	27.1
Dissolved Oxygen (mg L^{-1})	73	90	91
Atm. Pressure (mm Hg)	765.2	765.1	765

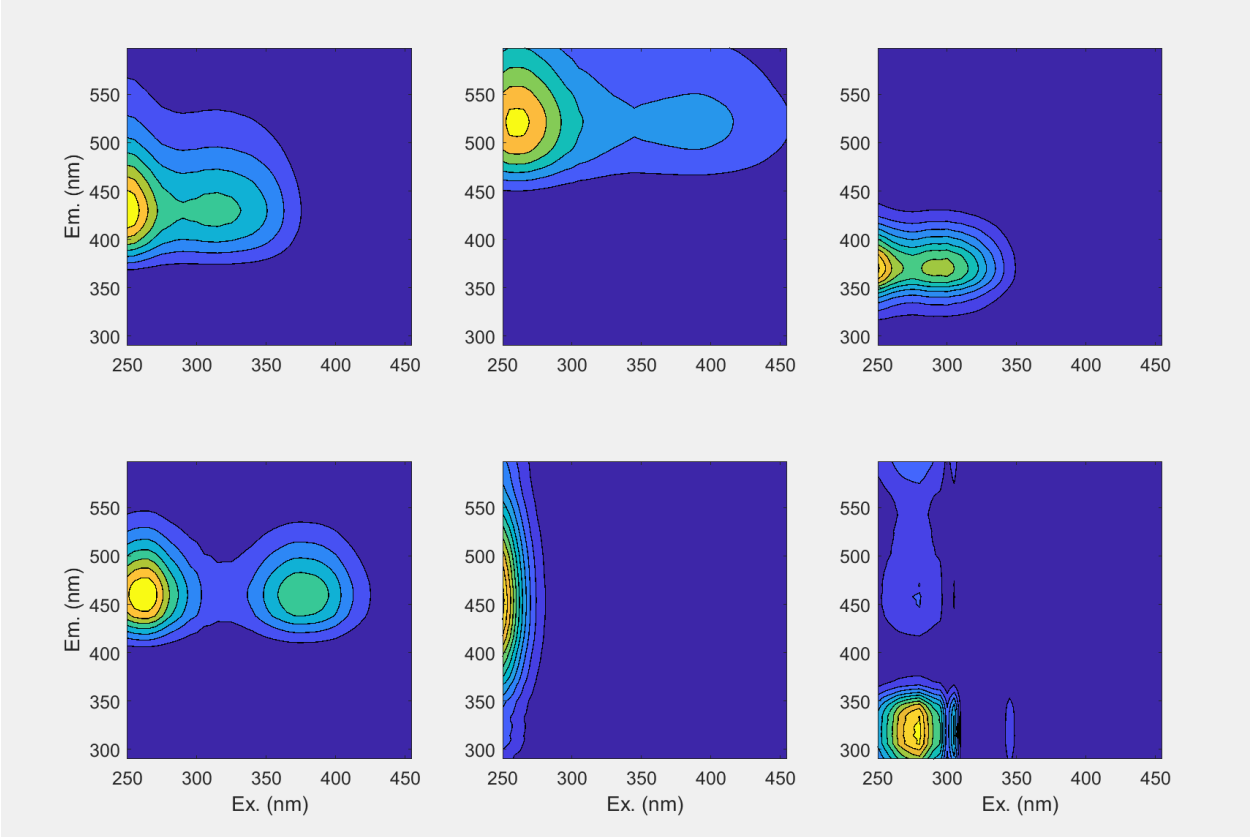

Fig. 1. Example Excitation Emission Matrices (EEMs) generated during the pilot study using data from Murphey et al. (2013). Plots are excitation (nm) v. emission (nm) with the z-axis indicating fluorescence intensity in Quinine Sulfate Units (QSU). Cooler colors indicate lower fluorescence intensity, with warmer colors indicating higher fluorescence intensity. These plots are commonly referred to as fluorophore fingerprints because they represent the fluorescence signature of specific DOM components. The excitation and emission wavelengths of peak fluorescence intensity depicted in these plots are then used to identify the DOM components. Image Credit: Ellie Gellerson

Fig 2. Station locations for the study, positioned along a salinity gradient in Penobscot Bay, ME. Also shown is the USGS monitoring station (NUMBER ALSD) where river flux data was collected from, as well as NOAA Buoy 44033, where current speed was collected from. Image credit: Google Earth.

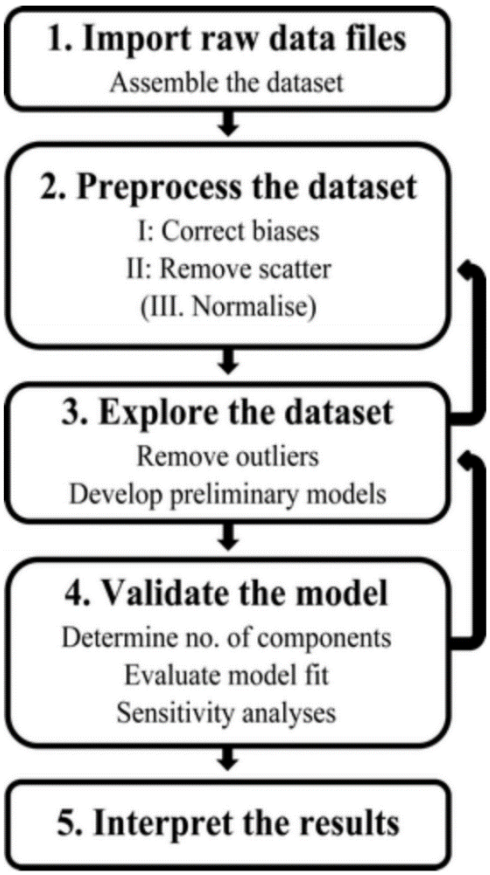

Fig. 3. General guide of steps that were taken to perform excitation-emission matrices and parallel factor analysis from data generated following spectral analysis of water samples (Murphey et al. 2013). Image credit: Murphey et al. (2013)

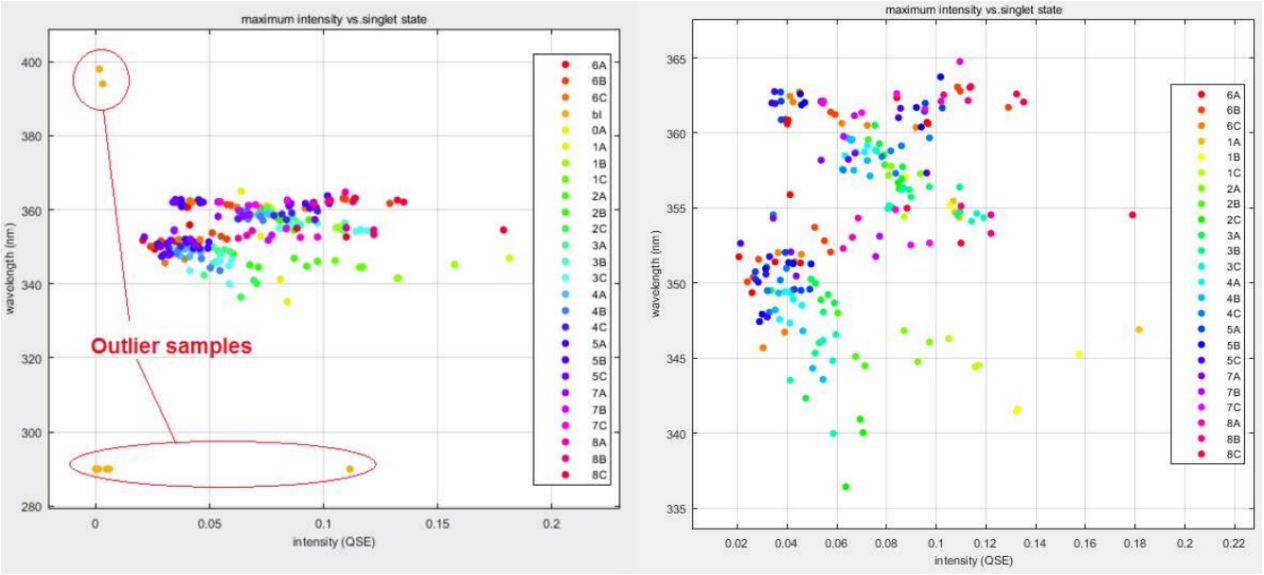

Fig. 4. Plot of maximum intensity (QSU) v. singlet state as generated by EEMlab, and data set following the removal of the outliers. Data is from the pilot study data set. This plot is used to manually identify outliers in the data set. The point circled in red can be identified as an outlier due to its clear separation from the cluster of all other data points. Image Credit: Paul Mico

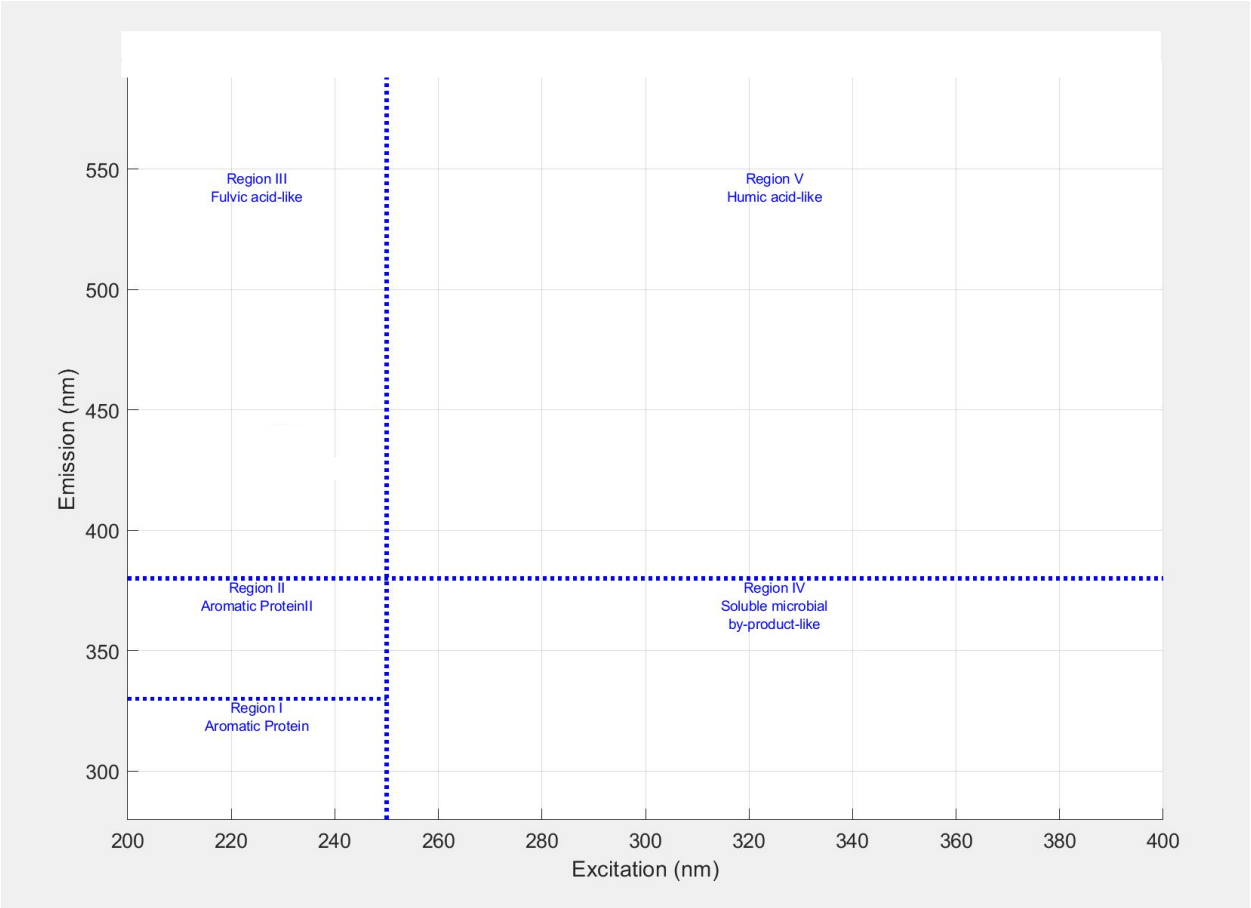

Fig. 5. Plot used by EEMlab to identify which class of DOM the component identified through EEM-PARAFAC belong to based off their unique fluorescence peak. The excitation and emission (nm) of the peak fluorescence of is plotted on this graph, and then depending on where that point falls determines the identify of that component. There are five classes of DOM a component could land in; Fulvic acid-like, humic acid-like, aromatic protein, aromatic protein II, and soluble microbial by-product like. More specific identities of components are then mad by comparing these peak values to literature.

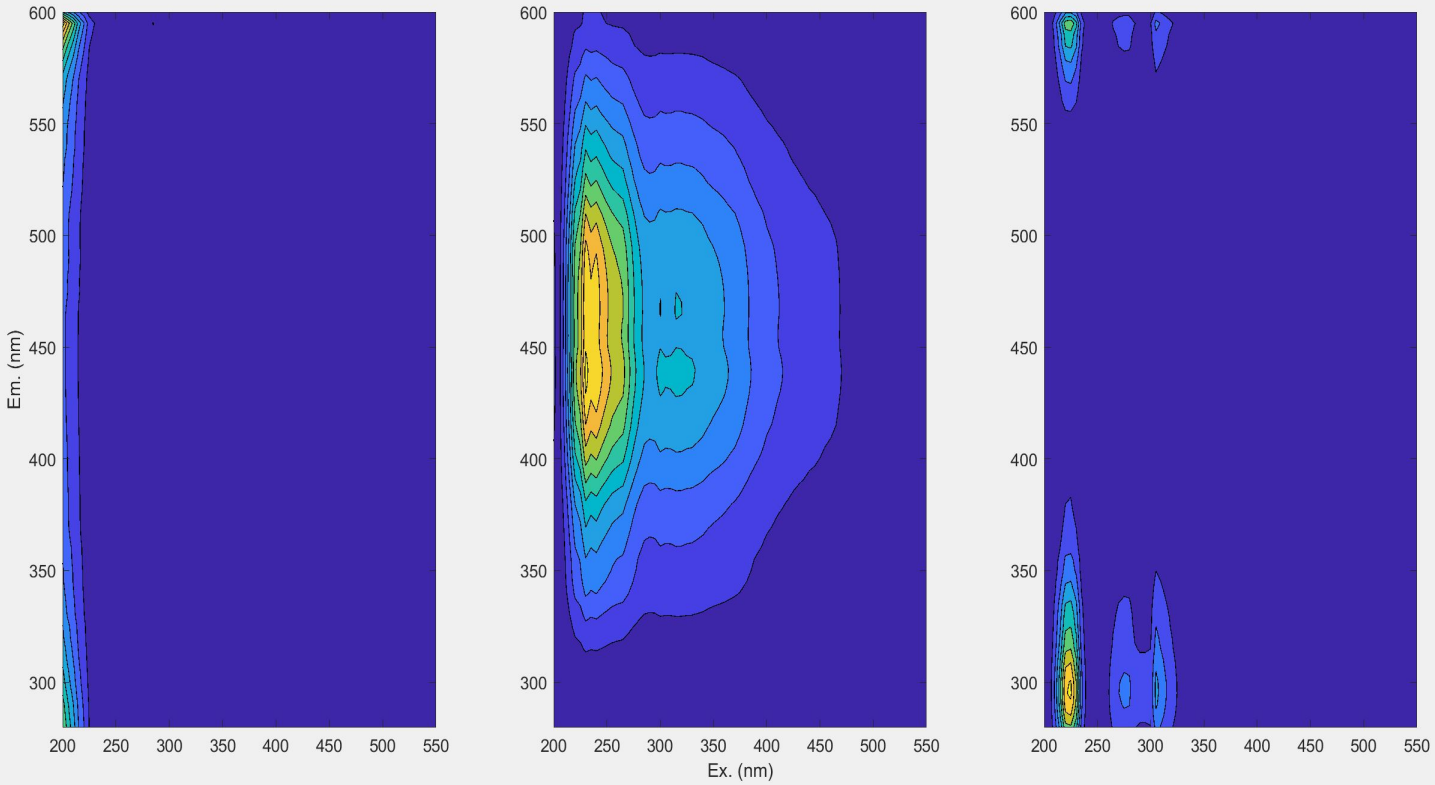

Fig. 6. EEM's for 3 DOM components identified through PARAFAC. Plots are excitation (nm) versus emission (nm), with the z-axis being fluorescence intensity in Raman Units (RU). Cooler colors indicate lower fluorescence intensity, with warmer colors indicating higher intensity. Components are identified as numbers one through three (left to right, top to bottom). Each EEM plot is unique to the DOM component it is associated with, thus being referred to as a fluorophore fingerprint. Image Credit: Ellie Gellerson

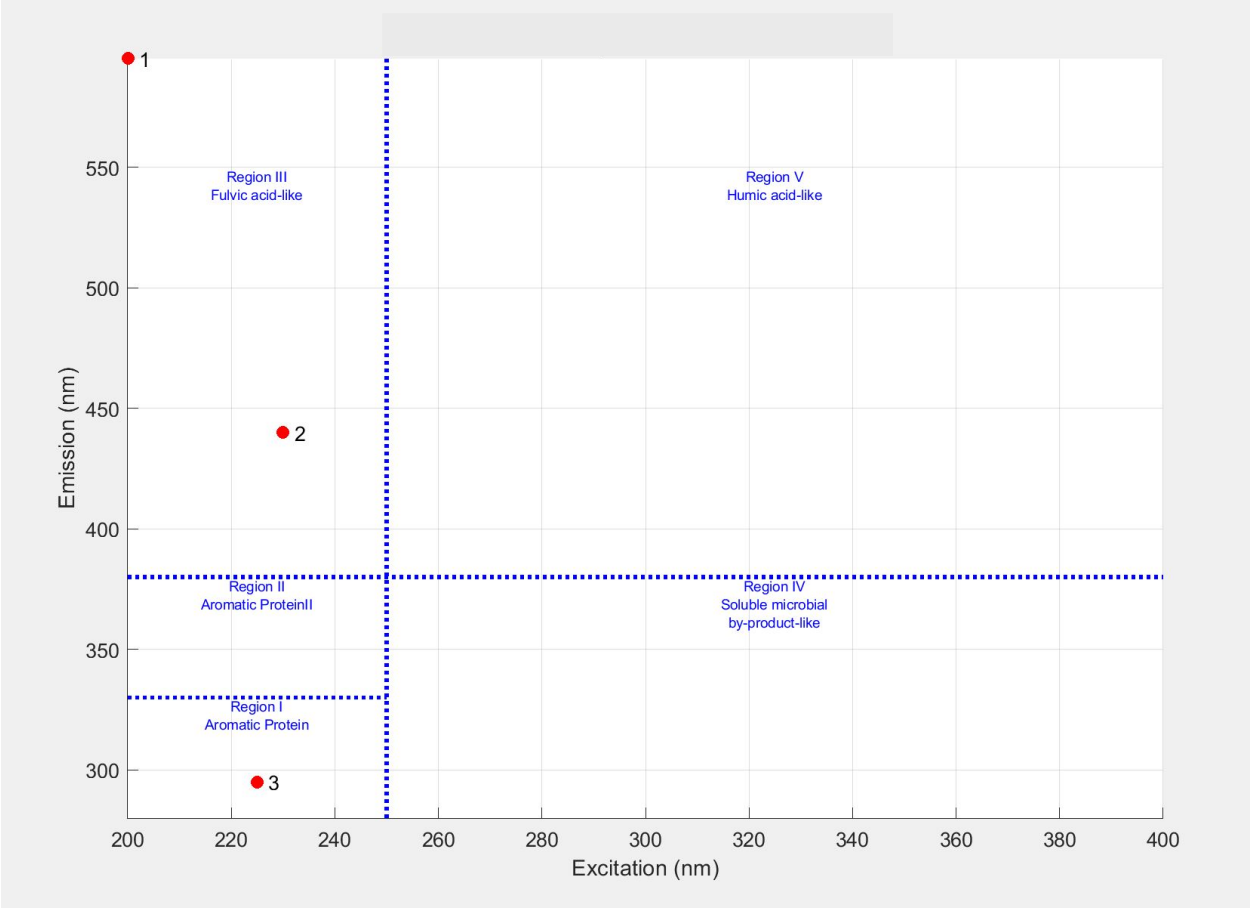

Fig. 7. Peak excitation (nm) and emission (nm) of 3 dissolved organic matter components derived through parallel factor analysis shown in Fig. 1. The location of the highest fluorescence intensity on an excitation emission matrix is identified as the peak. The location of this peak dictates which sub-group of dissolved organic matter the component belongs to, as shown above. Image credit: Ellie Gellerson

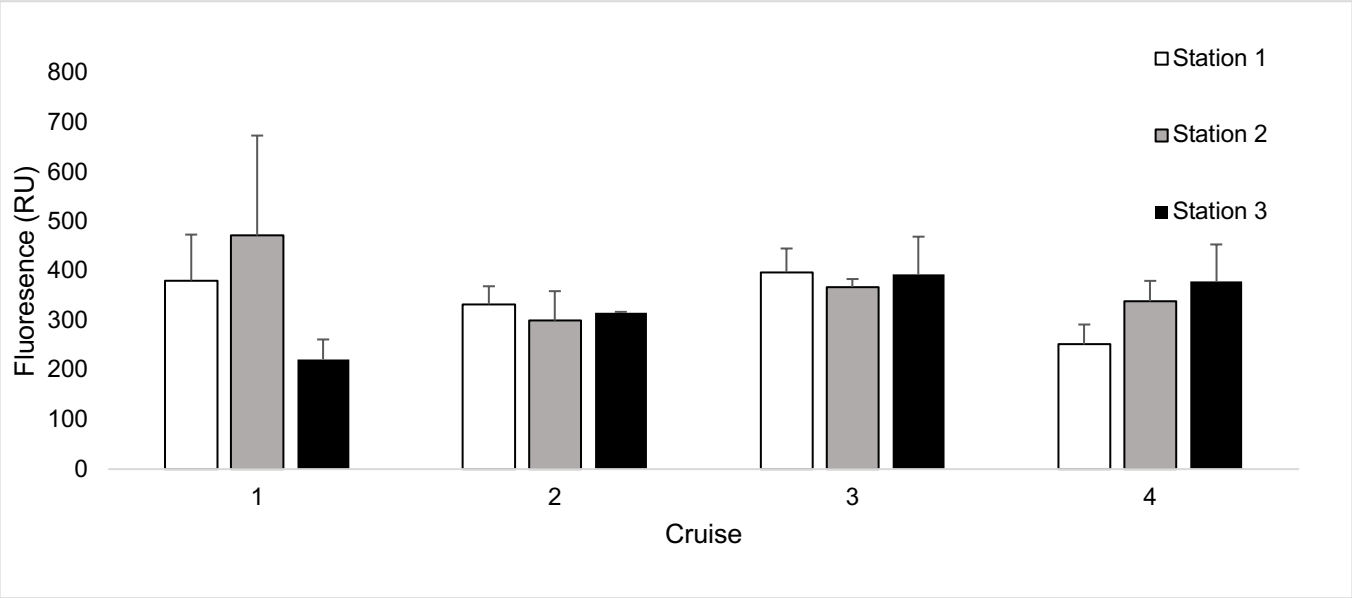

Fig. 8. Mean fluorescence signal (Raman Units) of DOM component 1 at different stations over individual cruise dates (Cruise: $F_{1,\ 36} = 1.091$, $n_{Cruise\ 1} = 9$, $n_{Cruise\ 2} = 9$, $n_{Cruise\ 3} = 9$, $n_{Cruise\ 4} = 9$, $p = 0.368$, Station: $F_{2,\ 36} = 0.644$, $n_{Cruise\ 1} = 9$, $n_{Cruise\ 2} = 9$, $n_{Cruise\ 3} = 9$, $n_{Cruise\ 4} = 9$, $p = 0.532$). No significant differences between cruises or stations were observed. Data are mean ± SD.

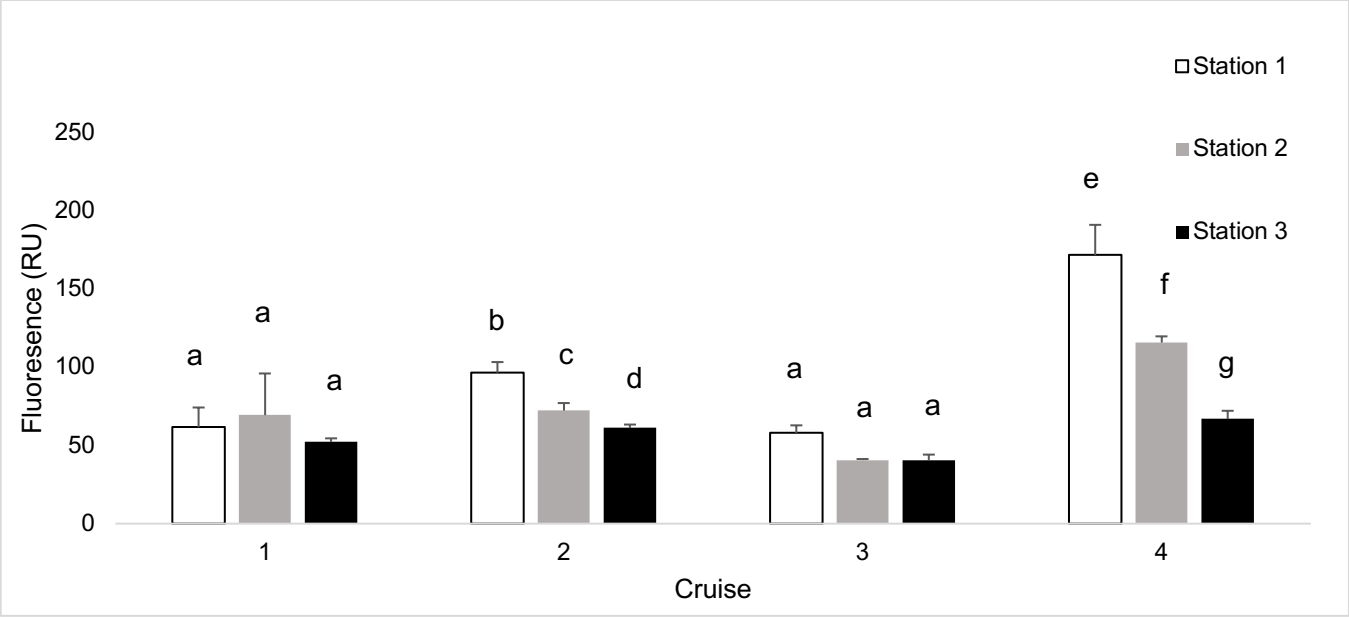

Fig. 9. Mean fluorescence signal (Raman Units) of DOM component 2 at different station over individual cruise dates (Cruise: $F_{1,\ 36} = 21.03$, $n_{Cruise\ 1} = 9$, $n_{Cruise\ 2} = 9$, $n_{Cruise\ 3} = 9$, $n_{Cruise\ 4} = 9$, $p < 0.001$, Station: $F_{2,\ 36} = 12.67$, $n_{Cruise\ 1} = 9$, $n_{Cruise\ 2} = 9$, $n_{Cruise\ 3} = 9$, $n_{Cruise\ 4} = 9$, $p < 0.001$). All measurements are significantly different with the exceptions for data from cruise 1 and cruise 3. Data with the letters are not significantly different from one another. Data are mean ± SD.

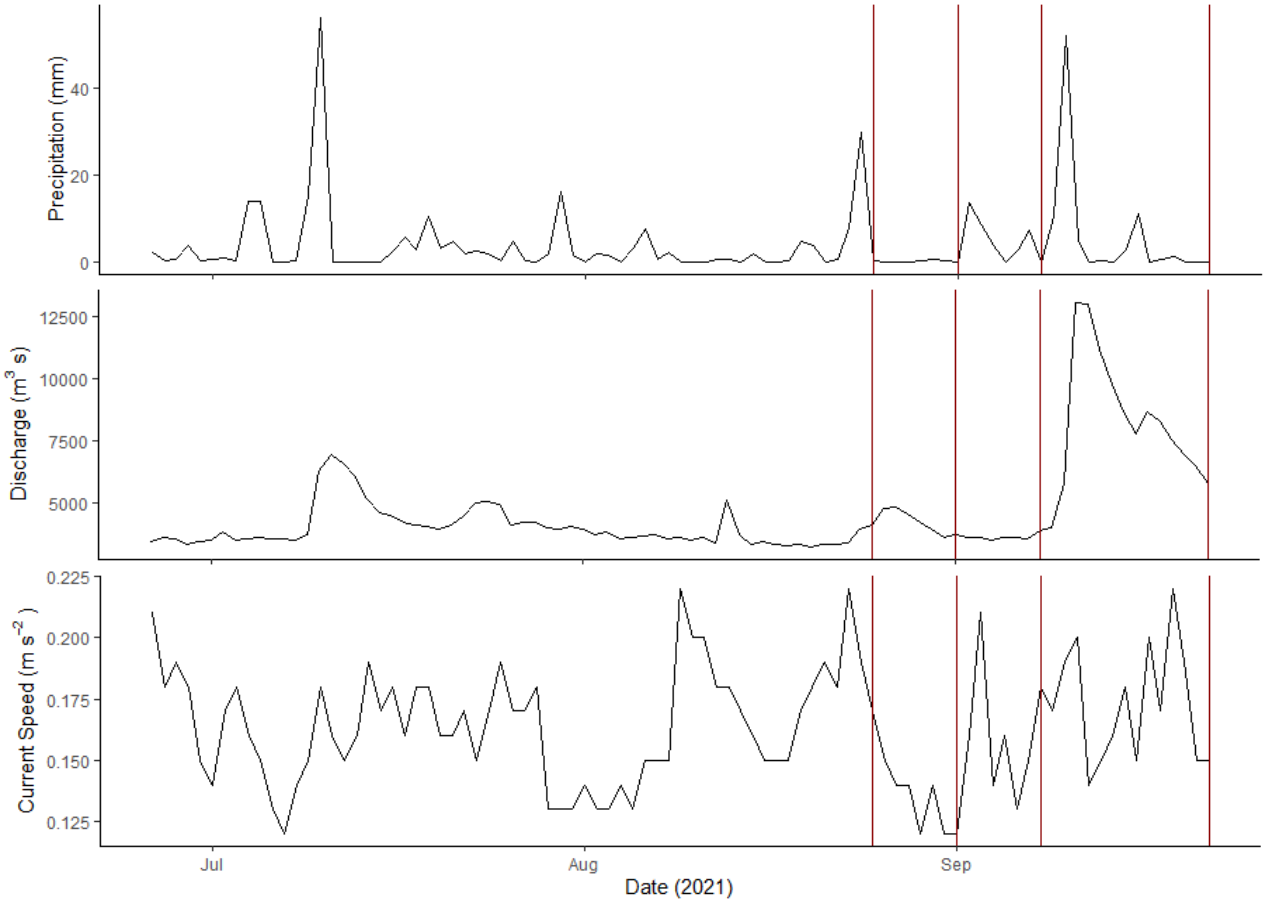

Fig. 10. Hydrologic data for the 60 days prior to Cruise 1 through Cruise 4 (June 22nd, 2021 – September 22nd, 2021). This data was utilized in the PCA analysis to see if weather events and hydrologic conditions play a role in controlling the abundance of DOM components identified in the study. Precipitation data (mm) is a daily average value for Penobscot County, ME sourced from NCEI. Discharge data (m^3 s^{-1}) is from USGS station 01034500 in West Enfield, Maine on the Penobscot River approximately 90 km north of cruise stations reported as a daily average. Current speed (m^2 s^{-1}) is reported in daily averages from the NERACOOS portal from NOAA Buoy 44033 in Penobscot Bay, ME approximately 35 km south of cruise station 3.

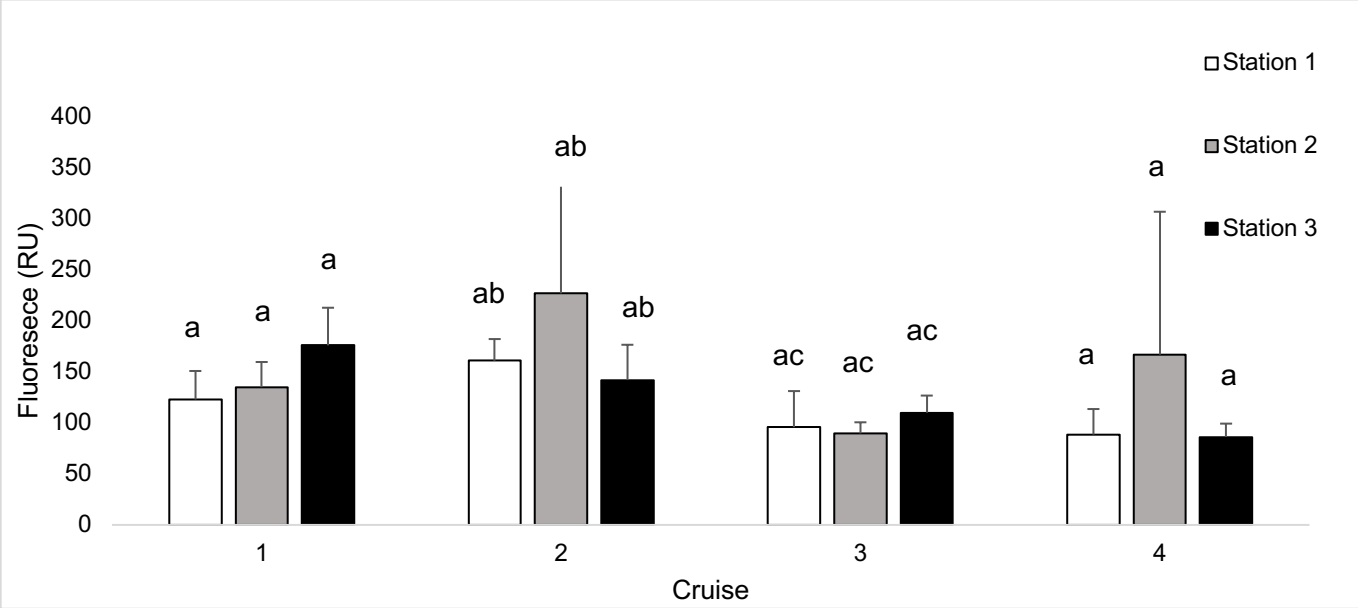

Fig. 11. Mean fluorescence signal (Raman Units) of DOM component 3 at different station over individual cruise dates (Cruise: $F_{1,\ 36} = 3.276$, $n_{Cruise\ 1} = 9$, $n_{Cruise\ 2} = 9$, $n_{Cruise\ 3} = 9$, $n_{Cruise\ 4} = 9$, $p = 0.0345$, Station: $F_{2,\ 36} = 12.67$, $n_{Cruise\ 1} = 9$, $n_{Cruise\ 2} = 9$, $n_{Cruise\ 3} = 9$, $n_{Cruise\ 4} = 9$, $p = 0.274$). Only cruises two and three were significantly different between one another, with no significant differences between the stations surveyed on each respective cruise. Stations with different letters indicate significant differences. Data are mean ± SD.

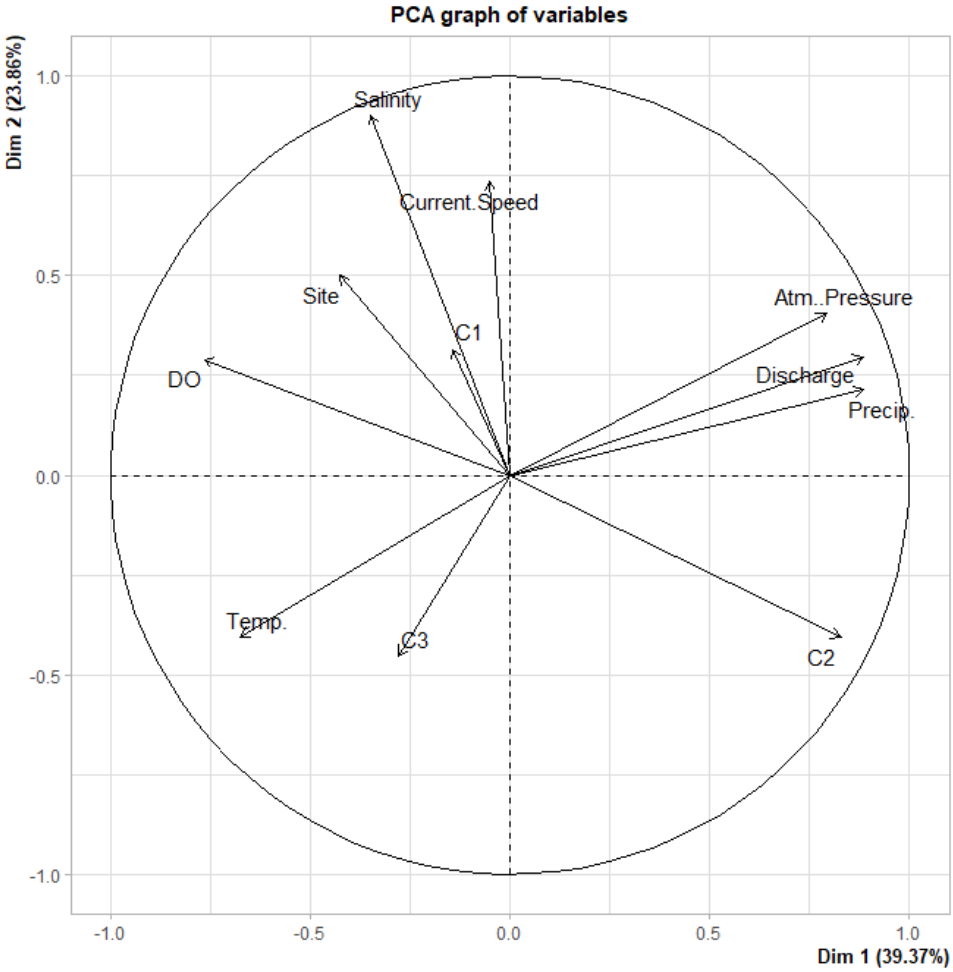

Fig. 12. Principal Component Analysis (PCA) of all environmental parameters collected during the study period in conjunction with relative abundance (RU) of the three DOM components. Vectors oriented in the same direction indicate positively correlated variables, while vectors in opposite directions indicate negatively correlated variables. Variables that are closer together are more closely related, and the length of a vector indicates how much variation in the data that variable can explain. With dimensions one and two, approximately 63.23% of the variation in the data set can be explained. Image Credit: Ellie Gellerson