

Fluorescence variability of marine and terrestrial colloids: Examining size fractions of chromophoric dissolved organic matter in the Damariscotta River estuary

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Abstract

Marine chromophoric dissolved organic matter (CDOM) imparts highly variable optical signatures in surface waters over short spatial and temporal scales, but the cause of that variability is poorly understood. A major fraction of dissolved organic matter in seawater is colloidal in size and can cycle quite rapidly, potentially contributing to the observed variability in CDOM. The relationship between marine colloids and CDOM optical variability was examined using flow field-flow fractionation (FIFFF) to partition the colloidal organic phase into a continuum of molecular sizes for optical characterization by excitation emission matrix spectroscopy (EEMS). Colloidal organic matter in surface seawater of the Damariscotta River estuary showed 2 major peaks in apparent abundance, spanning at ~1–5 kDa and ~15–150 kDa in size, respectively. The relative magnitude of these peaks changed systematically with the phase of phytoplankton blooms during 2003 and 2004, implying a relationship between colloid size distribution and bloom dynamics. Of the two colloidal sizes, the 1–5 kDa fraction was far more variable in apparent abundance than the larger colloidal matter. EEMS results reveal a compositional partitioning of protein-like and humic-like fluorescence between size fractions. Protein-like materials occurred primarily in the smallest colloid size fraction while humic-type materials resided mainly in the larger colloidal phase. These findings suggest that the fluorescence signature of bulk dissolved organic matter results from a collage of chromophores having optical characteristics that differ according to size of the molecular constituents. The colloidal-sized fluorescence characteristics of marine derived CDOM were contrasted with bulk CDOM to provide fundamental information on the distribution and forms of CDOM in Maine coastal waters. The findings here indicate that colloidal processes will have significant effects on the character and variability in the optical signature of surface seawaters.

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1. Introduction

Chromophoric dissolved organic material (CDOM) represents a major fraction of dissolved organic matter

(DOM) in seawater, and it can significantly hinder attempts to remotely sense chlorophyll in coastal waters. CDOM does not vary consistently with dissolved organic carbon (Nelson and Siegel, 2002), indicating that the chromophoric composition of DOM is not constant. The spatial and temporal variability in CDOM optical properties presumably reflects the balance between its production, transformation and removal

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from surface waters. Production of new dissolved material and its subsequent modification likely involves both biological and physical processes, including production and transformations by bacteria and algae, degradation due to photobleaching, and changing composition due to physical mixing and aggregation. Changes in CDOM composition associated with these processes will be superimposed over regional variations in the chemical composition of CDOM, particularly in nearshore waters where inputs of terrestrial humic substances can be significant.

The natural fluorescence properties of CDOM have been used to distinguish water masses in the marine environment (Baker and Spencer, 2004; Cabaniss and Schuman, 1987; Chen and Bada, 1992; Coble, 1996; Zika et al., 1993) and characterize CDOM in both the water column and sediment porewaters (Benamou et al., 1994; Burdige et al., 2004; Chen and Bada, 1994; Coble, 1996; Coble et al., 1998; De Souza Sierra et al., 1994). Excitation emission matrix spectroscopy (EEMS) is particularly well suited to analyze a mixture of fluorophores such as CDOM in seawater, utilizing multiple excitation and emission wavelengths to examine the luminescence of CDOM as a three-dimensional representation. Previous studies using three dimensional fluorescence techniques have distinguished several CDOM components with distinct optical properties in a variety of environments (Coble et al., 1990, 1998; Coble, 1996; De Souza Sierra et al., 1994; Mopper and Schultz, 1993; Parlanti et al., 2000).

Recent information on the fate and cycling of marine colloids has added an interesting dimension to the study of DOM optical variability. The definition of “dissolved” organic matter is operational, based on filter pore size cutoffs generally accepted by the bio-optical community (0.2 to 0.7 μm). Given that colloids are defined traditionally as particles 1–1000 nm in size, “dissolved” samples would include a subset of the colloidal fraction and indeed colloids comprise a significant fraction (e.g., 10–40%) of the marine DOM pool (Benner et al., 1992; Buesseler et al., 1996; Wells, 2002). The persistence of marine colloids in surface waters is largely dependent on the rates of microbial degradation (Benner et al., 1992) and co-aggregation to form sinking particles (Baskaran et al., 1992; Chin and Gschwend, 1992; Kepkay, 1994; Moran and Buesseler, 1992). In addition, recent work indicates that colloidal and dissolved fractions provide precursors for aquatic gel formation (Chin et al., 1998). The aquatic gel model implies not only that colloidal aggregation and degradation processes may be linked but also that microbial degradation of particulate gels may well induce the

continued removal of their colloidal precursors (Wells, 1998). Marine colloidal processes are believed to be rapid, so small shifts in colloid production and removal dynamics might significantly impact the abundance of dissolved chromophores and fluorophores in seawater.

Isolation of colloidal organic matter has been undertaken using a variety of methods. One of the more popular methods is cross-flow filtration (e.g., Wells, 2002), however this approach provides only a single size cut-off, and membrane performance and fouling can be problematic (Buesseler et al., 1996). Flow field flow fractionation (FIFFF) represents a more recent advance in size separation that uses an open channel to partition constituents of the colloidal phase into a continuum of macromolecular size. FIFFF has been applied to analysis of terrestrial humic and fulvic acids (Beckett et al., 1987) and more recently to marine samples (Vaillancourt and Balch, 2000; Wells, 2004; Zanardi-Lamardo et al., 2002).

We examined the size distribution of marine CDOM and its optical variability as a function of phytoplankton bloom dynamics in the Damariscotta River Estuary. By employing FIFFF with absorbance detection, a range of different size fractions of colloidal CDOM (CDOM_c) were collected for fluorescence analyses using EEMS. The optical variability and changes in apparent composition of CDOM_c were measured to determine whether the fluorescence signal of marine colloids varies with size and over time, and to provisionally assess the chemical variability of this under-explored pool of organic carbon. Evidence from fluorescence characteristics indicates that the chemical constituency of colloidal matter differs among colloidal size fractions. This study is part of a larger project studying the changes in colloid abundance and absorption coefficients as a function of colloid size and season (Floge et al., in preparation).

2. Materials and methods

Water samples were collected from the dock at the Darling Marine Center (Walpole, Maine) between January 2003 and June 2004. Using plastic gloves, glass bottles were opened and filled under the water surface to avoid contamination from the surface microlayer. Samples were immediately filtered in the adjacent lab using pre-cleaned plastic syringes and 0.22 μm syringe filters (Millex GV, Fisher Scientific). Salinities were measured with a refractometer (Fisher Scientific).

The theory of flow field flow fractionation has been described in detail elsewhere (Giddings, 1993). Briefly, a flow field is applied at right angles to the channel flow

within a shallow ($\sim 200 \mu\text{m}$) ribbon-like chamber (Fig. 1). Soluble fractions are driven through the membrane (1 kDa) on the accumulation wall, while colloidal components collect next to the accumulation wall. The resultant concentration gradient is opposed by diffusion (a function of colloidal size), resulting in colloids of different size being retained in different stream laminae above the accumulation wall. Smaller colloids will diffuse further than larger colloids, and thus when carrier flow is applied from the head of the column the smallest size fractions elute first followed by the continuum of colloids of increasing size.

The system void volume and channel thickness were determined using a 1 mg ml^{-1} solution of bovine albumin (SIGMA Chemical Company) dissolved in deionized water (Millipore). The estimated molecular weights of different colloidal fractions of CDOM were obtained by calibrating the FIFFF system with polystyrene sulfonate sodium salts (Polymer Standards Service) of varying molecular weights (from 1.3 to 350 kDa). Colloid size is related to the diffusion coefficient (D) which, in turn, affects retention time (λ) of these particles in the FIFFF systems according to the following expression:

$$\lambda = DV_0/w^2V$$

where V_0 is the channel void volume (ml), V is the crossflow rate (ml/s) and w is the channel thickness (cm)

(Giddings, 1993). The diffusion coefficient is related to molecular weight based on the assumptions used in random-coil models, and the following relationship can be applied:

$$D = AM^{-b}$$

where M is molecular weight, and A and b are constants for a particular sample-solvent system (Giddings, 1993). A molecular weight range of 1–400 kDa was analyzed for this study. Although the estimated molecular weights are indicated here, FIFFF fractograms instead are plotted according to retention times because the presumed different molecular architecture of natural colloids may well cause them to be retained differently than the standards used to “calibrate” the system.

Samples were analyzed after on-line preconcentration in the FIFFF channel to improve the channel outflow absorption signal used to characterize the colloid size distributions. 50 ml of sample material was pumped onto the column through the frit outlet, and focused at the head of the column (Lyven et al., 1997). The system flows were then applied, with colloid elution measured by UV absorption at 254 nm on the sample stream exiting the flow chamber. Separate studies demonstrated that colloid size distributions did not change with preconcentration volumes (Fløge, 2005), strong evidence that the sample preconcentration did not generate

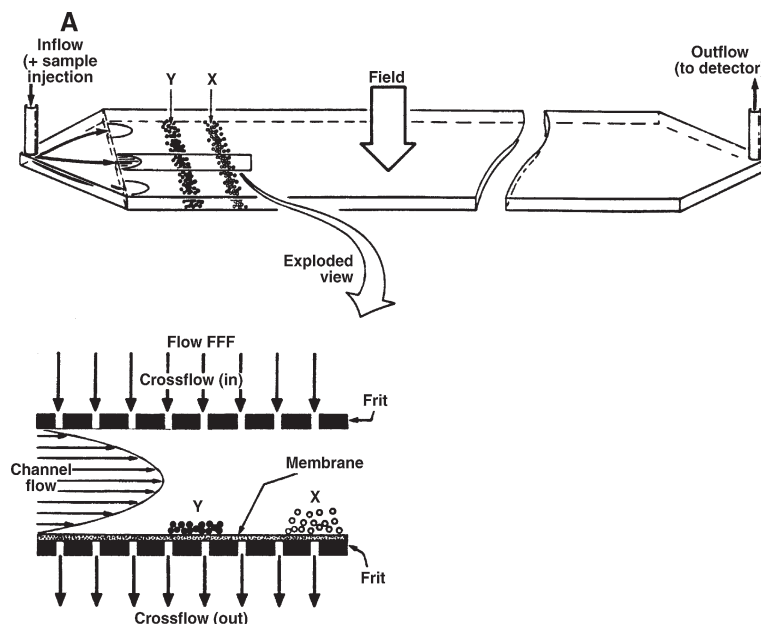


Fig. 1. Schematic of the flow-field flow fractionation channel, showing the perpendicular flow field and exploded view detailing the distribution of the flow profile and the porosity of the frit. Size separation is based upon the hydrodynamic diameter of the colloid (here, 1 kDa– $0.4 \mu\text{m}$), which affects the component's migration away from the membrane. This process separates CDOM $> 1 \text{ kDa}$ along the open channel whereby the smallest molecular weight material exits first followed by larger colloidal matter. Adapted from Giddings (1993).

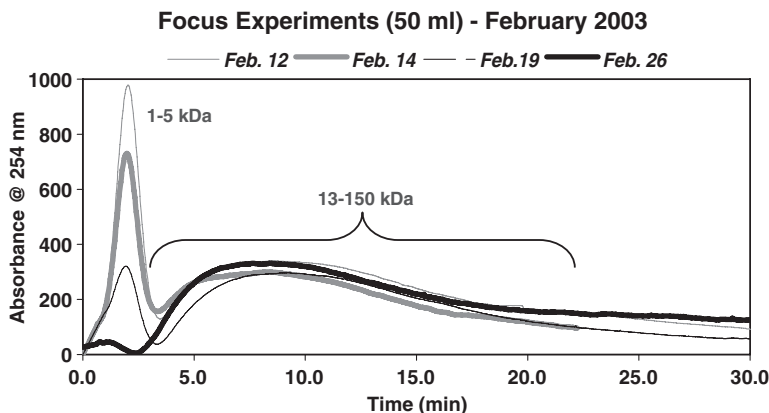


Fig. 2. Colloidal CDOM fractograms obtained by flow-field flow fractionation of surface seawater from the Damariscotta estuary. Overlaid fractograms display changes in CDOM_c over the course of a phytoplankton bloom. The first elution peak, corresponding to molecular weight standards of ~1–5 kDa, disappears rapidly as the bloom progresses. The second elution peak (~13–150 kDa) shows little variability over the course of the bloom.

artifacts in colloid size distributions by inducing aggregation. After absorbance detection, 500 μ l aliquots were collected from the sample outlet stream for EEMS analysis.

Excitation emission matrices (EEMs) of filtered seawater and FIFFF aliquots were generated on a Fluoromax-2 Spectrofluorometer (from JY-SPEX, Edison, NJ) using a low volume microcell (Hellma Industries). Experiments were run in ratio mode with a 0.5 s integration time and a 5 nm bandwidth for both excitation and emission. Analyses covered an excitation range from 220 to 390 nm in 10 nm increments and an emission range of 290–550 nm collected every 3 nm. The samples were analyzed in a quartz cell maintained at 20 °C with a temperature-controlled cell holder. The

Fluoromax-2 is equipped with a 150-W Xenon arc lamp, a single excitation monochromator (1200 grooves/mm) blazed at 250 nm and a single emission monochromator (1200 grooves/mm) blazed at 500 nm. It contains an air-cooled, red sensitive PMT. Fluorescence was corrected for instrument and lamp variability and normalized to quinine sulfate fluorescence intensity as previously described (Coble et al., 1993; method 1).

3. Results

A consistent pattern of CDOM_c size distributions was observed in all of samples from the Damariscotta River Estuary (Fig. 2). The first quantifiable peak eluted after ~2 min, representing the smallest colloidal

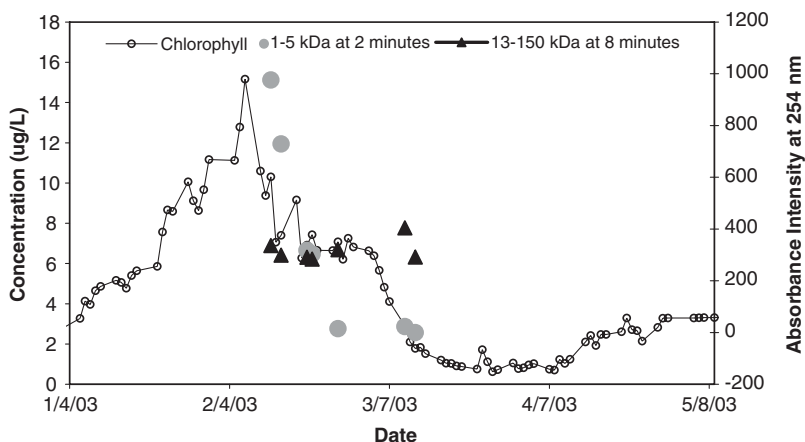


Fig. 3. Daily chlorophyll *a* data collected at the same dock and over the same time frame as the CDOM_c sample collections (Courtesy of M. J. Perry). The peak height for the ~1–5 kDa peak at 2 min is superimposed on the chlorophyll *a* data (second y-axis), and shows a clear trend of decreasing abundance over the course of the phytoplankton bloom. By comparison, the absorbance intensity for the ~13–150 kDa peak at 8 min remained relatively constant.

constituents. This colloidal retention time corresponds to materials ~1–5 kDa in size, based on the comparison to molecular weight standards. The second elution peak observed was much broader and at larger molecular weights (13–150 kDa).

Previous work has shown that colloidal organic material is produced during a phytoplankton bloom (Kep-

kay et al., 1993; Niven et al., 1994), and in light of this the changes in colloidal size fractions were examined against changes in phytoplankton abundance at the sampling site. Chlorophyll *a* concentrations measured off the DMC dock from January to May 2003 (courtesy of M.-J. Perry; <http://optics.dmc.maine.edu>) are plotted with the absorption maximum of the 1–5 kDa colloidal

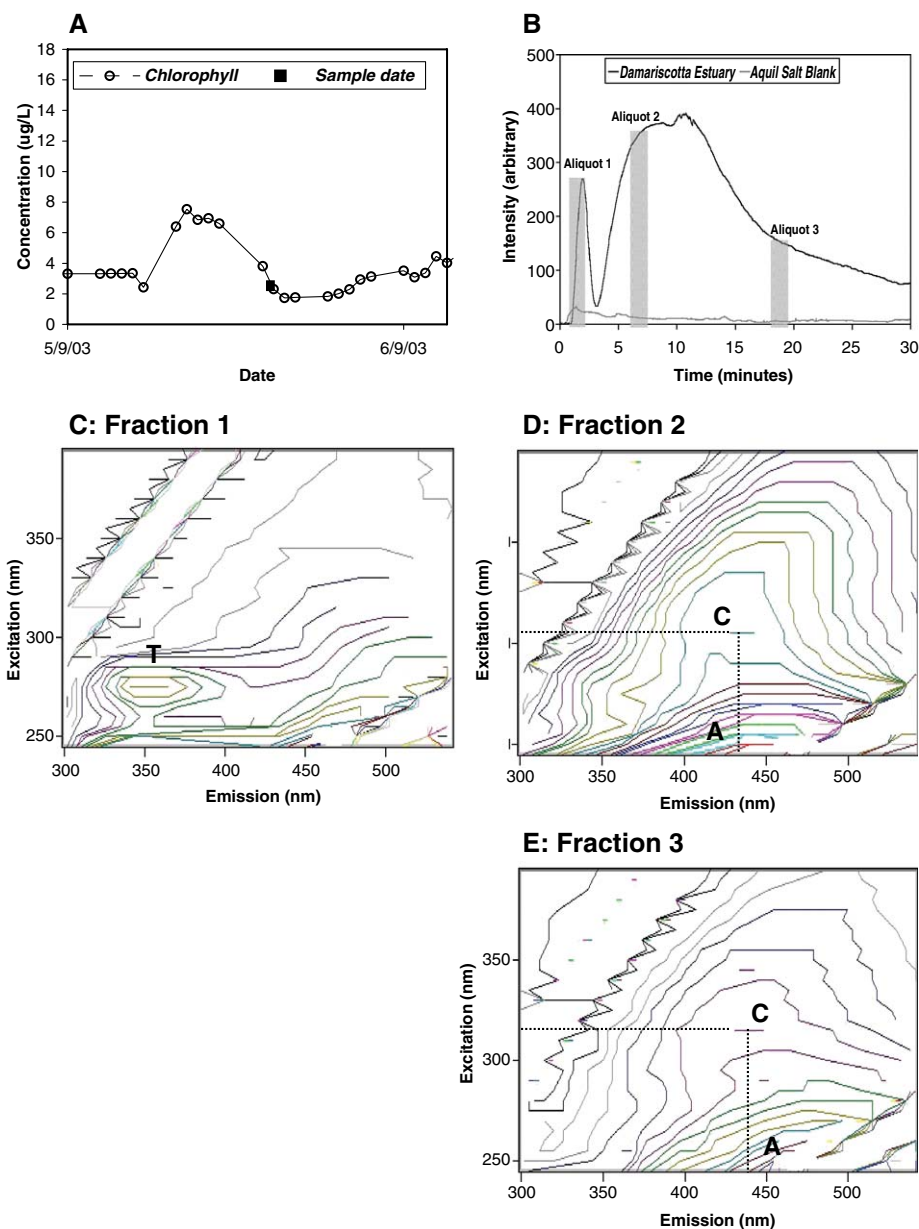


Fig. 4. A) Chlorophyll *a* data detailing bloom dynamics during mid-spring in the Damariscotta estuary. B) A fractogram profile showing the timing of three discrete fractions collected. C–E) Their respective EEMS fluorescent signatures. Peak designations A and C follow the conventions of Coble (1996). The protein-like signal in peak T ($Ex_{max}/Em_{max}=270/350$ nm) often seen in bulk seawater EEMS here clearly is associated with the smallest colloidal size fraction. The $CDOM_c$ in fractions 2 and 3 shows a significant blue-shift in the humic region associated with smaller colloidal sizes, as illustrated by the positions of the dotted lines in D and E.

peak from the month of February in Fig. 3. The apparent abundance of CDOM_c, as indicated by the absorption maximum at 2 min, decreased dramatically as the bloom expires. In contrast, the larger 13–150 kDa peak showed little if any change in intensity over the course of the bloom. Estimated abundances for both colloidal size fractions varied with a similar pattern over the course of three blooms at different times of year (Floge et al., in preparation).

EEMS analyses of discrete fractions from the FIFFF channel outflow collected at different elution times showed that the CDOM fluorescence characteristics varied across a range of colloidal sizes analyzed. Typical results are illustrated in Fig. 4 depicting a fractogram from a surface sample taken during late spring (Fig. 4A), coinciding with decreasing chlorophyll biomass at the end of a small phytoplankton bloom (Fig. 4B). The EEMS results for three retention times are shown; the initial small-sized colloidal fraction and two separate components of the larger colloidal phases. The contour plots of the fluorescence characteristics show a “protein-like” peak (Mopper and Schultz, 1993) that dominates the small, dynamic colloidal fraction (Fig. 4C, fraction 1). This peak has fluorescence characteristics similar to tryptophan, and has been previously identified as Peak T in bulk CDOM (Coble, 1996). This colloidal fraction largely lacks the fluorescence characteristics of marine or terrestrial humic matter.

In contrast, fractions from the early and later stages of the second colloidal retention peak (fractions 2 and 3) have a humic-like fluorescence signature, identified here using the labeling convention of peaks A and C (Coble, 1996). For fraction 2 (Fig. 4D), the peak A wavelength maxima (Ex_{max}/Em_{max}) occurs at 250/442 nm and peak C has an $Ex_{max}/Em_{max}=305/420$ nm. These peak positions are similar to peaks A and C observed for the bulk CDOM fluorescence characterized for this sample (data not shown). Fraction 3 (Fig. 4E) also shows A and C humic peaks, at $Ex_{max}/Em_{max}=250/458$ nm and 315/440 nm, respectively. The maxima of both peaks are shifted towards longer wavelengths relative to those in Fraction 2 and the bulk CDOM fluorescence for this sample. Peaks A and C for fraction 2 and bulk CDOM fluorescence for this study are similar to previously reported data for river and coastal regions in Maine (Coble, 1996; Mayer et al., 1999). To summarize, the fluorescence data clearly show differences in optical characteristics of CDOM_c among colloidal size fractions that imply a non-homogeneous composition of the colloidal organic phase, and that the abundance of CDOM_c changes over short time scales in association with the rapid increase and decline of phytoplankton populations.

4. Discussion

It is difficult to critically assess the cycling of marine CDOM in surface waters because its molecular constituents are unknown. While we have gained some understanding of the general effect that input (phytoplankton) and loss (photochemistry, microbial degradation) processes can have under laboratory conditions, the mechanistic basis for these changes, the fraction of the CDOM they affect, and the rates at which they operate in coastal and offshore waters are poorly understood. A central question is whether marine CDOM represents an optically (and thus chemically) uniform organic matrix, or instead, as suspected, it is an assemblage of different chromophore-containing molecular constituents spread non-homogenously through the broader dissolved organic milieu. The work here helps to address this question by ascertaining whether fluorescent CDOM constituents occur in the colloidal phase, and whether these fluorescence characteristics differ with colloidal size. If so, then physical as well as biological cycling of the bulk colloid carbon matrix may well contribute to the variability of marine CDOM in ways not predicted by simple chromophore reactivity.

We sought here to quantify the fluorescence characteristics of the more abundant colloidal constituents in the seawaters of the Damariscotta River estuary. This estuary has very low freshwater inflows and significant diurnal tidal pulsing up and down the inlet. As a consequence, bloom dynamics can be observed at a fixed point. The decline in phytoplankton biomass shown in Fig. 3 reflects then the bloom progression and is not the consequence of the lateral movements of patchy chlorophyll-containing surface waters past our sampling site.

The mass of colloidal CDOM is assumed here to be proportional to the magnitude of UV absorption at 254 nm. However, this view does not take into account how colloid shape and density may influence light scattering (apparent absorption), or that UV absorbing moieties may not be uniformly distributed within the organic phases comprising marine colloids. It is worth remembering then that the colloid size distributions shown here may not provide an accurate representation of colloidal mass distributions.

The two dominant colloidal size fractions (~1–5 kDa and 13–150 kDa) in the Damariscotta River estuary were not closely coupled, their relative abundances changing substantially over the sampling period. The majority of this change was attributable to fluctuations in the smaller colloidal material (~1–5 kDa), which shifted from high to low abundance over

a 2-week period coincident with decreasing chlorophyll biomass (Fig. 2). We have observed the reverse pattern (increasing abundance of small colloids) during the onset of phytoplankton blooms at this site (Floge et al., in preparation). Production of new colloidal organic carbon has been observed before and during phytoplankton blooms (Kepkay et al., 1993; Niven et al., 1994) and has been presumed to be associated with carbon exudation by phytoplankton and bacteria, release of cell products by viral lysis, and as a by-product of zooplankton and flagellate feeding (Niven et al., 1994 and references therein). These findings indicate that the very smallest of the colloidal phase may comprise a major fraction of this colloidal carbon release, and that this colloidal matter is reactive.

In contrast, the larger colloidal fraction (~13–150 kDa) was comparatively uniform in both its apparent abundance and size distribution over the same time period. This apparent stability may indicate that this colloidal fraction is chemically less labile or physically less affected by colloidal processes than the smaller colloid fraction. However, this stability also might conceal a tight coupling between dynamic production and loss processes; a balance not seen for the smaller colloidal fraction. The rapid decline in small colloid abundance with chlorophyll (Fig. 3) indicates these materials have comparatively short lifetimes, consistent with independent evidence that turnover of colloidal organic carbon can proceed at time scales of hours to days (Baskaran et al., 1992; Moran and Buessler, 1992, 1993). In any event, our findings show strong temporal variability in the persistence of these different colloidal CDOM size fractions.

Our EEMS data suggest that there are significant differences in the dominant molecular composition of small and larger colloidal phases, and that these differences are consistent with their (apparent) relative reactivities. The small colloid fraction (~1–5 kDa) contains by far the majority of the “protein-like” fluorescence signal ($E_{x_{max}}/E_{m_{max}}=275/350$ nm) among the size fractions examined, while the larger colloidal size fraction has an optical signature more characteristic of humic substances. Even so, the large colloidal matter (~13–150 kDa) is not optically homogeneous but contains a bathochromic shift (“blue shift”) in the peak humic fluorescence with decreasing colloid size, whereby the maximum in humic fluorescence moves to lower excitation and emission wavelengths (Fig. 4). A similar blue-shift in fluorescence has been noted in coastal waters as CDOM sources alter from terrestrial to marine (Coble, 1996; De Souza Sierra et al., 1994). This shift also has been attributed to changes in the pi bond

electron system and functional groups of CDOM related to biological and photochemical modifications (Moran and Zepp, 1997; Senesi et al., 1991), and ostensibly the production of new material (Coble et al., 1998). Because freshwater inputs to this site are small, particularly during winter months, it is likely that this size-related bathochromic shift in humic fluorescence reflects in-situ degradation (microbial and photochemical) or the release of new matter rather than the presence of terrestrially derived colloidal matter. Regardless, these findings strongly suggest that the composition of CDOM varies across the colloidal size continuum.

Although the “protein-like” fluorescence signature of the small colloidal fraction is not analytically diagnostic it is suggestive that this fraction might serve as a rich nitrogen source for bacteria, consistent with the apparently rapid turnover of this colloidal phase. This “protein-like” fluorescence of CDOM was observed to be very short-lived in estuaries, which was taken as evidence of its lability (Mayer et al., 1999) and microbial degradation has been shown to be a sink for colloidal organic carbon (Amon and Benner, 1996, 1994). Even so, evidence from carbon isotopic analyses of marine colloidal matter suggests that colloids >10 kDa are of more recent origin than the smaller colloidal organic phases (Santschi et al., 1995), implying a higher turnover rate for larger colloids than smaller colloids and truly soluble organic matter. The rapid loss of the small colloidal CDOM indicated here thus may reflect less on its chemical reactivity than on its proclivity for transport into larger size classes.

Colloid aggregation can be a major sink for marine colloidal matter under some conditions, transferring carbon into larger colloidal and particulate matter (Kepkay, 1994) or forming marine gels (Chin et al., 1998). Here, the different chromophoric character of small and larger colloidal phases indicates that aggregation was not a source of the larger colloids measured here, at least with respect to chromophorically labeled organic carbon. Nonetheless, we cannot rule out aggregation as being a significant process transferring the small colloidal phase into sizes >350 kDa. Similarly, while secondary photochemical reactions with DOM can cause the formation of lower molecular weight organic products (Moran and Zepp, 2000, 1997; Miller and Moran, 1997), the fluorescently distinct signature of the small colloid phase suggests it did not originate from the breakdown of larger colloidal matter. However, as noted above it is possible that photochemical processes contributed to a gradual decrease in size within the larger colloid fraction (~13–150 kDa).

Our findings provide the first clear optical evidence that marine CDOM is not a uniform matrix of recycled organic phases, but instead comprises subcomponents that can exist physically distinct from other constituents. Ascertaining the relative and absolute changes in the contributions of these fractions, and processes that control their cycling, will be key for achieving predictive insights to marine CDOM behavior.

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