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Marsh-Exported Dissolved Organic Matter Fate in Estuaries

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Marsh-Exported Dissolved Organic Matter Fate in Estuaries: Bioavailability and Photoreactivity

Submitted by:

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In fulfillment of
the Master of Science degree
in Earth and Atmospheric Sciences
at the City College of New York
Table of Contents

1. Abstract ......................................................................................................................... 3
2. Introduction .................................................................................................................. 4
   2.1. Overview and motivation ...................................................................................... 4
   2.2. Tidal marshes as sources of carbon to estuarine and coastal waters ................. 5
   2.3. Dissolved organic matter ..................................................................................... 6
   2.4. Linkages between DOM composition and optical characteristics ..................... 6
3. Methods ......................................................................................................................... 8
   3.1. Research sites ...................................................................................................... 8
   3.2. Collection of water samples, handling and storage ........................................... 9
   3.3. Incubations ......................................................................................................... 9
   3.4. Photobleaching experimental setup .................................................................. 10
   3.5. Measurements ...................................................................................................... 10
   3.6. CDOM absorption and fluorescence analysis .................................................... 12
   3.7. Heat treatment .................................................................................................... 12
   3.8. Refiltering ............................................................................................................ 14
4. Rhode River sub-estuary DOM quality ....................................................................... 17
   4.1. Results ................................................................................................................ 17
      4.1.1. Initial conditions ......................................................................................... 17
      4.1.2. Photobleaching ......................................................................................... 17
      4.1.3. Microbial degradation ............................................................................. 18
      4.1.4. Effects of photobleaching on microbial degradation ............................. 18
      4.1.5. Fluorescent DOM relative composition ............................................... 19
   4.2. Discussion ............................................................................................................ 25
      4.2.1. Initial conditions ...................................................................................... 25
      4.2.2. Photobleaching ....................................................................................... 26
      4.2.3. Microbial degradation ............................................................................ 27
      4.2.4. Effects of photobleaching on microbial degradation ............................. 28
      4.2.5. Fluorescent DOM relative composition ............................................... 29
5. Marsh DOM quality seasonality .............................................................................. 30
   5.1. Results ................................................................................................................ 30
      5.1.1. Initial conditions ...................................................................................... 30
      5.1.2. Photobleaching ....................................................................................... 30
      5.1.3. Microbial degradation ............................................................................ 31
      5.1.4. Effects of photobleaching on microbial degradation ............................. 32
   5.2. Discussion ............................................................................................................ 38
      5.2.1. Initial conditions ...................................................................................... 38
      5.2.2. Photobleaching ....................................................................................... 38
      5.2.3. Microbial degradation ............................................................................ 39
      5.2.4. Effects of photobleaching on microbial degradation ............................. 40
6. Summary and conclusions .......................................................................................... 41
7. References ...................................................................................................................... 43
1. Abstract

Dissolved organic matter (DOM) is a significant driver of estuarine productivity and nutrient cycling. The colored component of DOM, chromophoric dissolved organic matter (CDOM), impacts coastal optical properties, ocean color, and light attenuation. While marshes are largely considered sinks for carbon due to their high productivity and low soil carbon degradation rates, laterally they are sources of carbon as optically and chemically distinct DOM to surrounding aquatic ecosystems; these inputs are often essential in sustaining a net heterotrophic system. However, the photoreactivity and bioavailability of marsh-exported DOM is largely uncategorized, thus making it difficult to quantify its impacts on estuarine dynamics.

The photoreactivity and bioavailability of chromophoric and fluorescent dissolved organic matter (CDOM and FDOM) from various sources within the Rhode River, a brackish, eutrophic, sub-estuary during the summer, and seasonally across four Chesapeake Bay marshes, were examined. Microbial (14 d in the dark) and photochemical incubations (7 d in light followed by 7 d in dark) were performed, and changes to DOM optical properties were measured using spectrophotometry (CDOM absorption coefficients and spectral slopes) and spectrofluorometry (FDOM excitation-emission matrices analyzed using parallel-factor analysis, PARAFAC). 0.2 μm filters were used for “photochemical-only” treatments, whereas glass-fiber filters (GF/Fs) were used for microbial treatments. Initial results indicated the presence of bacterial cells in 0.2 μm filtrates; however, heating the filtrates in a water bath at 60°C for 30 min successfully killed the bacteria, while still preserving the DOM optical properties. Re-filtering both 0.2 μm or GF/F filtrates with a 0.2 μm filter at the end of a dark incubation resulted in the loss of 5-10% of the CDOM absorption coefficient at 300 nm (proxy for CDOM amount), indicating the removal of aggregates that may have formed over the 7 d incubation; studies that have utilized this method for estimating microbial bioavailability could be greatly overestimating the microbial degradation of CDOM, since reported losses due to microbial degradation are often of similar magnitudes.

Results showed that CDOM absorption coefficients, molecular weight, and total FDOM were greatest at the marsh site in the Rhode River, and that these parameters decreased down-estuary; CDOM absorption coefficients and total FDOM at marsh sites decreased in colder seasons, indicating lower export in colder months, while CDOM quality remained the same throughout. Photobleaching resulted in a net loss of CDOM and humic-like FDOM and a decrease in CDOM molecular weight, while microbial degradation resulted in a net loss, or no change, of CDOM absorption coefficients and a net increase in humic-like FDOM. Photoreactivity decreased down-estuary, with distance from terrestrial DOM sources, while microbial degradation increased. Seasonally, marsh-DOM photoreactivity increased in the colder seasons, most likely due to the lower previous exposure to UV-radiation in the winter compared to the summer; microbial degradation was much more variable seasonally. Photobleaching increased the bioavailability of marsh-derived CDOM and FDOM, resulting in higher rates of production of humic-like DOM, and a greater loss of CDOM. Jug Bay, a freshwater marsh down-stream of a major sewage treatment plant, had the greatest loss of CDOM by photobleaching and microbial degradation, and the smallest increase in humic-like FDOM due to microbial degradation compared to the other marshes. It also showed the greatest change in CDOM quality (e.g., molecular weight) with photobleaching, indicating that it is particularly photoreactive as well. This highlights the significance of human activity on marsh-exported DOM quality and export, and the potential impacts of these changes on estuarine carbon dynamics.
2. Introduction

2.1. Overview and motivation

Dissolved organic matter (DOM) is one of the most significant factors impacting aquatic productivity, nutrient cycling, and coastal optical properties. It fuels heterotrophic production in aquatic environments by providing carbon and nitrogen, it limits the amount of UV-radiation and visible light in coastal waters, and it makes up one of the largest reservoirs of carbon (in oceans, the carbon in DOM is estimated to about equal the amount of atmospheric CO$_2$). Despite its importance, DOM composition and transformation remain largely unknown, and some estimates propose that less than 10% of DOM molecular compounds have been categorized (Repeta, 2002). Wetlands export huge quantities of optically and chemically distinct DOM to aquatic ecosystems. Despite the importance of tidal marshes as major sources of dissolved carbon to estuaries and surrounding waters, carbon fluxes are not well-quantified in these ecosystems, and wetland contributions (or transformations) of carbon to adjacent waters are currently not included in biogeochemical models.

Rivers and estuaries export $0.2 \times 10^{15}$ g of dissolved organic carbon (DOC) annually (Meybeck, 1982), making estuaries one of the most important sources of DOM to marine ecosystems. However, only a relatively small portion of terrigenous DOM makes up the oceanic DOM pool (Hedges, 1992), indicating rapid cycling by both photochemical and biological processes in estuaries. One of the largest unknowns in coastal carbon cycling is the quantity and nature of DOM removal by microbial degradation in estuarine ecosystems. This is even further complicated when accounting for the various sources of DOM to estuaries and the impact of source on DOM quality and transformation. As a result, an essential piece in understanding the dynamics and functioning of temperate estuaries is missing, making it difficult to predict the impacts of climate change and coastal land-use changes on coastal carbon cycling and estuarine productivity.

The objective of my thesis research was to conduct a detailed optical characterization of DOM exported by various marsh-systems on Chesapeake Bay on the basis of bioavailability and photoreactivity. Chesapeake Bay is the largest estuary in the United States and drains a watershed that is heavily urbanized and has significant agricultural production, thereby contributing to eutrophic estuarine conditions and potential wetland loss. NOAA’s 2014 Fisheries and Economics of the U.S. Report indicated that the commercial seafood industry in Maryland and Virginia contributed $775 million in income and about 32,000 jobs to the local economy, making understanding estuarine productivity in this region essential. The overarching objective is supported by three main research objectives (ROs):

RO #1: Quantify the bioavailability and photoreactivity of DOM from different sources in the Rhode River sub-estuary, a eutrophic sub-estuary along the western shore of Chesapeake Bay surrounded by a 2300-ha watershed with mixed land use; sources of DOM in this system include marsh-exported DOM, watershed DOM, a mixture of terrestrial and estuarine DOM, and estuarine DOM. The Rhode River is located near the Smithsonian Environmental Research Center (SERC). It has numerous long-term ecological and biogeochemical studies conducted in it (Jordan, et al., 1983; Jordan, et al., 1991; Megonigal & Schlesinger, 1997; Tzortziou, et al., 2008), thus allowing for comparison of results to previous experiments.

RO #2: Quantify the bioavailability and photoreactivity of marsh-exported DOM from different brackish and freshwater marsh-systems throughout the Chesapeake Bay and analyze the spatial and seasonal variation in bioavailability and photoreactivity.

RO #3: Assess the influence of photochemical transformations on the bioavailability of DOM.
2.2. Tidal marshes as sources of carbon to estuarine and coastal waters

Wetlands are extremely productive ecosystems, responsible for a number of important processes such as carbon sequestration and storage (e.g., blue carbon), methane emissions, and nutrient cycling. Wetlands have some of the highest productivity rates of any ecosystem and their relatively low decomposition rates due to saturated, anoxic soil conditions result in the sequestration and storage of huge amounts of carbon. While wetland area makes up only about 6 to 7% of the total global area, they contribute to nearly 7 to 15% of global terrestrial productivity, and are estimated to store more than half of the global soil carbon (Tarnocai, Canadell, Schuur, Kuhry, Mazhitova, & Zimov, 2009). Tidal marshes are particularly productive; Chunara, et al. (2003), for example, estimated that tidal marshes and mangrove swamps store at least 44.6 Tg C yr⁻¹, globally, which is higher than the estimate for peatland carbon storage. While the saturated, anoxic soils of wetlands contribute to soil carbon storage, they also facilitate the production and subsequent release of methane (CH₄) to the atmosphere. Due to few available oxidants in wetland soils, methanogenesis is often the dominant (and final) pathway of carbon degradation. Estimates of CH₄ release by wetlands globally are around the order of 100 to 231 Tg yr⁻¹ CH₄, significantly offsetting potential carbon storage benefits (Schlesinger & Bernhardt, 2013).

The constant contact of wetland soils and vegetation to aquatic environments combined with anaerobic soils, which inhibit organic matter degradation, result in the export of DOM laterally to adjacent waters, either through leaching and diffusion from the soil into floodwater or through groundwater flow (Wetzel, 1992; Nixon, 1980; Jordan, et al., 1983; 1991; Schiff, et al., 1998; Tzortziou, et al., 2008; 2011). The export of DOM to rivers, lakes, and estuaries by wetlands exceeds in concentration the export by other ecosystems such as upland forests (Dalva & Moore, 1991) and variation in DOC fluxes between different river-systems has been attributed to wetland area within the watershed (Gergel, et al., 1999). While autochthonous DOM production in estuaries by phytoplankton is also a significant contributor to the estuarine DOM pool (Peterson, et al., 1986), in many aquatic ecosystems, terrestrial inputs of DOM and nutrients exceed autochthonous production (Schlesinger & Bernhardt, 2013), resulting in a net heterotrophic system. The DOM exported by marshes is chemically and optically distinct from DOM produced in surrounding aquatic systems (Thurman, 1985; Tzortziou, et al., 2008). For example, Helms, et al. (2008) found that wetland DOM had extremely high molecular weight. Tzortziou, et al. (2008) found that DOM exported from a brackish marsh had strong light-absorption and a high fluorescence signal compared to DOM in the surrounding estuary, and that these optical properties were typical of high-molecular weight, aromatic, and complex DOM.

Therefore, the scale and nature of DOM export from wetlands to estuarine ecosystems has substantial impacts on the biogeochemistry, optical properties, and biology of coastal waters. The loss of wetlands through sea-level rise coupled with human activities such as subsidence, decreased sediment loading, and other environmental stressors has enormous implications for coastal water quality and productivity. For example, Duan, et al. (2017) found a 58% decrease in DOC concentrations in the tributaries of the Mississippi River due to wetland loss, suggesting that the consistent source of DOC that wetlands provided to the surrounding aquatic system had been eliminated. Pendleton, et al. (2012) estimated that wetland loss has led to an increase the release of CO₂ by 0.15 to 1.02 Pg yr⁻¹ through both the lost potential of wetland carbon sequestration as well as changes in C soil wetland stocks. To fully understand the impacts of wetland loss on aquatic ecosystems such as estuaries, an understanding of marsh-DOM export and quality is essential.
2.3. **Dissolved organic matter**

DOM is the dissolved, soluble portion of organic matter, operationally defined as compounds that pass through a 0.2 μm filter (Tzortziou, et al., 2008) or that pass through a glass fiber filter (GF/F) with a nominal pore size of 0.7 μm (Fellman, et al., 2008), although other pore sizes are also used (e.g., 0.45 μm) (Karavanova, 2013). The use of other filter sizes for isolating DOM becomes problematic when comparing across studies, since the DOM fraction passing through the filter is dependent on the filter pore size. Collectively, DOM is composed of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorous (DOP), thus its importance in nutrient cycling.

The primary origin of DOM from terrestrial ecosystems is still somewhat debated by the community, since DOM has been observed to originate both from recent litter as well as from stable organic matter in the lower soil organic horizons. For plant litter, bacterial and fungal degradation of vascular plant detritus produces soluble organic compounds (e.g., intermediates of lignocellulose degradation), which make up a large portion of the DOM pool (Kaiser & Guggenberger, 2000). In addition, during the microbial degradation process, additional DOM is added to the DOM pool by the micro-organisms themselves (e.g., exopolysaccharides). Root exudates of carbohydrates and amino acids are also an important source of DOM in soils (Fellman, et al., 2008). Others believe that the predominant source of DOM is from the leaching and microbial decay of soil humus in the lower organic horizon, particularly since there is a much higher percentage of humus to soil litter (Kalbitz, et al., 2000).

DOM quality, which is strongly related to DOM source and DOM transformation processes, is dependent on the type of DOM compounds present. While specific compounds are largely uncategorized, groups of compounds can be determined using a number of methods (Repeta, 2002; Fellman, et al., 2010). DOM is known to be composed of aromatic DOM compounds, lignin, tannins and polyphenols, complexed amino acids (all of which are part of the hydrophobic fraction), and carbohydrates, small carboxylic acids, free proteins and peptides (all part of the hydrophilic fraction) (Inamdar, et al., 2011). DOM quality is related to the relative contributions of these different groups of compounds, and the light absorbing or fluorescing pools of DOM can be quantified using methods such as spectrophotometry and spectrofluorometry, which are both cost and time efficient.

2.4. **Linkages between DOM composition and optical characteristics**

DOM composition as it relates to carbon, nitrogen, and phosphorous functional groups, classes of compounds such as amino acids, carbohydrates and lignin phenols, and labile vs. refractory pools is relatively well-categorized (Repeta, 2002); however, these methods are often time-consuming, costly, and require large sample volumes (Fellman, et al., 2010), making it difficult to apply these techniques across a variety of ecosystems. Optics provides an alternative method for the characterization of DOM, thus allowing for a more comprehensive analysis of the colored and fluorescing pools of DOM across many systems. The pool of DOM that absorbs light selectively, chromophoric dissolved organic matter (CDOM), can be analyzed using absorption parameters such as the absorption slope from 275 – 295 nm ($S_{275-295}$), 350 – 400 nm ($S_{350-400}$), and the slope ratio ($S_R$, defined as the ratio of $S_{275-295}$ to $S_{350-400}$). The molecular weight of DOM has been shown to be negatively correlated to $S_R$ (Helms, Stubbins, Ritchie, Minor, Kieber, & Mopper, 2008) and the extent of photochemical degradation to be positively correlated to $S_{275-295}$ (Helms, et al., 2008; Tzortziou, et al., 2008). Hernes & Benner (2003) found the absorption coefficient at 350 nm ($a_{CDOM,350}$) to be associated with dissolved lignin concentration. The specific UV-absorbance at 254 nm (SUVA_{254}), which is CDOM absorbance
at 254 nm divided by DOC concentration, has been used as a measure of CDOM aromaticity (Weishaar, Aiken, Bergamaschi, Fram, Fujii, & Mopper, 2003).

Fluorescence spectroscopy can also be used as a proxy for DOM composition. Fluorescence excitation-emission matrices (EEMs), in which emission scans measure fluorescence intensity for a range of excitation wavelengths, provides detailed information on the fluorophores present in fluorescent DOM (FDOM), a subset of CDOM. Coble (1996) identified 5 peaks in EEMs from samples collected from a variety of freshwater, coastal, and marine environments (Table 1). Collectively Peak B and Peak T constitute the protein-like peak often used in the literature. The use of parallel factor analysis (PARAFAC) to examine EEMs in recent years has allowed for a more comprehensive classification of DOM fluorophores and composition (Stedmon & Markager, 2005; Yamashita, et al., 2008); PARAFAC decomposes the EEM signal into components that make up that signal and determines the contribution of those components to the total fluorescence (Stedmon & Bro, 2008). Some common components determined through EEM-PARAFAC are similar to fluorescence peaks identified by Coble (1996) (Table 1).

Table 1. Commonly identified PARAFAC components and their corresponding peaks identified in Coble (1996). Adapted from Fellman, et al., 2010.

<table>
<thead>
<tr>
<th>Component</th>
<th>Excitation and emission maxima (nm)</th>
<th>Peak Name (Coble, 1996)</th>
<th>This Study</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine-like</td>
<td>Ex: 270-275 Em: 304-312</td>
<td>B</td>
<td>C4</td>
<td>Amino acids; more degraded peptide material</td>
</tr>
<tr>
<td>Tryptophan-like</td>
<td>Ex: 270-280 (&lt;240) Em: 330-368</td>
<td>B, T</td>
<td>C4</td>
<td>Amino acids; intact or less-degraded peptide material</td>
</tr>
<tr>
<td>Ultraviolet A</td>
<td>Ex: 290-325 (&lt;250) Em: 370-430</td>
<td>M</td>
<td>C3</td>
<td>Low molecular weight, associated with biological activity</td>
</tr>
<tr>
<td>(UVA) Humic-Like</td>
<td>Ex: &lt;260 Em: 448-480</td>
<td>A</td>
<td>C1</td>
<td>High molecular weight, aromatic humic</td>
</tr>
<tr>
<td>UVC Humic-Like</td>
<td>Ex: 320-360 Em: 420-460</td>
<td>C</td>
<td>C1</td>
<td>High molecular weight humic</td>
</tr>
<tr>
<td>UVC Humic-Like</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The composition of DOM (i.e., the molecular and optical characterization) impacts its bioavailability and photoreactivity, and vice-versa; for example, humic and aromatic DOM is more photoreactive (Lu, Bauer, Canuel, Yamashita, Chambers, & Jaffè, 2013), whereas protein-like DOM has been shown to be more bioavailable (Fellman, et al., 2008). Therefore, DOM composition can be used to determine the dominant degradation processes under certain environmental conditions, as well as the processes that have already affected the DOM. While photochemical degradation of terrestrial DOM has been shown to lead to an increase in DOM bioavailability (Tranvik, et al. 1997; Raymond & Bauer, 2000; Bracchini, et al., 2006), its influence on the bioavailability of marsh-exported DOM has not been as widely studied. Recent studies also acknowledge the contribution of labile DOM from wetlands (Fellman, et al., 2008); however, the relative importance of environmental factors (salinity and microbial communities) and source material (related to marsh vegetation type) is largely unknown, and therefore, so is the spatial and temporal variability in microbial and photochemical degradation rates of DOM in marsh-estuarine systems.
3. Methods

3.1. Research sites

Figure 1. (a) Sampling sites on the Rhode River sub-estuary, and; (b) marsh sampling sites on Chesapeake Bay. Marsh sites are denoted in green.

To quantify the bioavailability and photoreactivity (as well as their interplay) of DOM from different sources (RO #1 and #3), incubation experiments were performed on samples collected from various sites at low tide in the Rhode River sub-estuary (Figure 1a), during late June to July 2016. The Kirkpatrick brackish marsh, also known as the Global Change Research Wetland (GCRReW), is an intensive study site sampled monthly as part of the NASA MarshCycle Project (Lead PI: Maria Tzortziou) and is the principal source of marsh DOM to the Rhode River. Additional sampling was conducted at the Fish Weir, SERC Dock, Canoe Shed, and Rhode River Mouth (RR Mouth). The Canoe Shed site is located upstream of Muddy Creek, and is surrounded mostly by forested area and mud flats, and thus DOM from this site is considered “non-marsh terrestrial” or watershed DOM. The Fish Weir is located at the intersection of Muddy Creek and the Rhode River, and therefore, is a mixture of watershed DOM and estuarine DOM. The influence of GCRReW may also be significant at the Fish Weir depending on the tidal stage (e.g., high tide). The SERC dock is predominantly estuarine DOM, with very little marsh influence. Lastly, the Rhode River Mouth is where the West River opens to the main stem of Chesapeake Bay; it is therefore the most down-estuary endmember of all the investigated sites.

To quantify the bioavailability and photoreactivity (as well as their interplay) of marsh-exported DOM across a range of environmental characteristics (ROs #2 and #3), photobleaching and microbial incubation experiments were performed on samples collected from various freshwater and brackish marshes on Chesapeake Bay during low tide (Figure 1b) over the summer and fall of 2016 and the winter of 2016 to 2017. GCRReW is a high-elevation tidal marsh with predominantly *Spartina patens*, *S. cynosuroides*, *Distichlis spicata*, *Iva frutescens*, and *Scirpus olneyi*; it’s total soil organic carbon (TOC) is about 80%. Taskinas is dominated by *Spartina patens* and *Distichlis spicata*; it’s TOC is about 23%. Jug Bay and Sweet Hall are freshwater marshes. Jug Bay is located on the Patuxent River and is highly influenced by urban and suburban development (Swarth, et al., 2012); Jug Bay is also close to a wastewater treatment
plant, making it eutrophic. The predominant vegetation species are *Leersia oryzoides*, *Hibiscus moscheutos*, *Peltandra virginica*, *Phragmites australis*, *Polygonum arifolium*, and *Typha × glauca* (high marsh) and *Nuphar lutea*, *Pontederia cordata*, and *Z. aquatic* (low marsh) (Swarth, et al., 2012). The sampling site for Jug Bay is located near a low marsh region. It has an average TOC of 6%. Sweet Hall is dominated by *Peltandra virginica*, *Carex stricta*, *Leersia oryzoides*, *Polygonum punctatum*, and *Polygonum arifolium* (Perry & Atkinson, 1997), and it has an average TOC of 58%.

3.2. Collection of water samples, handling and storage

Samples were collected for the incubation experiments from the study sites shown in Figure 1 over the summer and fall of 2016, and winter of 2016-2017 (Table 2). All water samples were collected no more than 30 min before or after low tide. Samples were stored in the refrigerator and began being filtered on the same day as collection. The exceptions were Sweet Hall and Taskinas, which had to be transported to SERC from the Virginia Institute of Marine Science, and thus these were filtered within three days of collection. We used glass-fiber filters (GF/F, nominal pore size of 0.7 μm) to remove particulate material but retain bacteria in the filtrate (used for the bacterial incubations); it is important to note that only a portion (35-43% according to one study, Lee, et al., 1995) of the total bacterial cell count passes through a GF/F; the filter preferentially removes larger cells (e.g., diameters greater than 0.8 μm). A portion of the GF/F filtrate was then filtered through 0.2 μm pore-diameter membrane filters to remove most of the bacteria and separate only the DOM component (used as a dark control for assessing only the photochemical reactivity of DOM) (Lu, et. al., 2013). Filtered water was stored in the dark at 4°C for less than 1 week before further analysis. Incubations were started within 1-2 days of sample filtration. Samples were measured for absorption spectra or fluorescence within two days of the incubation time point, and were stored in a refrigerator at 4°C until sample measurement. The exception was for Incubation 16-8 (October 2016), in which travel to and from SERC prevented immediate measurements; absorption and fluorescence measurements for this incubation were conducted within 2 weeks of the incubation measurement time point, and samples were stored in a refrigerator at 4°C between the incubation time point and measurement.

Table 2. Dates and sampling sites for incubation experiments performed from July 2016 to January 2017.

<table>
<thead>
<tr>
<th>Incubation ID/Date</th>
<th>Sampling Site(s)</th>
<th>Date of Sampling</th>
<th>Heat Treatment? (See §3.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-6/Jul 2016</td>
<td>Canoe Shed and RR Mouth</td>
<td>6/28/16</td>
<td>No</td>
</tr>
<tr>
<td>16-6/Jul 2016</td>
<td>Fish Weir and Jug Bay</td>
<td>6/29/16</td>
<td>No</td>
</tr>
<tr>
<td>16-7/Jul 2016</td>
<td>Jug Bay and Taskinas</td>
<td>7/20/16</td>
<td>No</td>
</tr>
<tr>
<td>16-7/Jul 2016</td>
<td>Dock and GCREW</td>
<td>7/21/16</td>
<td>No</td>
</tr>
<tr>
<td>16-8/Oct 2016</td>
<td>Taskinas and Sweet Hall</td>
<td>10/18/16</td>
<td>No</td>
</tr>
<tr>
<td>16-8/Oct 2016</td>
<td>GCREW and Jug Bay</td>
<td>10/19/16</td>
<td>No</td>
</tr>
<tr>
<td>17-1/Jan 2017</td>
<td>Taskinas and Sweet Hall</td>
<td>1/4/17</td>
<td>Yes</td>
</tr>
<tr>
<td>17-1/Jan 2017</td>
<td>GCREW and Jug Bay</td>
<td>1/5/17</td>
<td>Yes</td>
</tr>
</tbody>
</table>

3.3. Incubations

Water samples from all sites (Figure 1) were collected and filtered for 2-week dark and light incubation experiments. There were four treatments for the incubations: dark incubation of 0.2 μm (assuming no bacteria, thus, "control", "C"), dark incubation of GF/F (microbial-only degradation, "MD"), light incubation of 0.2 μm (photochemical-only degradation "PB"), and
light incubation of GF/F (combined microbial and photochemical degradation "MD + PB"). Exactly 100 mL of the GF/F filtrate was distributed either into combusted 120 mL amber bottles (dark treatments) or Teflon bottles (light treatments). For the control and photochemical-only treatments, exactly 100 mL of the 0.2 μm filtrate was distributed into the combusted amber bottles for dark treatments or into Teflon bottles for the light treatments. Starting in January 2016, the 0.2 μm samples that were used for the control and photochemical-only treatments underwent a heating treatment before incubating the samples, to kill any remaining bacteria (see §3.7).

All four treatments were incubated for 2-weeks. Four replicate bottles were used for the microbial-only treatment, two for the control, and three each for the photochemical-only and combined treatments. Dark treatments (control and microbial-only) remained in the dark over the course of the 2-week incubation and were inverted once per day to reduce settling any settling of aggregates that may have formed over the course of the incubation (see §3.8). Measurements were taken at three time points: day 0, day 7, and day 14. Light treatments (photochemical-only and combined) were placed over low-level continuous UV light for 1 week and then in the dark for 1 week, and inverted once per day. While undergoing UV-degradation, bottles were rotated positions each day to ensure equal irradiation. The combined treatments were placed in the dark for the second week to quantify the impacts of photochemical degradation on bioavailability (RO #3).

3.4. Photobleaching experimental setup
For the photochemical incubation experiments, a UV-transmitting acrylic Plexiglas sheet was placed about one inch above two Q-labs UVA340 lamps. Teflon bottles with 100 mL of the sample filtrates were placed on top of the UV-transmitting sheet, with the center of the bottles centered on the lamp tube, as shown in Figure 2. 100 mL of filtrate filled the Teflon bottles to a depth of about 4 cm. UV spectral irradiance (290-650 nm) and PAR (400-700 nm) was measured at the surface of the Plexiglas using a fiber optic spectroradiometer, as described by Neale & Fritz (2001), and a QSL 2100 probe, respectively. Total UV irradiance was 17.2 W m\(^{-2}\); the UV exposure over 24 h was about the same as the UV-exposure over a clear summer day in the Rhode River (Neale, et al., 2005).

![Figure 2. UV exposure experimental setup. Teflon bottles were filled with 100 mL of filtrate and placed on the UV-transmitting plastic for 7 d. Samples were rotated each day to ensure consistent UV-exposure across samples.](image)

3.5. Measurements
The following parameters were measured for all incubations on day 0, day 7, and day 14 of the incubation: CDOM absorption spectra (\(a_{\text{CDOM}λ}\)) and DOM fluorescence excitation-emission matrices (EEMs). The absorption of CDOM at 300 nm (\(a_{\text{CDOM}300}\)) was used as an indicator of CDOM concentration and quality, since higher concentrations of aromatic CDOM have higher absorption in the UV wavelengths. The following spectral slopes derived from \(a_{\text{CDOM}λ}\) were also calculated: \(S_R\), \(S_{275-295}\), and \(S_{350-400}\). Later incubations also included
measurements of bacterial counts by Blake Clark using a flow cytometer at day 0, 7, and 14. We conducted a parallel factor (PARAFAC analysis) on fluorescence excitation-emission matrices measured. Our model output four components: 3 humic-like and 1 protein-like (Figure 3) (Table 3).

![Figure 3. Four fluorescence components identified by our PARAFAC model. Components identified as (a) visible-emitting humic (VIS-Humic); (b) VIS-Humic; (c) UV-emitting humic (UV-Humic); and (d) Protein-Like.](image)

**Table 3. PARAFAC components identified in this study and their probable sources.**

<table>
<thead>
<tr>
<th>Component Number</th>
<th>Component Name</th>
<th>Excitation maximum (nm)</th>
<th>Emission maximum (nm)</th>
<th>Probable sources (Fellman, et al., 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>VIS-humic</td>
<td>245 (350)</td>
<td>456</td>
<td>“Terrestrial”, commonly found in wetlands and forests</td>
</tr>
<tr>
<td>C2</td>
<td>VIS-humic</td>
<td>245 (400)</td>
<td>514</td>
<td>“Terrestrial”, commonly found in wetlands (Wagner, et al., 2015)</td>
</tr>
<tr>
<td>C3</td>
<td>UV-humic</td>
<td>240 (310)</td>
<td>392</td>
<td>Associated with biologic activity; commonly found in wastewater, wetland and agricultural environments</td>
</tr>
<tr>
<td>C4</td>
<td>Protein-like</td>
<td>275</td>
<td>322</td>
<td>Fresh or degraded peptide material or proteins; phytoplankton, bacterial processing</td>
</tr>
</tbody>
</table>
Our C1 (VIS-humic) component contained both Peak A and C (Coble, 1996) (Table 1), and is associated with “terrestrial” sources of DOM (Yamashita, et al., 2010); it has a high contribution of high-molecular weight compounds relative to low-molecular weight compounds and has a high aromaticity. C2 (VIS-humic) was not defined by Coble (1996), but has been observed in wetland ecosystems (Wagner, et al., 2015). It has similar properties to C1, but has higher aromaticity, is of higher molecular weight, is more depleted in N, and has higher contributions of CHOS groups (Wagner, et al., 2015). C3 (UV-humic) is equivalent to Peak M identified by Coble (1996) (Table 1), and tends to be lower-molecular weight and more aliphatic compared to C1 and C2. Our C4 component was the combined signal of tryptophan and tyrosine (Table 1), commonly referred to as “protein-like.”

3.6. CDOM absorption and fluorescence analysis

CDOM absorption was measured using a CARY-IV dual-beam spectrophotometer, from 270 to 750 nm, at 2 nm intervals. 1 cm path-length, acid-washed and deionized water (DI)-rinsed, quartz cuvettes were used. Measurements were baseline corrected using DI, with a blank run at the beginning and end of the run, and every 5 samples. Duplicate measurements were performed on each sample. 

\[ a_{CDOM\lambda} = 2.303 \frac{OD}{l_g} \]

\( S_{275-295} \) and \( S_{350-400} \) were estimated following Helms, et al. (2008), using the log-transformed linear regression. \( S_R \) was then calculated by taking the ratio of \( S_{275-295} \) and \( S_{350-400} \).

FDOM excitation-emission matrices (EEMs) were measured using a SPEX Fluoromax-3 spectrofluorometer. Fluorescence was corrected for absorption within the sample (inner-filter effect) using the absorption spectra measured spectrophotometrically. Excitation intervals were 5 nm and emission were 2 nm. An DI EEM was measured for each set of sample EEMs run; after the inner-filter corrections, the DI EEM was subtracted from the sample EEM and then converted to Raman Units, using the area under the Raman scattering peak (excitation: 250 nm and emission: 370 to 428 nm).

3.7. Heat treatment

In January 2017, we began heating the control and photochemical-only treatments bottles in a water bath at 60°C for 30 min and then allowed the samples to return to room temperature before incubating, to prevent bacterial growth in our 0.2 μm samples. Although most studies have assumed samples filtered through a 0.2 μm filter are sterile and contain no bacteria (Zhang, et al., 2013; Lu, et al., 2013; Stedmon & Markager, 2005), our results from samples collected in October 2016 showed that while the initial bacterial counts of 0.2 μm-filtered samples were orders of magnitude lower than GF/F-filtered samples, at the end of a 1-week incubation, there was an increase in bacterial counts in the 0.2 μm filtered samples, indicating bacterial growth. We found no significant difference (p = 0.1, 0.7, 0.02, and 0.04 for GCReW, Jug Bay, Sweet Hall, and Taskinas, respectively) in bacterial count between the two pore sizes after 1-week of incubation (Figure 4). The heating of the 0.2 μm filtrates successfully killed bacteria and prevented bacterial growth over the course of 5 days (Figure 5). No consistent nor significant changes (p = 0.84, 0.76, 0.56, 0.96, 0.92, and 0.89, for C1, C2, C3, C4, \( a_{CDOM300} \), and \( S_R \), respectively) in DOM optical properties were observed by heating the samples, indicating no significant changes to DOM quality (Figure 6).
Figure 4. Microbial counts for incubations performed on filtrate from 0.2 μm (dark grey) and GF/F (light grey).

Figure 5. Microbial counts from heating experiment incubations conducted in January 2017 on samples filtered through 0.2 μm, not heated (black) and heated (red).
Figure 6. Optical properties for the January 2017 heating experiment incubations on Day 0 for 0.2 μm filtered samples not heated (dark grey) and heated (red).

3.8. Refiltering

Because DOM is most often defined as organic compounds that pass through a 0.2 μm filter, it is important to justify the use of GF/F (nominal pore size of 0.7 μm) for incubation experiments. Previous incubation experiments have measured biogeochemical and optical properties on 0.2 μm filtrate, inoculated the 0.2 μm filtrate with bacteria, and incubated the mixture. At the end of the incubation, the samples are then refiltered through a 0.2 μm filter, and re-measured. This approach was not suitable for our sites. Refiltering the 0.2 μm control filtrate at the end of the incubation (even without the addition of a bacterial inoculum) resulted in a loss of DOM (up to a 10% loss in \( a_{\text{CDOM}300} \) over the course of 1-week) (Figure 7). This loss by refiltering is of similar magnitude to reported losses by microbial degradation; as a result, studies utilizing this method could be greatly overestimating the microbial degradation, even by a factor
of 2. The portion of DOM that was lost by refiltering is the protein-like component, indicating the potential removal of protein-like aggregates that could have formed over the course of the incubation. Other incubation studies have conducted optical and biogeochemical measurements on samples containing a bacterial inoculum (often GF/F filtrate) without re-filtering (Stedmon & Markager, 2005; Lu, et al., 2013). Our measurements of DOM filtrate from 0.2 μm filters and GF/F across all sites on Day 0 showed no significant difference in any of the optical properties (p = 0.97, 0.93, 0.95, 0.17, 0.66, and 0.91) for C1, C2, C3, C4, aCDOM, and S_R respectively), indicating no significant difference in humic DOM between the two pore sizes (Figure 8), despite the fact that the GF/F filtrates looked to have a slightly higher contribution of the protein-like PARAFAC fluorescence component in all sites (Figure 8d). However, there was no significant difference (p = 0.17) between the filter sizes, so the GF/F filtrates were deemed sufficiently similar to the 0.2 μm filtrates to justify their use for microbial treatments.

Figure 7. Absorption of CDOM at 300 nm (proxy for CDOM concentration) for sites from Incubation 16-4 on Day 14 before refiltering (black) and immediately after refiltering (grey): (a) GCReW and (b) the Dock; and, (c) Percent of CDOM lost from refiltering for sites from Incubation 16-4 on Day 14: (c) GCReW and (d) the Dock.
Figure 8. Optical properties showing the difference between 0.2 µm filtered and GF/F filtered samples on Day 0 (all sites and all incubations).
4. Rhode River sub-estuary DOM quality

4.1. Results

The following results are from photochemical and microbial incubation experiments conducted in July 2016 on sites in the Rhode River sub-estuary (Figure 1a).

4.1.1. Initial conditions

Marsh-exported DOM (collected from GCReW at low tide) had the highest overall fluorescence and absorption followed by DOM collected from the watershed (Canoe Shed) (Figure 9). DOM absorption and fluorescence gradually decreased along the salinity gradient from the Fish Weir site to the Dock and to the Rhode River Mouth.

All humic-like fluorescence components showed a steep decrease in concentration with distance from terrestrial sources (Figure 9a). C4 (protein-like component) was more consistent across sites, but still decreased slightly with distance from terrestrial sites (data not shown). C1 and C2 (VIS-humic components) decreased faster down-estuary than C3 (UV-humic component) and C4 (protein-like component) (Figure 9b-d). C1, C2, C3 and C4 ranged from 1.6 to 0.2 RU, 0.57 to 0.08 RU, 1.0 to 0.2 RU, and 0.2 to 0.1 RU, respectively, with the highest values at GCReW and the lowest at the Rhode River Mouth.

$a_{\text{CDOM}}300$ decreased from the marsh/watershed sites to the down-estuary sites, and $S_R$ increased (Figure 9e-f); for GCReW and the Rhode River Mouth, $a_{\text{CDOM}}300 = 36.7$ and 6.3 m$^{-1}$ and $S_R = 0.9$ and 1.5, respectively. The $S_R$ increase with distance from terrestrial sources was the result of both the increase in the slope from 275-295 nm and the decrease in the slope from 350-400 nm with distance from terrestrial sites (Figure 9g-h).

4.1.2. Photobleaching

The more “terrestrial” DOM samples (GCReW, Canoe Shed and Fish Weir) had the greatest relative loss of humic-like DOM fluorescence and CDOM absorption by photobleaching, while changes in protein-like fluorescence were highly variable across sites.

The 0.2 μm filtrate light treatments showed a significant decrease in all humic-like components, with an average loss of 71, 36, and 78% (s.d. = 5.9, 11.2, and 4.7%) over 7 d for C1, C2 and C3, respectively. The loss of C1 (VIS-humic component) was highest in “terrestrial” sources (Canoe Shed, 79% loss) and lowest down-estuary (Rhode River Mouth, 62% loss) (Figure 10a). The loss of C1 at GCReW (71%) was lower than both the Canoe Shed and Fish Weir (79 and 74%). The loss of the C2 (longer-wavelength VIS-humic component) also decreased with distance from terrestrial sites, though much more variably (Figure 10b). C3 (UV-humic component) decreased about equally in all sites except for GCReW, with a loss of 83, 80, 78, and 79% for the Canoe Shed, Fish Weir, Dock, and Rhode River Mouth, respectively (Figure 10c). GCReW lost only 70% of C3 over the 7 d. The protein-like component (C4) showed much more variation than the humic components, with some sites increasing with photobleaching and others decreasing, though only the Fish Weir and Dock showed a significant change (a gain of 11.4% and loss of 27%, p = 0.002 and 0.03, respectively) (Figure 10d).

CDOM absorption at 300 nm decreased in all sites, with little variation between sites, with an average loss of about a 52% (s.d. = 2.9%) over 7 d. However, the Dock showed a smaller loss of $a_{\text{CDOM}}300$ compared to the other sites (about 45% over 7 d) (Figure 10e). $S_R$ increased significantly for all sites, with the greatest increase occurring in the Canoe Shed (83%), and with steadily lower increases down-estuary (Figure 10f). The $S_R$ increase was the result of both the
increase $S_{275-295}$ (Figure 10g) and the decrease in $S_{350-400}$ (Figure 10h), which occurred in all sites except the Rhode River Mouth, where $S_{350-400}$ showed no significant change.

4.1.3. Microbial degradation

Microbial degradation produced humic-like DOM for all sites, but at a much lower magnitude compared to the loss by photobleaching. The down-estuary site (Rhode River Mouth) produced more humic-like DOM than the marsh site (GCReW).

C1 (shorter-wavelength VIS-humic component) increased for all sites after 14 d of the dark incubation with GF/F filtrate (Figure 11a), with an average loss of 4.9% (s.d. = 1.1%), though the change was only marginally statistically significant at the Canoe Shed and Dock (p < 0.05) and not statistically significant at the other sites. The increase in C1 was greater with distance from the marsh (an increase of 3.8% and 6.5% for the GCReW and the Rhode River Mouth, respectively). C2 (longer-wavelength VIS-humic component) increased for all sites over the 14 d period (Figure 11b), with an average increase of 5.2% (s.d. = 2.9%). C2 increased by 6.3% and 8.7% (p = 0.1 and 0.02) for GCReW and the Rhode River Mouth, respectively. C3 (UV-humic) also increased in all sites (Figure 11c), with an average increase of 7.2% (s.d. = 3.2%) over all sites. The greatest increases occurred in the Canoe Shed and Rhode River Mouth (10.7 and 9.7%, p = 0.1 and 0.0007, respectively), whereas the Dock showed the smallest increase in C3 after 14 d (2.4%, p = 0.09). The C4 (protein-like component) had the greatest variation in increase across sites (Figure 11d). The Canoe Shed, Fish Weir and Rhode River Mouth had an extremely large increase in C4 over the 14 d dark incubation (47, 109, and 36%, p = 0.008, 0.3, and 0.07, respectively). GCReW and the Dock showed the smallest increase in C4 over the 14 d dark incubation (6.5 and 0.83%, p = 0.4 and 0.6, respectively).

Regarding CDOM absorption, the only sites that showed a significant change were GCReW and the Rhode River Mouth (loss of 3 and 11% in $a_{CDOM300}$ over 14 d, p = 0.001 and 4.6E-5, respectively). $a_{CDOM300}$ at the Dock increased marginally significantly over the 14 d dark period (2.5%, p = 0.05). The other sites showed no significant change in $a_{CDOM300}$ (Figure 11e). $S_R$ increased across all sites except the Rhode River Mouth. $S_R$ increased at GCReW, the Canoe Shed, the Fish Weir, and the Dock, on average by 3.8% (s.d. = 4.7%). However, the increase was statistically significant only for GCReW (increase of 2.9%, p = 0.0003) and the Dock (increase of 9.4% and p = 0.01). An opposite pattern was observed for $S_R$ at the Rhode River Mouth, with a decrease of 4.6% over 14 d (p = 0.003). $S_{275-295}$ decreased in all sites except the Rhode River Mouth over the 14 d dark incubation, with an average loss of 3.6% (s.d. = 2.2%) (Figure 11g). The greatest loss occurred for the Canoe Shed and the lowest for GCReW (6.6 and 1.5%, p = 0.0008 and 0.0003, respectively). Unlike at the other sites, $S_{275-295}$ at the Rhode River Mouth increased by 8.7% (p = 0.0003) over the 14 d dark incubation.

4.1.4. Effects of photobleaching on microbial degradation

Photobleaching increased the magnitude of humic-like DOM production by microbial degradation, particularly in the marsh and watershed DOM sites (GCReW and Canoe Shed). $S_q$ and $S_{275-295}$ decreased for all sites, while $S_{350-400}$ increased.

C1 increased over the 7 d dark microbial degradation (after 7 d of photobleaching), increasing, on average, by 16.5% (s.d. = 2.7%) (Figure 12a). The increase was greater in the marsh and watershed DOM sites (GCReW and Canoe Shed) compared to the down-estuary site (Rhode River Mouth), though this difference was not significant. C2 was more variable (Figure 12b). The only marginally significant change was an increase of 7.8% (p = 0.02) for the Canoe Shed. C3 increased the most of the humic-like FDOM components over the 7 d microbial
incubation after photobleaching, with an average increase of 28.5% (s.d. = 10.3%) (Figure 12c). The greatest increase in C3 was for the watershed DOM sites (Canoe Shed and Fish Weir: ΔC3 = 34 and 38%, p = 0.001 and 0.08, respectively) and in the down-estuary site (Rhode River Mouth: ΔC3 = 36%, p = 0.05). The protein-like component was much more variable and showed no significant changes for any site (Figure 12d).

a_{CDOM-300} increased for some sites (Fish Weir and Dock: Δa_{CDOM-300} = 3.9% and 6.8%, p = 0.2 and 0.0002, respectively) and decreased for others (GCREW, Canoe Shed, and Rhode River Mouth: Δa_{CDOM-300} = −9.1%, −1.4%, and −3.2%, p = 0.001, 0.02, and 0.05, respectively). There was no down-estuary trend in a_{CDOM-300} (Figure 12e). S_R decreased for all sites by, on average, 4.2% (s.d. = 3.1%), though there were no significant differences in S_R loss between sites (Figure 12f). The greatest loss in S_R occurred in the Canoe Shed (7.3%, p = 0.002), and the lowest loss occurred in GCREW (1.12%, p = 0.1). S_{275-295} either showed no significant change (GCREW, Fish Weir, and Rhode River Mouth), or decreased (Canoe Shed and Dock, ΔS_{275-295} = −3.7 and −4.1%, p = 0.002, and 0.01, respectively). S_{350-400} was much more variable; the only site that showed a significant change was the Canoe Shed (an increase of 3.8%, p = 0.005). The other sites showed no significant change.

4.1.5. Fluorescent DOM relative composition

Photobleaching followed by microbial degradation of marsh and watershed DOM resulted in a fluorescence signature more similar to the initial down-estuary FDOM. Initially (0 d), the DOM composition showed a relative decrease of the VIS-humic components and a relative increase in the protein-like component with distance from terrestrial sites (Figure 13a). The relative contribution of the UV-humic component showed little variation across sites. After 7 d of the light 0.2 μm treatment, the relative contribution of the humic-like components was lower than the initial (0 d) and showed much smaller variation across sites (Figure 13b). Because of this decrease, the C2 (VIS-humic) and C4 (protein-like) components made up a much greater percentage of the total fluorescence in the photobleached treatment. Microbial degradation only (14 d of the GF/F filtered dark incubation) (Figure 13c), resulted in little difference in the composition of the DOM compared to the initial, other than a slight increase in the relative contribution of the protein-like component across all sites. The combination of microbial and photochemical degradation smoothed out differences in DOM composition across the salinity gradient (Figure 13d). GCREW after being photobleached and microbially-degraded (Figure 13d), had a similar FDOM signature to the down-estuary initial DOM signature (e.g., the Dock or the Rhode River Mouth), though the C3: UV-humic component made up a significantly lower percentage of the total fluorescence.

Total DOM fluorscence decreased from the marsh (GCREW) to the down-estuary end-member (Rhode River Mouth) (Figure 13e). In addition, total DOM fluorescence was much lower in the two treatments that were exposed the UV-radiation (Figure 13f and h) compared to the microbial-only treatment (Figure 13g). The greatest absolute change due to photobleaching occurred in C1 (VIS-humic) and C3 (UV-humic). C2 (VIS-humic) and the protein-like component were more photo-refractory. Microbial degradation (Figure 13g) increased the total fluorescence slightly, specifically in C3 (UV-humic). Microbial degradation after photobleaching slightly offset the changes in photobleaching, by increasing the fluorescence slightly (Figure 13h compared to f).
Figure 9. Optical parameters for GF/F (0.7 μm) filtrates initially (0 d) for two incubations performed in July 2016. Sites are ordered starting with the marsh (GCReW LT), and then ordered by distance from terrestrial site (Canoe Shed) to the most saline estuarine site (Rhode River Mouth).
Figure 10. Change in optical properties after 7 d of light incubations performed on 0.2 μm filtrate. Sites are ordered from marsh/terrestrial to the most saline estuarine site. The change for each parameter was calculated by dividing the difference between the 7 d and initial (0 d) values by the initial value, multiplied by 100%.
Figure 11. Change in optical properties after 14 d of dark incubations performed on GF/F filtrate. Sites are ordered from marsh/terrestrial to the most saline estuarine site. The change for each parameter was calculated by dividing the difference between the 14 d and initial (0 d) values by the initial value, multiplied by 100%.
Figure 12. Change in optical properties over two 7 d dark incubations performed in July 2016 on GF/F filtrates from the Rhode River sub-estuary sites after a 7 d light incubation. The change was calculated by dividing the difference between the values at the end of the 7 d dark incubation and the start of the 7 d dark incubation (which was also the end of the 7 d light incubation) by the values at the start of the 7 d dark incubation, multiplied by 100%.
Figure 13. (Top) Relative contributions of each PARAFAC component to the total fluorescence for each incubation treatment and (Bottom) Fluorescence of each PARAFAC component in Raman Units for each incubation treatment: (a/e) Initial (0 d) GF/F filtrate; (b/f) GF/F dark incubation after 7 d; (c/g) 0.2 μm light incubation after 7 d; and, (d/h) GF/F dark incubation after 7 d, after a 7 d light incubation. Sites are ordered from marsh/terrestrial to the most saline estuarine site.
4.2. Discussion
4.2.1. Initial conditions

Our results showed a steeper gradient (change by a factor of 0.88 and 0.87, respectively) in the two visible humic-like fluorescence components (C1 and C2) across the salinity gradient from the marsh to the Rhode River mouth compared to the UV-humic (C3) and protein-like (C4) components (change by a factor of 0.79 and 0.48, respectively). These results indicate either the preferential loss of the longer wavelength and higher molecular weight, fluorescent, humic-like components relative to C3 and C4, additional sources of C3 and C4 down-estuary resulting in a lower net loss, or most likely, both. Since C1 and C2 have higher aromaticity, and therefore, are expected to be more photoreactive (Wagner, et al., 2015), their loss down-estuary could be the result of preferential photo-degradation; in addition, C3 and C4 are both associated with microbially-processed and biological material (Coble, 1996; Fellman, et al., 2010), whereas C1 and C2 are associated with terrestrial sources (Coble, 1996; Fellman, et al., 2010; Yamashita, et al., 2010; Wagner, et al., 2015). Thus, C3 and C4 fluorescence down-estuary could be supplemented by autochthonous production in the estuary. An increase in C3/C1 and C4/C1 down-estuary is also suggestive of a higher contribution of lower-molecular weight DOM down-estuary, since the UV-humic (C3) component is associated with lower molecular weight humic-like DOM, and the protein-like component (C4) even more so (Coble, et al., 1996; Wagner, et al., 2015).

Consistent with the fluorescence results, the CDOM absorption coefficient ($a_{CDOM,300}$) decreased and $S_R$ increased with distance from terrestrial sites, which indicates a decrease in both CDOM concentration and in DOM molecular weight and aromaticity down-estuary. This is in agreement with other studies of CDOM dynamics across transects from a terrestrial to an estuarine (or coastal ocean) end-member (Hernes & Benner, 2003; Tzortziou, et al., 2008; Helms, et al., 2008; Yamashita, et al., 2008; Dalzell, et al., 2009; Fellman, et al., 2011). The change in $S_R$ observed from GCReW to the Dock ($S_R = 0.92$ to 1.3) was remarkably consistent with Tzortziou et al. (2011), in which the $S_R$ for a transect conducted in the Rhode River during the summer (June to July) in 2008 was 0.95 at GCReW (Station A1) and 1.3 at the Dock (Station A14). This indicates that the marsh is exporting DOM of similar quality, and/or that the transformation and autochthonous production of DOM down-estuary is occurring in similar magnitudes, inter-annually. However, estimates of $a_{CDOM,300}$ from Tzortziou, et al. (2011) using their values of $a_{CDOM,440}$, the spectral slope, and the equation: $a_{CDOM,300} = a_{CDOM,440}e^{-(S_{300 nm} - 440 nm)}$ from Helms, et al. (2008) produced $a_{CDOM,300}$ values of 26.3 m$^{-1}$ and 7.67 m$^{-1}$ for GCReW (Station A1) and the Dock (Station A14), respectively. The estimated value for GCReW is lower than our value of 36.7 m$^{-1}$, though the Dock is consistent inter-annually with a value of 7.70 m$^{-1}$. So, while the quality of DOM being exported by the marsh is consistent inter-annually, the magnitude of marsh-DOM export varies.

Our measurements show that during ebbing tide, the Rhode River marsh (GCReW) exports high molecular weight (low $S_R$) and strongly-absorbing (high $a_{CDOM,300}$) CDOM to the surrounding estuarine water (Tzortziou, et al., 2008; Tzortziou, et al., 2011). The CDOM-enriched water is then diluted with estuarine water characterized by lower CDOM absorption and higher CDOM absorption spectral slope ratio, down-estuary; in addition, photobleaching of CDOM contributes to the loss in $a_{CDOM,300}$, as is indicated by the higher $S_R$ and $S_{275-295}$ with distance from terrestrial source. For samples collected along a transect in the Delaware Estuary, Helms, et al. (2008) observed higher $S_R$ values compared to predicted $S_R$ values from a two-endmember conservative mixing model for samples within the estuary; the higher $S_R$, compared to that expected from conservative mixing, was attributed to photobleaching. They thus proposed
that the optical changes down-estuary are the result of: the mixing of low $S_R$ (terrestrial) DOM with high $S_R$ (autochthonous or photobleached terrestrial) DOM, increasing $S_{275-295}$ down-estuary (photobleaching impacts), and decreasing $S_{350-400}$ down-estuary (microbial impacts); this is consistent with the initial optics of the Rhode River sub-estuary sites in our incubation experiments. Our results are also consistent with measurements by Tzortziou et al. (2011), which concluded that CDOM changes along the full extent of the Rhode River estuarine gradient are consistent with photodegradation as the dominant transformation process of the photo-labile marsh-DOM components over short time-scales.

4.2.2. Photobleaching

The loss of the VIS-humic DOM fluorescence component C1 (up to 80% loss over 7 d) with photobleaching is consistent with the literature. Moran, et al. (2000) showed that the greatest loss by photobleaching in estuarine DOM occurred in the Peak C fluorescence region identified in Coble, et al. (1996), which is the same spectral region (excitation = 320-360 nm, Em = 420-460 nm) as the secondary longer-wavelength peak in our PARAFAC-identified C1 (excitation = 245 nm (350 nm), emission = 456 nm). Lu, et al. (2013) also observed a decrease in a fluorescence component (excitation: <250 nm (330 nm), emission: 442 nm) during photobleaching that is consistent with our C1 component. However, in our study, the greatest loss by photobleaching occurred in C3 (UV-humic), and the least in C2 (longer-wavelength VIS-humic). While the literature supports the preferential loss of long-wavelength (e.g., Peak C) FDOM to shorter-wavelength FDOM (e.g., Peak A) with photobleaching (Stedmon & Markager, 2005; Helms, et al., 2013), our PARAFAC components are a combination of A and C peaks, and thus, cannot be directly related as such. The greatest loss of the humic-like components occurred in the marsh and watershed sites (GCReW, Canoe Shed, and the Fish Weir), and the lowest occurred in the down-estuary site (Rhode River Mouth), indicating that the terrestrial sites have higher contributions of aromatic, photoreactive FDOM. Even though the Canoe Shed had a greater percent loss in humic-like FDOM compared to GCReW, because the fluorescence at GCReW was higher initially (0 d) in absolute terms, the loss was about the same (a loss in C1, C2, and C3 of 1.1, 0.2, and 0.7 RU compared to 0.9, 0.2 and 0.7 RU for GCReW and the Canoe Shed, respectively). The greater photoreactivity of the Canoe Shed (“non-marsh terrestrial”) compared to GCReW (”marsh”), could be due to previous UV-exposure at both sites. The Canoe Shed site is surrounded by forest, and thus is much more shaded compared to GCReW, in which samples were collected in the middle of the tidal creek with no shaded vegetation; thus the previous exposure to UV-radiation at the Canoe Shed is less, resulting in a higher photoreactivity. This has been observed in other systems, particularly in streams, where shaded streams have been shown to have DOM with a higher photoreactivity (Lu, Bauer, Canuel, Yamashita, Chambers, & Jaffè, 2013). While the loss of C1 and C2 by photobleaching decreased down-estuary, the loss in C3 by photobleaching was relatively constant across all sites, suggesting either different rates of photobleaching for C3 vs. C1, or the production of fresh, non-photobleached C3 down-estuary, offsetting the loss in photoreactivity with increased photobleaching down-estuary. Given that C3 is associated with marine humic-like DOM, it is possible that autochthonous production of C3 down-estuary (as described in §4.2.1) is indeed contributing to the un-photobleached UV-humic DOM pool, resulting in relatively constant rates of C3 loss by photobleaching.

Moran, et al. (2000) found that the loss of absorption by photobleaching was highest at 350 nm for estuarine DOM; however, Tzortziou, et al. (2007) found that the spectral region with the greatest loss by photobleaching is dependent on the irradiation wavelengths used. For an irradiation wavelength of 395 nm, absorption loss was greater at longer wavelengths, and for
shorter irradiation wavelengths (e.g., shorter than 332 nm), absorption loss was greater at the shorter UV wavelengths (Tzortziou, et al., 2007). This is consistent with our observed loss of a-CDOM300, for irradiation wavelengths centered around 340 nm. This could also explain why the greatest loss in fluorescence was observed for our C3 component, which is the shorter-wavelength humic-like component. The loss of fluorescence with photobleaching has been shown to be greater than the loss of CDOM absorption (Zhang, Liu, Osburn, Wang, Qin, & Zhou, 2013), which is also consistent with our results, since C1 and C3 was lost at a higher percentage by photobleaching than was aCDOM-300.

Tzortziou, et al. (2007) found that the loss of CDOM absorption and fluorescence by photochemical degradation was greatest in the freshwater and marsh sites of the Rhode River sub-estuary (equivalent to our Canoe Shed and GCRW sites) and lowest in the most down-estuary end-member (similar to our Rhode River Mouth site). The higher photoreactivity of DOM at the marsh and watershed-DOM sites is typical of terrestrial DOM, which tends to have components that have higher aromaticity and are more photoreactive (Lu, Bauer, Canuel, Yamashita, Chambers, & Jaffé, 2013). The increase in S_R with photobleaching is consistent with the preferential degradation of longer-wavelength CDOM (Helms, Stubbins, Ritchie, Minor, Kieber, & Mopper, 2008). The relative increase in S_R over the 7 d light incubation decreased with down-estuary (e.g., with higher initial S_R values), since lower molecular weight CDOM (higher S_R) is less susceptible to photobleaching than higher molecular weight CDOM (lower S_R). Thus, as DOM is exposed to UV-radiation down-estuary it becomes photobleached, and the amount of change due to photobleaching decreases, further demonstrating the importance of previous exposure history in photoreactivity. \( S_{275-295} \) and \( S_{350-400} \) trends are much more variable with no clear down-estuary trend.

4.2.3. Microbial degradation

The increase in C1 after 14 d of GF/F dark incubation is consistent with other microbial incubations conducted on fjord (Stedmon & Markager, 2005) and estuarine and marine DOM Rochelle-Newall & Fisher, 2002). Rochelle-Newall & Fisher (2002) found that the increase in humic-like DOM fluorescence (excitation: 355 nm, emission: 450 nm) in dark incubations was strongly and positively correlated to bacterial biomass. They proposed that the CDOM production observed in their incubations was the result of microbial processing of non-colored DOM derived from phytoplankton. Therefore, it is important to note that while photo-degradation only impacts the colored component of DOM by converting highly absorbing CDOM to more “bleached” and less strongly absorbing CDOM, microbial degradation acts on both the colored and non-colored DOM pools. Rochelle-Newall & Fisher (2002) also observed, however, few significant correlations between algal biomass and the rate of CDOM production, and instead showed an increased rate of CDOM production by bacteria with light exposure. This could explain the more pronounced increase in C1 with distance down-estuary that was observed in our 14 d GF/F dark incubation, since down-estuary DOM tends to be more photobleached (higher \( S_{275-295} \)), thus allowing for the increased production of humic-like DOM by microbes.

Santos, et al. (2014) attributed the increase in CDOM absorption during microbial degradation for samples collected from estuarine sites that were predominantly algal-derived, to the transformation of lower-molecular weight algal-derived DOM to humic-like DOM. Nelson, et al. (2004) found that CDOM production in marine samples increased with the addition of DOM derived from phytoplankton or zooplankton. These studies indicate that the substrate quality largely impacts CDOM production, thus explaining our down-estuary gradient, as well as the observed variability, for our microbial incubations. C3 had the greatest increase with microbial degradation out of all the fluorescence components. This is consistent with our initial
data and photobleaching data, which suggested the production of C3 down-estuary. However, the rate of increase of C3 is not much greater than the rate of increase of C1 or C2. The increase in the protein-like fluorescence component across all sites during the 14 d GF/F dark incubation is also consistent with other microbial incubation results. Cammack, et al. (2004) observed a strong positive correlation between the tryptophan-like fluorescence component and bacterial production, respiration, and community respiration in Quebec lakes. Moran et al. (2000) found an increase in amino acid fluorescence (Peak T from Coble, et al., 1996) in dark control incubations. This indicates the production of protein-like FDOM by bacterial processing of DOM in all sites.

While the various DOM fluorescence components either increased or showed no change for all sites, $a_{CDOM,300}$ decreased (GCReW and the Rhode River) or showed no change. The change in $S_R$ was consistent with the increase in the protein-like component (with the exception of the Rhode River Mouth); as the protein-like DOM (generally low-molecular weight) was produced over the 14 d incubation, the overall DOM molecular weight decreased, as indicated by the increasing $S_R$. The Rhode River Mouth had the most significant loss of $a_{CDOM,300}$. At the same time, $S_R$ for the Rhode River Mouth also decreased by about 10% over the 14 d, which is consistent with results from other microbial incubations (Helms, et al., 2008; Moran, et al., 2000).

4.2.4. Effects of photobleaching on microbial degradation

Photobleaching resulted in the increasing bioavailability of DOM, especially for the more terrestrial, humic-like, and aromatic DOM. Previous studies found similar results for DOM collected from different environments (e.g., brackish and marine estuarine, estuarine dominated by vascular-plant DOM, arctic headwater stream DOM) (Santos, et al., 2014; Moran, et al., 2000; Cory, et al., 2015, respectively), though few studies have examined the impacts of photobleaching on marsh-exported DOM. The effect of the increase in C1 (VIS-humic) was greater in the marsh and watershed DOM sites (GCReW and the Canoe Shed) compared to the down-estuary sites, since estuarine DOM that has already been significantly photobleached is less photoreactive. The greatest increase from microbial degradation with photobleaching occurred in C3 (UV-humic). For microbial degradation alone, the production of C3 was only slightly higher than the production of C1 (7.2% compared to 4.9% over 14 d); however, after photobleaching, the production of C3 by microbial degradation was, on average, almost double the change in C1 (28.5% compared to 16.5% over 7 d), suggesting that while photobleaching stimulates the production of both C1 and C3 by microbial degradation, the increase in C3 is greater.

The decreasing $S_R$ is indicative of the production of high molecular weight, humic-like DOM, and is consistent with the decrease in $S_{350-400}$ (Helms, Stubbins, Ritchie, Minor, Kieber, & Mopper, 2008). After photobleaching, CDOM absorption decreased with microbial degradation, to a much greater extent than with microbial degradation alone. This is supported by Miller & Moran (1997), who collected samples from a coastal southeastern U.S. salt marsh dominated by Spartina alterniflora and found no change in $a_{CDOM,350}$ during microbial degradation alone, and a 4% decrease during microbial degradation after photobleaching. On the other hand, Cory, et al. (2015) collected samples from an arctic headwater beaded stream with predominantly terrestrial soil DOM sources and found an increase in CDOM and FDOM during microbial incubations after photobleaching, indicating the importance of DOM source when evaluating the impacts of photobleaching on microbial degradation. This is particularly important since so few studies have examined this interplay for marsh-exported DOM.
4.2.5. Fluorescent DOM relative composition

The down-estuary gradient observed in FDOM initially is the product of both photochemical and microbial degradation. Photochemical degradation preferentially degrades aromatic and humic-like DOM (specifically, C1 and C3, and to a lesser extent, C2). Photobleaching has significant impacts on the FDOM composition and, in general, results in the UV-humic component being relatively constant across all sites. The over-reduced UV-humic component observed in the combined photochemical and microbial incubation at GCReW (compared to the initial down-estuary sites) could be because of the intensive photobleaching. In the natural environment, attenuation by other water constituents would likely be higher, thus reducing the impacts of photobleaching. Microbial degradation, on the other hand, might be of similar or even greater significance (due to the presence of particulates in the water column).

Since our incubations demonstrated that microbial degradation produces humic-like DOM, particularly C3 (UV-humic), this might explain the difference observed between the lab incubated GCReW fluorescence DOM signature and the initial down-estuary sites, which we would expect to be similar if down-estuary DOM is the result of the combined impacts of the photobleaching and microbial degradation of marsh-exported DOM. Observed differences could also be because of autochthonous production of DOM in the down-estuary end-members, which would not be produced in the photobleaching and microbial degradation of marsh-exported DOM in our incubation experiments. For example, the contribution of phytoplankton-derived DOM to the pool down-estuary could provide additional labile substrates for the microbial production of humic DOM. Autochthonous DOM production would also likely contribute protein-like sources of FDOM to the DOM pool; these contributions combined might result in a larger percentage of protein-like DOM as well as humic-like (particularly UV-humic, which had the greatest amount of production with microbial degradation) to the total fluorescence.

5. Marsh DOM quality seasonality

5.1. Results
The following are results collected from three incubations performed in July 2016, October 2016, and January 2017, on DOM collected at low tide from the following marsh sites: GCReW, Jug Bay, Sweet Hall, and Taskinas (Figure 1b).

5.1.1. Initial conditions
The amount of CDOM exported from the Chesapeake Bay marsh systems showed a seasonal pattern, with significantly lower CDOM amount (i.e., lower absorption and fluorescence magnitude) in the colder months. However, CDOM quality (i.e., absorption slopes, $S_R$, and ratio of fluorescence components) remained relatively constant seasonally. Variability in CDOM amount and quality across marsh systems was lower than seasonal variability.

Overall the freshwater Sweet Hall marsh system had higher concentrations of humic-like DOM (1.22 RU and 31.0 m\(^{-1}\) for C1 and \(a_{CDOM}300\), respectively, averaged over the two incubations from October 2016 and January 2017); Jug Bay seemed to have the lowest, with C1 and \(a_{CDOM}300\) values of 0.96 RU and 17.4 m\(^{-1}\), respectively. The ratio of the protein-like component to the shorter-wavelength-emitting VIS-humic component (C4/C1) was significantly higher in Jug Bay (\(p = 0.008\) and 0.004) than in GCReW and Taskinas, respectively. The other DOM fluorescence components showed little variation across sites. Jug Bay also had significantly (\(p = 0.005\)) steeper $S_{350-400}$ averaged over the three incubations compared to GCReW and marginally significantly (\(p = 0.08\) and 0.07) steeper $S_{350-400}$ compared and Taskinas, respectively.

The seasonal variation in marsh-exported CDOM amount was much more pronounced than spatial variation. For all marsh sites, CDOM absorption and fluorescence decreased from the summer to the winter months; averaged over all sites, samples collected in January 2017 had significantly (\(p = 0.009\)) and marginally significant (\(p = 0.015\)) lower values of C1 (VIS-humic) compared to the July 2016 and October 2016 incubations, respectively (Figure 14a). While CDOM absorption also seemed to decrease from the summer to the winter months, this decrease was not statistically significant. DOM samples collected in January 2017 had significantly (\(p = 0.0008\) and \(p = 0.002\)) lower concentrations of the UV-humic-like component (C3) than in July 2016 and October 2016, respectively (averaged over all marsh sites) (Figure 14c). Interestingly, the CDOM optical properties that are proxies for CDOM quality (rather than CDOM amount) did not show any statistically significant differences seasonally. $S_R$ averaged over all sites and all seasons was 0.85 (s.d. = 0.05) (Figure 9f), with both the lowest and highest $S_R$ measured in July 2016, the lowest ($S_R = 0.78$) at Jug Bay and the highest ($S_R = 0.92$) at GCReW. $S_{275-295}$ and $S_{350-400}$ did not show any significant variation by season either (Figure 14g-h).

5.1.2. Photobleaching
The amount of photobleaching varied by marsh as well as by season. Photobleaching resulted in a greater change in quality in samples collected in the winter than in the summer.

There was a decrease in all the humic-like components with photobleaching. The loss in C1 (VIS-humic) was, on average, 80.1% (s.d. = 4.3%) over 7 d for all sites and all seasons (Figure 15a). There was no significant variation in the loss of humic-like components, seasonally. GCReW had significantly (\(p = 0.0005\) and 0.006) lower losses of C1 averaged over the three incubations than Jug Bay and Taskinas, respectively, and on average, GCReW lost 71,
78, and 76% of C1 for July 2016, October 2016, and January 2017, respectively. The same trends in significant differences between seasons and marshes were observed for the other two humic-like fluorescence components (GCReW had a significantly lower loss than both Jug Bay and Taskinas). On average, C2 decreased by 53% (s.d. = 5.8%) and C3 decreased by 80.3% (s.d. = 5.8%) over 7 d for all sites and all seasons. The protein-like component (C4) was much more variable, sometimes increasing with photobleaching, sometimes decreasing, and sometimes showing no change (Figure 15d); however, there was a consistent decrease in C4 with photobleaching at Jug Bay, regardless of season. While this loss in C4 seemed to increase in the colder months, there was no significant difference among months. GCReW and Taskinas showed similar trends to one another, with increasing C4 with photobleaching in July 2016 and January 2017, and decreasing in October 2016.

On average, a_CDOM300 decreased by 57.7% (s.d. = 4.6%) over the 7 d light incubation (Figure 15e). There was no significant difference between the loss of a_CDOM300 between marshes. However, the percent loss of a_CDOM300 was significantly higher, on average, in January 2017 than it was in July 2016 and October 2016 (p = 2e-6 and 2e-4, respectively), though this was more variable when looking at the seasonal variations for specific sites. GCReW and Taskinas, for example, showed very similar trends in the seasonal loss of a_CDOM300 by photobleaching.

On average, S_R increased by 102% (s.d. = 16.0%) for all sites and all seasons (Figure 15f). Similar to the other CDOM optical properties, the increase in S_R was significantly higher in January 2017 than in July 2016 (p = 0.001), though not significantly higher compared to October 2016. Jug Bay had a significantly higher percent increase in S_R with photobleaching than both GCReW and Taskinas (p = 3e-5 and 0.016, respectively, averaged over all three incubations); this difference is even more pronounced when comparing within each season. S_R at Jug Bay increased by 109, 109 and 134% after 7 d of photobleaching in July 2016, October 2016, and January 2017, respectively, which is higher than the average percent increases of 89, 103, and 112% for all sites. Similar, but more pronounced, trends were observed in S_275-295 (Figure 15g). S_275-295 increased by about 69.7% over the 7 d light incubation. January 2017 incubations had a significantly higher percent increase in S_275-295 with photobleaching than July 2016 and October 2017 (p < 1e-7 and p = 2e-7, respectively). Jug Bay had a significantly lower percent increase than GCReW, Sweet Hall (for October 2016 and January 2017 only), and Taskinas (p = 0.06, 0.009 and 0.006, respectively). S_350-400 showed the opposite trend, and decreased on average, by 15.7% (s.d. = 6.6%) (Figure 15h). There were no significant seasonal trends for S_350-400 other than the percent loss in S_350-400 being significantly lower in January 2017 than October 2016 (p = 0.004). However, the loss in S_350-400 for all the marshes were significantly different from one another, with Jug Bay having the highest percent loss (a loss of 23.5, 25.1, and 24.8%, for July 2016, October 2016, and January 2017, respectively) and GCReW having the lowest percent loss (a loss of 10.3, 10.8, and 5.8%, for July 2016, October 2016, and January 2017, respectively).

5.1.3. Microbial degradation

Microbial degradation had a much lower impact on CDOM than photobleaching, and in general, resulted in an increase in the humic-like DOM fluorescence components and a decrease in CDOM absorption and S_350-400. No consistent seasonal trends were observed in the impacts of microbial degradation on CDOM optical properties. The Jug Bay marsh had the most “bioavailable” CDOM in terms of change to CDOM absorption.

Humic-like DOM components increased for all sites except Jug Bay over the 14 d microbial incubations. The VIS-humic component (C1) significantly increased in all sites except Jug Bay by, on average, 3.4% (s.d. = 1.9%), for all three incubations. The relative increase in C1
was significantly greater in January 2017 compared to July and October 2016 (p = 0.01 and 4e-5, respectively) (Figure 16a). Jug Bay showed no significant change in C1 for July 2016, October 2016, or January 2017 (p = 0.6, 0.09, and 0.1, respectively). Similar trends were observed for the longer wavelength VIS-humic component (C2); the increase (not including Jug Bay) was on average, 4.0% (s.d. = 2.3%) over the 14 d incubation. Jug Bay showed no significant change in C2, except for January 2017, where it decreased by 2.2% (p = 4e-4). There were no other significant variability trends in C2. The UV-humic component (C3) showed almost the same trends as the other two humic-like components, with an average gain (not including Jug Bay) of 3.6% (s.d. = 2.2%) (Figure 16c). The protein-like component (C4) showed no significant changes for any site in any incubation (Figure 16d).

\( \text{a}_{\text{CDOM},300} \) showed opposite trends to DOM fluorescence, with an average loss of 2.8% (s.d. = 4.6%) (Figure 16e). Jug Bay had a significantly greater percent loss of \( \text{a}_{\text{CDOM},300} \) over the 14 d microbial incubation compared to GCReW, Sweet Hall, and Taskinas (p = 0.01, 0.0006, and 0.0007, respectively). \( S_R \) significantly increased or showed no change over the 14 d microbial incubation for all sites (Figure 16f). Jug Bay had the largest percent increase in \( S_R \) (13.8, 1.3, and 10.9%, p = 0.02, 0.2, and 1e-4 for July 2016, October 2016, and January 2017, respectively), and averaged over the three incubations, had a significantly higher increase in \( S_R \) compared to GCReW, Sweet Hall (for October 2016 and January 2017 only), and Taskinas (p = 0.003, 0.05, and 0.002, respectively). With the exception of Jug Bay in the July 2016 incubations, \( S_{275-295} \) decreased or remained the same for all sites (Figure 16g), but this decrease was very small (< 3%). There were no significant trends seasonally or across marsh sites in the change in \( S_{275-295} \). However, in the Jug Bay July 2016 incubation, \( S_{275-295} \) increased by 6.7% (p = 0.01). \( S_{350-400} \) decreased for all sites and all incubations (Figure 16h). The average loss was 4.4% (s.d. = 3.1%). Averaged over the three incubations, Jug Bay had a significantly higher loss in \( S_{350-400} \) than GCReW, Sweet Hall (for October 2016 and January 2017 only), and Taskinas (p = 0.04, 0.002, and 0.005, respectively). There were no consistent seasonal trends for \( S_{350-400} \).

5.1.4. Effects of photobleaching on microbial degradation

Exposure to light increased, overall, the humic-like DOM production during microbial degradation. There were no distinct seasonal patterns or cross-system variability for the impacts of photobleaching on microbial degradation, other than the significantly greater loss of CDOM absorption properties in Jug Bay.

C1 (VIS-humic) increased over the 7 d dark incubation after the 7 d light incubation, by, on average, 17.9% (s.d. = 8.5%) (Figure 17a); this is much higher compared to the increase in the microbial-only incubations (an average increase of 3.4% over 14 d). There was a large range in C1 production, however, with both the lowest and the highest percent increases occurring in the October 2016 incubation, for DOM collected from Sweet Hall (5.9%, p = 0.2) and Taskinas (29.8%, p = 0.008), respectively. There was no consistent variation among marshes, except that Sweet Hall had a smaller percent increase in C1 averaged over the 3 incubations than both GCReW and Taskinas (p = 0.01 and 0.02, respectively). C2 decreased for most sites, with an average loss of 4.8% (s.d. = 3.5%) (Figure 17b). However, there was also an increase in C2 for GCReW and Taskinas in the October 2016 incubations. There were no significant trends in C2, either seasonally or across marsh sites. The UV-humic component (C3) increased for all sites, with an average increase of 18.8% (Figure 17c); this was also much higher than the microbial-only incubations (an increase of 3.6% over 14 d). Similar to C1, both the lowest and the highest percent increases were measured during the October 2016 incubation, for DOM collected from Sweet Hall (2.4%, p = 0.5) and GCReW (41%, p = 0.04), respectively. GCReW had a significantly higher percent increase in C1 compared to Jug Bay and Sweet Hall (for October
2016 and January 2017 only), averaged over the three incubations \((p = 0.01 \text{ and } 0.01\), respectively), though this trend is only apparent in the October 2016 and January 2017 incubations. The protein-like component (C4) was much more variable (Figure 17d), with most incubations showing no significant changes in C4, except for GCReW and Taskinas in October 2016, which had marginally significant increases of 29.7 and 21.7\% \((p = 0.04 \text{ and } 0.05\), respectively.

\(a_{\text{CDOM}300}\) decreased or showed no significant changes for all sites and all incubations (Figure 17e), with an average decrease of 6\% \((\text{s.d.} = 6.3\%)\) over the 7 d dark incubation (compared to an average decrease of 2.8\% over 14 d for microbial-only incubations). Jug Bay had a significantly greater loss in \(a_{\text{CDOM}300}\) compared to GCReW, Sweet Hall (for October 2016 and January 2017 only), and Taskinas averaged over the three incubations \((p = 4e^{-4}, 0.001, 1e^{-7}\). This average loss in \(a_{\text{CDOM}300}\) for Jug Bay for all three seasons (loss of 13.7\%, \text{s.d.} = 3.1\% over 14 d) was much higher than the loss for the Jug Bay microbial-only incubations (loss of 6.3\%, \text{s.d.} = 7.3\% over 14 d). The between-marsh trend observed was less apparent when comparing sites within individual incubations, and the significance between GCReW and Jug Bay decreased in both the July 2016 and the January 2017 incubations, with the former being only marginally significant and the latter showing no significant difference \((p = 0.04 \text{ and } p = 0.4\), respectively). \(S_R\) decreased for all incubations (compared to the overall increase observed in the microbial degradation only treatment) for all sites except Jug Bay in July 2016 (Figure 17f). The average decrease (not including the Jug Bay July 2016 incubation) was 5.3\% \((\text{s.d.} = 3.2\%)\). \(S_R\) for the Jug Bay July 2016 incubation increased by 11\% \((p = 0.008)\). There were no significant trends seasonally or across marsh systems. \(S_{275-295}\) decreased or showed no change for all sites except Jug Bay, which increased, and the average change was about \(+/-\ 3.3\%.\) There were no significant trends seasonally. \(S_{350-400}\) increased or showed no significant change for all sites and incubations except Jug Bay in the July 2016 incubation, which decreased by about 6\%. The average increase in \(S_{350-400}\) (not including the Jug Bay July 2016 incubation) was 4.6\% \((\text{s.d.} = 4.1\%)\). Once again, there were no significant seasonal trends in \(S_{350-400}\).
Figure 14. Optical parameters for GF/F (0.7 μm) filtrates initially (0 d) for incubations performed in July 2016, October 2016, and January 2017 for marsh sites.
Figure 15. Change in optical properties after 7 d of light incubations performed on 0.2 μm filtrate for incubations performed in July 2016, October 2016, and January 2017. The change for each parameter was calculated by dividing the difference between the 7 d and initial (0 d) values by the initial value, multiplied by 100%.
Figure 16. Change in optical properties after 14 d of dark incubations performed on GF/F filtrate for incubations performed in July 2016, October 2016, and January 2017. The change for each parameter was calculated by dividing the difference between the 14 d and initial (0 d) values by the initial value, multiplied by 100%.
Figure 17. Change in optical properties over 7 d of dark incubations after 7 d of light incubations performed on GF/F filtrate for incubations performed in July 2016, October 2016, and January 2017. The change was calculated by dividing the difference between the values at the end of the 7 d dark incubation and the start of the 7 d dark incubation (which was also the end of the 7 d light incubation) by the values at the start of the 7 d dark incubation, multiplied by 100%.
5.2. Discussion

5.2.1. Initial conditions

The decrease in the DOM fluorescence and $a_{\text{CDOM}300}$ for all marsh sites from July to October to January indicated a decrease in the concentration of DOM exported by the marsh from the summer to the winter. At the same time, the ratios of fluorescence components, $S_R$, $S_{275-295}$, and $S_{350-400}$ remained relatively constant, indicating similar DOM quality across seasons. This is consistent with Tzortziou, et al. (2008), in which CDOM and DOC export from the Kirkpatrick Marsh (GCRew) was greatest during the summer (July) and was also consistent with monthly measurements collected at the mouth of GCRew as part of the NASA MarshCycle project (unpublished). Other studies have also shown increased marsh-export of DOM in the summer months (Stedmon & Markager, 2005; Fellman, et al., 2008; Osburn, et al., 2016). This seasonal variability could be related to a certain extent to seasonal variability in above ground marsh biomass (an important source of DOM export to the estuary), with marsh biomass peaking in the summer and senescing in the fall. Fellman, et al. (2008) attributed the higher DOM concentrations observed in the summer for a bog and fen site to the biota; marsh vegetation in the summer takes up water from the soil, thus concentrating the soil DOM. The relatively consistent quality across seasons further supports this. One of the main hypotheses of a new NSF-funded project in the Chesapeake Bay is that DOM storage in tidal marsh soils reduces seasonal and inter-annual variation in the quality and quantity of DOM export from temperate marshes, relative to the strong seasonal and inter-annual variation in the ultimate source of these compounds (i.e., plant production).

5.2.2. Photobleaching

The loss of all three humic-like fluorescence components with photobleaching is consistent with previous studies (Tzortziou et al., 2007; Helms, et al., 2008; Cory, et al. 2015; Aullo-Maestro, et al., 2016), and also consistent with the observed loss in CDOM absorption. While there was little variation in the percent loss of the humic-like components seasonally or across marsh systems, the percent change in $S_R$ (proxy for DOM quality) showed some seasonal variability, with overall largest percent increase in $S_R$ observed for the samples collected in the fall and winter, though there were no significant differences in DOM quality initially (see §5.2.1). One potential explanation is the difference in the natural exposure to UV radiation experienced by the samples prior to collection; since the January incubation samples were exposed to less natural UV-radiation prior to sampling due to increased cloudiness and shorter days in the winter, they could potentially have more photoreactive CDOM, given that they have less previous exposure to sunlight. The dilution of this aromatic, marsh-derived CDOM with estuarine CDOM would not necessarily reduce the photoreactivity, given that the concentration of high aromaticity, humic-like DOM still dominates the signal. This is consistent with measurements of photoreactivity conducted by Lu, et al. (2013) and Cory, et al. (2015), which argue that UV-radiation is the main factor dictating photoreactivity (for DOM of similar source material). Tzortziou, et al. (2007) also concluded that previous exposure of CDOM to solar radiation during transport from the head to the mouth of the Rhode River estuary could partly explain the observed decrease in the photoreactivity of CDOM along the estuarine gradient. It is also important to note that while the photoreactivity of the winter CDOM is greater than the summer CDOM, photobleaching is a more important process of DOM loss in the summer, due to overall higher exposure to UV-radiation.

While some studies showed an increase in the protein-like fluorescence with photobleaching (Cory, et al., 2015; Zhang, et al., 2013), others showed a decrease (Moran, et al.,
This is consistent with the variation observed in our sites, in which some sites and months showed increases while others showed decreases. The consistent loss of the protein-like component (C4) across all seasons in Jug Bay indicates that this type of DOM is relatively photoreactive. The increasing protein-like component observed for some sites could be the result of the photo-degradation of humic-like DOM, and the photo-dissolution of humic-protein complexes, resulting in an enhancement of the protein-like signal due to a reduction quenching (Wang, et al., 2015). However, the presence of humic-protein complexes will depend on the source of the DOM, and thus the change in the protein-like fluorescence with photobleaching is expected to be extremely variable. Jug Bay showed a significantly higher loss in C3 than the other marsh sites than any other marsh and a significantly higher loss of S350-400. We also observed a strong correlation positive between the loss of C3 and the loss of S350-400 ($R^2 = 0.50, p = 0.0001$, data not shown); this relationship to S350-400 was not as strong for the other humic-like components ($R^2 = 0.37$ and $0.13$ for C1 and C2, respectively). This suggests that the preferential photo-degradation of the shorter-wavelength (e.g., UV-humic) DOM also results in the preferential loss of absorption in the shorter-wavelength UV, thus reducing S350-400. This is consistent with results from Tzortziou, et al. (2007), which found that irradiation in shorter-UV wavelengths resulted in the preferential loss of CDOM absorption and fluorescence in the shorter-wavelength UV.

5.2.3. Microbial degradation

Microbial degradation resulted in the opposite shifts in DOM fluorescence compared to photochemical degradation. As discussed in §4.2, although small (within 5% change in most cases) the increase in the VIS- and UV-humic components (C1 and C3) with microbial degradation is consistent with other studies (Moran, et al., 2000; Rochelle-Newall & Fisher, 2002; Lu, et al., 2013; Cory, et al., 2015), particularly for the UV-humic component, which is associated with biological processing. C1 and C3 showed little change in July 2016 and October 2016 at Jug Bay; this could indicate either the lack of production of the humic-like components as seen in the other sites, or, more likely, the utilization and degradation of this FDOM by microbes, thus offsetting its production.

Changes in CDOM absorption by microbial degradation are more variable, with most studies showing no change or an increase in CDOM absorption with microbial degradation (Miller & Moran, 1997; Rochelle-Newall & Fisher, 2002; Nelson, et al., 2004; Santos, et al., 2014), although most of these studies were conducted on down-estuary or marine DOM, which had already been highly impacted by photobleaching. Some studies found a decrease in CDOM absorption and molecular weight with microbial degradation, specifically for terrestrially-derived DOM (Moran, et al., 2000; Santos, et al., 2014; Cory, et al., 2015). This is consistent with our results for the change in $a_{CDOM}300$, which decreased throughout the 7 d incubation. Increasing $S_R$ with microbial degradation indicates the production of lower molecular weight DOM; this is consistent with the loss of CDOM observed for our samples, though not necessarily with the increase in the humic-like DOM fluorescence components. However, this increase in $S_R$ is relatively small (~%5). Microbial degradation is impacted by numerous factors, such as nutrient availability, microbial community, source material, impacts of photobleaching, and temperature (which was constant in our experiments); thus, the effects of microbial degradation on CDOM and FDOM are highly variable compared to photobleaching, and are difficult to tease apart.
5.2.4. Effects of photobleaching on microbial degradation

Across all marsh sites and seasons, photobleaching resulted in increasing bioavailability of DOM; the same trend has been observed in numerous studies, but for DOM collected from different environments, such as the Ria de Aveiro estuary (Santos, et al., 2014), the Satilla estuary dominated by inputs of vascular plants (Moran, et al., 2000), and an arctic headwater stream (Cory, et al., 2015). Few studies have examined the impact on marsh-exported DOM, and even fewer have looked at seasonal trends or variability depending on marsh characteristics. C1 and C3 humic-like components increased with microbial degradation after photobleaching to a much greater extent than by microbial degradation alone (e.g., an average increase in C1 by 17.9% over 7 d vs. 3.4% over 14 d). C2 was also lost at a greater rate (even in sites that showed no loss with microbial degradation alone), indicating that photobleaching is somehow allowing for the microbial utilization of the C2. The loss in aCDOM300 increased for almost all sites with the photobleaching treatment prior to the microbial treatment. This was particularly true for Jug Bay, which lost 13.7% over the 7 d period after photobleaching (compared to 6.3% over 14 d in the microbial degradation only treatment). Jug Bay showed a smaller gain of humic-like DOM over the 7 d microbial treatment than the other sites; paired with high loss of CDOM and increasing S275-295 (indicative of the loss of longer-wavelength CDOM), this would suggest that the humic-like DOM being produced by Jug Bay is simultaneously being utilized, thus resulting in a small net change. While SR increased and S350-400 decreased in the microbial degradation only treatment, the opposite trend occurred during the microbial degradation treatment that had been photobleached first. The decrease in SR and S350-400 is consistent with the literature (Helms, Stubbins, Ritchie, Minor, Kieber, & Mopper, 2008), and indicates the production of higher-molecular weight, aromatic, humic-like DOM. The lack of seasonal variations in microbial degradation and the impact of photobleaching on bioavailability in consistent with the relatively stable quality seasonally, since microbial degradation is largely driven by DOM source and quality.
6. Summary and conclusions

- Heating 0.2 μm filtrates at 60°C for 30 min successfully killed bacteria without changing DOM optical properties, thus reducing the impact of microbes in photochemical-only and control treatments.
- Re-filtering of both GF/F and 0.2 μm filtrates with 0.2 μm filters at the end of an incubation resulted in a loss of the CDOM absorption coefficient by 5-10%, which is similar in magnitude to reported CDOM losses by microbial degradation; studies that have utilized this method could be greatly overestimating bioavailability (even by a factor of 2).
- CDOM absorption and humic-like FDOM decreased down-estuary, with distance from terrestrial sources of DOM.
- CDOM molecular weight decreased down-estuary, as indicated by both the CDOM quality measurements as well as the ratios of the fluorescence components.
- Seasonal variability of both CDOM quantity and quality was more pronounced than between-marsh variability.
- Marsh CDOM and FDOM amount decreased from the summer to the winter, but the quality remained relatively constant throughout.
- Photobleaching resulted in a decrease in CDOM and humic-like FDOM and a decrease in CDOM molecular weight.
- Photobleaching had the greatest impact on marsh and watershed samples and the lowest on down-estuary samples, for both quantity and quality.
- The impacts of photobleaching on CDOM quality was greater in marsh sites collected during the winter months than in the summer.
- Microbial degradation produced humic-like FDOM (opposite impact than photobleaching) and decreased CDOM amount (same impact as photobleaching), but at much lower rates than photobleaching.
- The production of C1 (VIS-humic) and the loss of CDOM amount by microbial degradation increased down-estuary.
- The between-marsh variability in microbial degradation was greater than the seasonal variability.
- Jug Bay had the highest rates of CDOM quantity loss and the lowest production rates of humic-like FDOM.
- Photobleaching increased the bioavailability of CDOM and FDOM, with the production of humic-like FDOM and the loss of CDOM more than doubling.
- The impacts of photobleaching on CDOM and FDOM bioavailability were greater in marsh and watershed sites than down-estuary sites.
- Photobleaching impacts on microbial degradation showed little seasonal variability, but Jug Bay had significantly greater changes in optical properties than the other sites.

Marshes export humic-like, aromatic, and strongly absorbing and fluorescing DOM to adjacent waters, particularly in the summer when marsh plant biomass is peaking and marsh soil DOM pools become more concentrated. This DOM, once in the estuary, is photobleached and microbially degraded, with photobleaching acting as the main mechanism of DOM removal. Photobleaching is a relatively straightforward process; its influence is mainly determined by the absorptivity, molecular weight, and aromaticity of the DOM and UV-radiation limitations. Thus, in the summer when high-molecular-weight, aromatic DOM is exported in large quantities from
marshes to adjacent estuarine systems and UV-radiation is at a maximum, photo-degradation is an extremely important process for DOM removal (RO #1 and #2).

Because microbial degradation has been shown to both degrade and produce absorbing and fluorescent DOM, changes to DOM optical properties by microbial degradation are the net effect of both processes, and can vary significantly depending on the available substrate, temperature, and other factors. However, microbial degradation of DOM (whether in a lab setting or naturally down-estuary), in general, produces humic-like FDOM. This microbial production of humic-like DOM is enhanced after exposure to light (RO #3). As a result, microbial degradation often offsets the loss of humic-like FDOM by photobleaching, and thus the down-estuarine gradient in DOM optical properties is the combination of these processes (RO #1); additional autochthonous inputs of lower-molecular weight DOM (e.g., by phytoplankton) down-estuary, as well as mixing, also contribute to this gradient.

While many studies have examined the quality of DOM in a variety of ecosystems, few have focused on marshes, and even fewer have compared marshes with different characteristics or across seasons. Our study has shown that DOM source plays an extremely important role in the transformation processes the DOM undergoes once exported, and that these vary both seasonally and between marsh-systems. Jug Bay, which is the most human-influenced site in terms of nutrient inputs, as well as the site with the most distinct vegetation, had both extremely photoreactive and bioavailable DOM compared to the other marshes. The lack of production or the quick utilization of humic-like FDOM in Jug Bay by microbial degradation coupled with the high photoreactivity of the DOM could result in lower concentrations of aromatic and high molecular weight DOM that persist in the estuary. This has implications on how nutrient loading and eutrophic conditions in estuaries might influence optical properties and biogeochemical cycling; a shift to more eutrophic marsh-systems with vegetation associated with more photo- and bio-labile DOM could lead to higher rates of DOM degradation in estuaries and no source of refractory DOM, potentially reducing allochthonous inputs of humic-like DOM down-estuary, and ultimately, to marine environments. Our approach could be applied to other DOM sources (e.g., DOM leachate experiments) to more directly examine the impacts of vegetation type on DOM quality. This could be coupled with other techniques, such as remote sensing, to better predict or model the export and fate of DOM in different marsh-systems based on the characteristics of those marsh-systems, which would be a particularly useful approach in systems undergoing biogeochemical changes due to anthropogenic influences and climate change.
7. References


