Responses to Reviews for Biogeosciences Discuss., 12, 7301-7333, 2015

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"Colored dissolved organic matter in shallow estuaries: relationships between carbon sources and light attenuation"

(Note: title changed in response to reviewer comment, seen below)

by: William K. Oestreich, Neil K. Ganju, John W. Pohlman, and Steven E. Suttles

Response format:

Anonymous Referee comments (normal font, left justified)

Author response (italics, indented)

Followed by figures relevant to response, as well as a marked-up version of the manuscript

Responses to Anonymous Referee #1 comments:

The manuscript addresses the reliability of fluorescent dissolved organic matter (FDOM) measurements at a fixed excitation : emission pairing as a proxy for light absorption by colored dissolved organic matter (CDOM). This is an important analytical issue in estuarine and coastal waters, where FDOM measurements can be rapidly and cheaply in situ, but CDOM measurements require collection of discrete samples for analysis in the laboratory or the deployment of more involved, power hungry and labor sapping in situ probes.

The data collected appears of decent quality. However, there are some significant changes that need to be made to the data presentation and interpretation before publication is possible. These are addressed below.

Based on the comments from Referee #1, we have modified the manuscript and figures significantly. This includes recalculating spectral slopes for Barnegat Bay and Chincoteague Bay over the wavelength range 340-440 nm in order to facilitate comparisons to West Falmouth Harbor.

Furthermore, methods have been clarified, and regressions for Figure 3 have been adjusted to exclude outliers. Additional plots have also been created, discussed, and added to a supplementary information section at the Referee's request. Finally, minor text and figure presentation suggestions have been taken into account with edits made accordingly.

1) Add the FDOM Ex:Em wavelength pairing to the abstract so that the reader knows straightaway that FDOM here refers only to this pairing as utilized on probes and in situ sondes.

This is an important point to communicate to the reader before the presenting the results of this study. Therefore, this information that originally was only presented in the methods section, has been added to the abstract as well. Starting at page 7302, line 11, the text now reads:

"Land use surrounding these estuaries ranges from urban to developed, with varying sources of nutrients and organic matter. Measurements of fDOM (excitation and emission wavelengths of 365nm (±5nm) and 460nm (±40nm), respectively) and CDOM absorbance were taken along a terrestrial-to-marine gradient in all three estuaries."

2) CDOM spectral slope needs to be calculated over consistent wavelength ranges with the same data resolution (data points per nm) for the data to be comparable. Spectral slope changes with wavelength (see Helms et al. cited in the manuscript). Either reduce the wavelength range to 340-440nm for the whole analysis or remove the WFH data from the comparison.

Spectral slope has been recalculated for both Barnegat Bay and Chincoteague Bay data for only the range 340-440nm in order to facilitate comparison to the data from West Falmouth Harbor. These recalculated values are now included in Table S1 (formerly Table 2), alongside the spectral slope calculated over 340-720nm. They also are reflected in the Results section, with the text for Section 4.1 changed to read as follows:

"The estuary-wide average spectral slope (over the range 340-440 nm) for West Falmouth was higher than for Barnegat and Chincoteague, with Savg equal to 0.021, 0.016, and 0.018, respectively (Table S1). At West Falmouth Harbor, spectral slope ranged from 0.013 – 0.044, with a standard deviation of 0.010. At Barnegat Bay, S ranged from 0.011 – 0.019, with a standard deviation of 0.002. At Chincoteague Bay, S ranged from 0.014 – 0.023, with a standard deviation of 0.003. Spectral slope values for Barnegat and Chincoteague were slightly higher over the range 340-440 nm as compared to S calculated over the range 340-720 nm (Table S1)." (Pg 7309, lines 18-25).

Comparison of spectral slopes over the ranges of 340-440nm and 340-720nm shows that the values for spectral slope do change slightly depending on the wavelength range employed, as pointed

out by the Referee. However, these changes are quite small, and spectral slopes observed for both Barnegat Bay and Chincoteague Bay are still significantly less steep than those for West Falmouth Harbor.

The fact that these spectral slope values change only slightly depending on wavelength range does bring up a significant point though. In the original discussion of spectral slope values, we had indicated the smaller wavelength range as a possible factor in explaining the wider range of spectral slopes observed in West Falmouth Harbor. The fact that spectral slopes for the range 340-440nm at Barnegat Bay and Chincoteague Bay are still less variable than of those for West Falmouth Harbor indicates that wavelength range does not necessarily explain the variability in spectral slopes at West Falmouth Harbor. The discussion of spectral slopes (Pg 7312, lines 14-25; pg 7313, lines 1-10) has been adjusted to reflect this. This section now reads as follows:

"All values observed for spectral slope were within ranges reported for similar estuaries and coastal waters (Keith et al., 2002; Green and Blough, 1994). At Barnegat Bay and Chincoteague Bay, the range of calculated spectral slopes was quite small (Table S1). At West Falmouth Harbor, however, there was significantly more variability in spectral slope. This is likely due to a combination of at least two factors. For one, the relatively low DOC concentrations from West Falmouth Harbor contributed to more instrumental variability in spectral slope values at this estuary. Significantly lower fDOM and absorbance measurements were recorded at West Falmouth Harbor compared to Barnegat Bay and Chincoteague Bay (Table S1)."

3) How were offsets from zero determined for the WFH CDOM spectra and were they applied for the other data? Samples are routinely zeroed at or above 600 nm (e.g. see Helms et al again) as there should be very limited CDOM light absorption at these long wavelengths. This may be important in the trends seen in Fig 3 and discussed next.

Offsets from zero were determined for the WFH CDOM spectra by running a blank sample (Milli-Q water) at 440nm (the high end of the measured wavelength spectrum). For BB and CB, offsets from zero were determined by running a blank sample (Milli-Q water) before measurement at each wavelength (340-720nm). This was not clearly explained in the original methods section, and therefore, text has been added to clarify this methodology (Pg 7307, lines 7-9):

"The estimated photometric accuracy of the spectrophotometer was 0.003 absorbance units. Offsets from zero were determined for the WFH CDOM spectra by running a blank sample (Milli-Q water) at 440nm (the high end of the recorded spectrum). For BB and CB, offsets from zero were determined by running a blank sample before measurement at each wavelength (340-720nm). Absorbance measurements were converted to absorption coefficients as follows"

While the methodologies for WFH and BB/CB were slightly different in range of measurements and zeroing, these data are still comparable. The difference is range of measurements is addressed by

comparing only over the 340-440nm range (as suggested in comment 2). The slight difference in zeroing is not a concern, as both methodologies provide offsets from zero, just with more frequent blank runs through the instrument for BB and CB.

4) Figure 3. The whole paper indicates that CDOM and FDOM do not correlate well.

Looking at Fig 3 these seems completely inaccurate for all but the WFH samples and

two other outliers. The two outliers are: 1) The BB-S sample with CDOM of ~15 and

FDOM of <20. 2) the BB-N sample with FDOM >60 and CDOM <2. Looking at the rest

of the data on this plot, these two samples are obviously outliers. They may have been confused with other samples or contaminated at some point in the sample processing. They look as if they may have been switched (i.e. the BB-S has the CDOM value of BB-N or vice versa). Whatever the case, the best thing to do at this point is to remove them from the correlations and/or delete the completely. Having analyzed hundreds, if not thousands of samples of this sort, the lack of coherence of these two samples with the rest of the dataset screams analytical error. For this reason, I would favor deletion. Once these samples are removed from the regressions the R2 for all the BB-N, BB-S and CB data would fall on a very tight line. From a visual appraisal of the data, it appears that a single regression for all three datasets would be insignificantly different from regressions of all 3 sample sets indicating that within NE estuaries with terrigenous DOM inputs, a single CDOM:FDOM regression can be applied. The WFH data falls above this combined BB and CB line. This could simply be an analytical error as the sample absorbance spectra were not zeroed at 600-700nm (see comment 3). Here I am not sure how the authors should proceed. As the CDOM data obtained was not measured out to 700nm, they have no way of checking if they had a blank issue for those runs. All of these samples are at low CDOM. The analytical noise could therefore have contributed to the lack of a relationship between FDOM and CDOM within these samples. Although the above reservations cause me to question the data a little, the fact the samples all have low CDOM is consistent with the groundwater dominated WFH estuary. Therefore, if caveats are added that CDOM was low and may not have been fully corrected, then the data can be discussed and the difference between WFH and the other estuaries attributed to groundwater inputs. More references for CDOM and FDOM from groundwater systems, estuarine and otherwise should be added though.

The Referee makes several important points in this comment. First of all, the concern over what to do with the two outlier samples (BB01 and BB15) is necessary to address. We have chosen to keep these samples in presentation of the figures, as they display the very strong or weak outlier signals that can be observed in these dynamic systems. The waters of this estuary are rich in diverse DOC sources, and the deviation of these data points shows us that the fDOM-CDOM relationship is not straightforward in estuaries with diverse sources and transport mechanisms. We doubt analytical error for these data points, as multiple authors were present for sample processing to ensure no deviation from the methods outlined here. Even if the two samples had been switched, as suggested by the referee, these samples still would be outliers from the trend observed in Figure 3.

However, because they obviously deviate from the general trends for Barnegat Bay North and South, respectively, they have been removed from the regression analyses in an updated Figure 3. With these outliers removed, BB-S, BB-N, and CB all take on a fairly uniform CDOM-fDOM relationship, as suggested by the Referee. However, WFH samples still fall along a significantly different line. This notion supports the idea of a fairly uniform CDOM-fDOM relationship, with significant variability depending on estuary inputs. This is in line with the claims investigated in this paper (and conclusions of other studies), including the following statement: "The significant variability within a somewhat consistent overall trend between fDOM and absorption by CDOM in these estuaries was expected based on the results of previous studies (Hoge et al., 1993; Del Castillo et al., 1999; Clark et al., 2004)" (Pg 7315, lines 19-21). Considering the different fDOM-CDOM ratio observed at WFH, as well as the deviation from the general trend observed for specific samples at Barnegat Bay, we contend that there is significant variability in the fDOM-CDOM relationship for shallow estuaries such as those studied here.

In addition, the low CDOM levels observed at West Falmouth Harbor are not surprising, which inspires confidence in this data. As the Referee has pointed out here, low CDOM is consistent with the findings of previous studies on low CDOM from groundwater sources (Shen et al., 2015; Chen et al., 2010; Huang and Chen, 2009) and the fact that WFH is groundwater-dominated (Ganju, 2011). Discussion of this concept has been added. Additionally, further discussion of the potential influence of analytical noise due to low CDOM has been added. Finally, a caveat has been added for the limited wavelength range over which CDOM absorbance was measured at WFH.

To accommodate the corrections outlined here, as well as the comment from Referee #2 on the discussion of Figure 6, Section 5.4 has been edited significantly. This discussion section now reads as follows (Pg 7315, lines 19-27; pg7316, lines 1-7).

"The significant variability within a somewhat consistent overall trend between fDOM and absorption by CDOM in these estuaries was expected based on the results of previous studies (Hoge et al., 1993; Del Castillo et al., 1999; Clark et al., 2004). West Falmouth Harbor in particular showed a different absorption coefficient to fDOM ratio as compared to the general trend for Barnegat and Chincoteague Bays (Fig. 3). It should be noted that the CDOM absorbance signal was generally low for all WFH samples, meaning analytical noise in the data could affect this ratio. Furthermore, the fact that WFH samples were zeroed at 440 nm only for absorbance measurements could enhance such noise. However, the low signals observed for WFH inspire confidence in the data, considering that West Falmouth Harbor is marked by strong groundwater influence (Ganju, 2011). In studies of both estuarine and other systems, CDOM levels have been measured at low levels in groundwater as compared to other sources (Shen et al., 2015; Chen et al., 2010; Huang and Chen, 2009).

Even with these caveats taken into consideration, the variability in this study can be explained in part by the differing DOC sources within the estuaries. In this study, 13C-enriched DOC sources correspond to a higher absorption coefficient per unit fluorescence (Fig. 6). While the relatively uniform CDOM-fDOM relationship for Barnegat Bay results in clustering of Barnegat Bay points in the center of Figure 6, this relationship is highlighted by both the Barnegat Bay outliers and the higher CDOMabs/fDOM observed for the more 13C-enriched samples at West Falmouth Harbor. Points such as the outliers at Barnegat Bay are indicative of how the fDOM-CDOM relationship can be altered in an estuary with such diverse sources and transport mechanisms. This assertion of variable fDOM-CDOM relationship depending on source is supported by the findings of Tzortziou et al., 2008, which suggested that marsh-exported DOC has a lower fluorescence per unit absorbance as compared to humic DOC (associated with a freshwater

source). For our study, 13C-enriched DOC (likely Spartina source) was associated with a lower fluorescence per unit absorbance. 13C-depleted DOC (terrestrial source) was associated with a higher fluorescence per unit absorbance. While other studies have focused on differences in the fluorescence-absorbance relationship as a function of molecular weight (Belzile and Guo, 2006; Stewart and Wetzel, 1980), the combination of CDOM optical and isotopic analyses presented here provide a connection between CDOM source and optical characteristics, as suggested by Tzortziou et al., 2008."

Note: The following references have been added for Shen et al., 2015; Chen et al. 2010, and Huang and Chen, 2009:

Chen, M., Price, R. M., Yamashita, Y., and Jaffe, R.: Comparative study of dissolved organic matter from groundwater and surface water in the Florida coastal Everglades using multi-dimensional spectrofluorometry combined with multivariate statistics, Applied Geochemistry, 25, 872-880, 2010.

Huang, W., and Chen, R. F.: Sources and transformations of chromorphic dissolved organic matter in the Neponset River Watershed, J Geophysical Research, 114, G00F05, 2009.

Shen, Y., Chapelle, F. H., Strom, E. W., and Benner, R.: Origins and bioavailability of dissolved organic matter in groundwater, Biogeochemistry, 122, 61-78, 2015.

5) A plot of CDOM absorbance versus salinity should be added and discussed.

This plot has been created and is presented in attached Figure R1. We have chosen not to include this plot as a part of the manuscript, as it provides little insight not already displayed in Figure 2 of the current manuscript. Discussion of Figure 2 is already included in the paper as well. Furthermore, there is extensive discussion in the paper of the somewhat consistent relationship between fDOM and CDOM absorbance, with notable outliers (linked to organic matter source) and some difference between estuaries. Adding the plot of CDOM absorbance vs. salinity does not present new information not already explained in the discussion mentioned here or Figure 2.

6) A plot of Spectral Slope versus salinity should be added and discussed as a qualitative indicator of endmembers along with d13C data.

This plot has been created and is presented in attached Figure R2. While an additional tool for determining end-members would be highly desirable, this plot does not appear to show any significant relationships or provide additional insights on this issue. For this reason, we have chosen not to include this plot as an additional figure in the manuscript.

7) Try a plot of spectral slope versus d13C.

This plot has been created and is presented in attached Figure R3. Once again, this plot does not appear to display significant relationships between spectral slope and isotopic signature. This plot does not appear to provide assistance in delineating end-members for this study, and therefore has not been included in the manuscript.

8) Page 73713. Spectral slopes become steeper, not larger.

This error in terminology is important to note and has been changed accordingly (Pg 7313, lines 6-9). The text now reads, "More specifically, previous studies have shown that DOM comprised of primarily fulvic acids has steeper spectral slopes than DOM comprised of primarily humic acids (Carder et al., 1989)."

9) Figs 4 and 5, the maps require some color, indication that part of the map is land, part ocean, etc. not just an abstract outline.

We agree that the maps of Figures 4 and 5 are not clear, and may be especially confusing to readers that are not familiar with the estuaries discussed in this paper. Colors have been added to make the distinction between land and ocean and improve these figures.

Response to Anonymous Referee #2 Comments:

Review of "Colored dissolved organic matter in shallow estuaries: the effect of source on quantification". Please note that I performed this review without considering the comments of other reviewers to provide an unbiased evaluation of the manuscript.

Summary: The manuscript entitled "Colored dissolved organic matter in shallow estuaries: the effect of source on quantification" by Dr. Oestreich and Co-authors evaluates the role of different sources of organic matter on light attenuation in estuaries. Using data from multiple locations within 3 different shallow estuaries, the authors suggest that the ratios of fDOM to CDOM vary substantially, dependent on the sources of organic matter and thus challenge the commonly used approach to estimate light attenuation though fDOM.

Overall I would consider the manuscript to be a reasonable contribution to the journal Biogeosciences. At current, however, the manuscript falls short on convincing me of the conclusions

drawn (please find my detailed comments on this below). Considering these circumstances, I suggest the manuscript to be revised before being evaluated again.

We appreciate the consideration of the manuscript as a reasonable contribution to the journal Biogeosciences. Significant changes have been made to improve the manuscript according to the comments from Referee #2. For one, some figures have been edited to include statistical measures of the relationships presented. Tables containing redundant information have been moved to the new Supplementary Information section. Minor, yet important, edits have been made to the text according to these comments as well. Finally, the issue of how to deal with the outliers in Figure 3 is again discussed.

Major Comments

i) Data Quality: Challenging methods that others use needs to be based on a high number of good quality data points, which are then analyzed carefully. Unfortunately the data presented here does not give this impression. For example, the data presented in figure 3 and the plotted relationships really look like they are driven by some, few outliers. I can only hypothesize what caused these, but I would strongly recommend going back to the data and finding out what happened there. To me this Figure looks like a general relationship for all sites, except WFH. Also, I suggest the authors consider removing the strongly deviating points and/or applying alternative methods, such as for example robust regressions that are insensitive to outliers to reevaluate the data.

We agree that the relationship presented in Figure 3 is influenced by two outlier sampling locations: BB01 and BB15. Because they clearly deviate from the general trend for their respective estuaries, these points have been removed from the regression analyses now incorporated for this data (meaning both Figure 3 and the caption have been adjusted accordingly). However, they have not been removed from the figures entirely, as they display the very strong or weak outlier signals that can be observed in these dynamic systems. The complete justification for this can be found in the response to a similar comment from Referee #1.

Regression of the relationships represented in Figure 3, with these outliers removed from the analyses, displays exactly what the Referee has suggested here: all sites display a similar relationship except for West Falmouth Harbor. This observation is in line with the rest of the observations and conclusions presented in this paper. The different optical properties of DOM seen in West Falmouth Harbor, including the relationship presented in Figure 3, is understandable considering the different inputs known for West Falmouth Harbor (as compared to Barnegat and Chincoteague Bays), including significant groundwater influence (Ganju 2011). The concept of differing optical properties depending on DOM source is a central focus of this paper, and is displayed by the relationships presented in both Figures 3 and 6. This concept is further supported by the anomalous optical measurements observed for outlier samples BB01 and BB15, which are likely examples of the strong signals that can be observed for certain inputs in these systems.

The updated caption for Figure 3 now reads, "Figure 3. Absorption coefficient at 340nm versus fluorescence measurement for all sampling sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), South Barnegat Bay (BB-S), and Chincoteague Bay (CB). Dashed lines indicate the best linear fit to the data, with associated R2 and p-value. Two outliers (indicated by "") removed from the regressions for Barnegat Bay."*

ii) Given that many different relationships are presented in Figures 2 and 3, please provide objective measures on how good these are, such as p values, whether they are significantly different from each other, etc.

We agree that the statistical measures suggested here can contribute to better communicating the relationships presented in Figures 2 and 3. To address this, linear regressions have been performed for each data set presented in these two figures. Associated R^2 and p-values are now included alongside each regression line on the plots. In the case of Figure 3, BB-N, BB-S, and CB have been combined into one regression, as per comment 4 from Referee #1. Outliers have been removed from the regression analyses shown for Figure 3, though they are still presented in the Figure (denoted by an asterisk). Captions for Figures 2 and 3 have also been updated to reflect these changes.

The updated caption for Figure 2 now reads, "Figure 2. Fluorescence measurement versus salinity for all sample sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), South Barnegat Bay (BB-S), and Chincoteague Bay (CB). Dashed lines indicate the best linear fits to the data, with associated R2 and p-value."

iii) Mixing model: Whereas I am in general a fan of simple mixing models, I was wondering if you considered including any uncertainty into the mixing calculations (?). Here some more advanced methods, such as a baysian mixing model would be possible.

We agree that uncertainty is important to consider with this mixing model. However, the analytical error for each measurement (see methods) is less than the size of the symbol. This is also the case for salinity measurements. For this reason, uncertainties are not displayed in Figures 4 and 5.

As for the suggestion of a Bayesian mixing model, we believe performing such a model is beyond the scope of this study. We believe the simple mixing model employed here (Kaldy et al., 2005) is better suited for this study. However, in an attempt to further clarify the way in which this model was used, both Figures 4 and 5 and their captions have been slightly altered. The conservative mixing model has been changed from individual points to a continuous line connecting calculated points, as the system (not individual points) are being modeled. The captions have been altered to read as follows:

"Figure 4. (a) Measured δ13C-DOC values and salinity for West Falmouth Harbor are plotted against an isotopic conservative mixing model for location. Deviations from the model suggest contributions of DOC that is distinct from the assumed end-members. (b) Spatial plot of isotopic signatures measured at West Falmouth Harbor."

And:

"Figure 5. (a) Measured δ13C-DOC values and salinity for both North and South Barnegat Bay are plotted against an isotopic conservative mixing model for location. Deviations from the model suggest contributions of DOC that is distinct from the assumed end-members. (b) Spatial plot of isotopic signatures measured at Barnegat Bay."

iv) Overall the manuscript has sections which appear to be the ones of a student paper. Please make sure the overall quality is high. One example is that all data from Table 2 and 3 is presented in the plots already. Please avoid giving the reader redundant information. If appropriate at all, move these tables to a supplementary.

The manuscript has been edited according to the suggested changes and minor comments listed below, as well as additional editing by each of the authors and an internal organizational review. We are confident that these edits have increased the overall writing and organizational quality of the manuscript. This includes moving Tables 2 and 3 to a supplementary information section (note: this means the original Table 4 has been relabeled as Table 2; the original Tables 2 and 3 are now Tables S1 and S2, respectively). While the information provided in these tables is presented in various plots, the tables conglomerate all of the relevant information from each sampling location, rather than requiring the reader to pull such information from each individual plot. That being said, we agree that redundant information of this type is not necessary for the main text, hence the move to the supplementary information section.

Minor comments and suggested changes:

Title: The second part of the title does not make sense to me. What source and what quantification? Please revise. One option would be to modify to "the effect of different carbon sources on light attenuation". Another option is more torwards "the effect of land-use on light attenuation".

As the referee points out, the title could be more descriptive of the work presented. Several options for title were considered, and the following title was chosen: ***"Colored dissolved organic matter in shallow estuaries: relationships between carbon sources and light attenuation."*** *This*

title incorporates the suggestions provided here, while communicating that the manuscript concerns both effects and identification of relationships.

P7302L7: suggest remove "and models" as this is redundant. Models just calculate.

This is a good correction- the original text was redundant. The suggested change has been made.

P7305, L26: The percentages do not add up to 100%, what is the remaining?

We acknowledge that not including all components is confusing. The remaining 36% is forested area. This correction has been added to the site description for Chincoteague Bay (Pg 7305 line 26).

P7306 and P7307: Abs samples are filtered, whereas Florescence is not. Even if F is corrected for turbidity, any idea what the effect of this is? Also, how long were the samples stored and how, before Abs analysis was done? Together, this may cause some of the inconsistencies.

We are not sure of the effect of not filtering samples before making fluorescence measurements. However, we contend that this is not a matter of concern for this study. The work presented here is meant to analyze to usage of fluorescence (fDOM) data as typically collected in situ as a proxy for absorbance by CDOM. fDOM is often measured in situ in the manner employed for this study. Of course, the necessary corrections (described in Downing et al., 2012) for temperature, turbidity, and inner filter effects are necessary after data collection. For CDOM absorbance measurements however, one must ensure that only the dissolved portion of organic matter is being measured, hence the filtering of samples. In this way, we were able to compare easily collected in situ fDOM data to actual CDOM absorbance measurements. Filtering of samples for fluorescence measurements would not yield the comparison we aimed to analyze in this study.

As for storage times, fluorescence and absorbance analyses were performed simultaneously at West Falmouth Harbor and for land-approached sites at Barnegat and Chincoteague Bays. For samples collected via boat at BB and CB, absorbance samples were stored in one-liter Nalgene sampling bottles (as discussed in Methods Section 3.1) due to the inability to operate the spectrophotometer on deck. These samples were then analyzed for absorbance on shore during the same day, after inverting the sampling bottles to ensure mixing. Given that these measurements were all performed either simultaneously or within several hours of one another, and that the fluorescence data has been corrected for turbidity, temperature, and inner filter effects, we are very confident in the quality of the data.

P7308: description of Delta 13C analysis very clear.

We are glad that this description is clearly stated, as this was an important part of the study methods.

P7309, L22-24: last sentence belongs to discussion.

This is a good point, as the statement compares results to the findings of other studies. This statement has been moved to the very beginning of the discussion section on spectral slopes (Pg7312, line 14).

P7310, L14: how is the 'strongest' quantified here?

In this statement, "strongest" was meant to indicate the locations with the steepest decrease in fDOM signal over the salinity gradient. This usage has been clarified in the updated text by substituting in "steepest relationship (most rapidly decreasing fDOM signal with increasing salinity)" for "strongest" (Pg 7310, line 14).

P7311, L16-17: this belongs to the discussion.

Once again, the statement mentioned here discusses the agreement of our results with information from another study (Ganju, 2011), and therefore belongs in the discussion. It has been moved to the end of Section 5.3- the discussion of isotopes and mixing in the estuaries (Pg 7315, line 17).

P7312, L1-11, as well as Fig6: Not sure what this comparison tells. Looks to me as if a global relationship could even be established. . .

The comparison presented in Figure 6 is meant to display any relationship between isotopic signature and the fDOM-CDOM ratio. Because we establish the variability in the fDOM-CDOM relationship earlier in the paper (Figure 3), DOC source is investigated as a possible explanatory factor. While there is certainly not an exponential relationship between these variables (as a reminder, the x-axis of Figure 6 is presented on a natural log scale), there is still a discernable, positive relationship, as seen in Figure 6. The generally higher CDOM absorbance per unit fluorescence observed for West Falmouth Harbor (Figure 3) corresponds with the generally ^{13}C-enriched isotopic signatures observed for WFH. The relatively lower CDOM absorbance per unit fluorescence observed

for Barnegat Bay (North and South) corresponds with the relatively less ^{13}C-enriched isotopic signatures measured at this estuary. While the trend is not necessarily observed when considering only the majority of Barnegat Bay samples alone, the outliers with respect to fDOM-CDOM relationship emphasize this trend. Site BB01 displays both the lowest fDOM-CDOM absorption ratio and the least ^{13}C-enriched isotopic signature. Site WF02 displays both the highest fDOM-CDOM absorption ratio and the most ^{13}C-enriched isotopic signature. As discussed in Section 5.4, DOC source appears to be correlated with fDOM-CDOM absorption ratio, and potentially provide an explanation for deviations from the somewhat uniform fDOM-CDOM relationship observed for CB, and most samples at BB.

P7313, L3: please revise, as the sentence doesn't read well.

We agree that this sentence could be much clearer. It has been edited to read, "Some of the variability in spectral slopes observed at West Falmouth Harbor may be attributed to the physical complexity and short residence time of the estuary, especially when considering that DOM source is known to affect DOM optical properties (Helms et al., 2008; De Souza Sierra et al., 1994)." (Pg 7313, line 3)

P7317, L5pp: isn't past tense more appropriate here?

Yes, we agree that past tense is more appropriate for the conclusions presented in the lines indicated by the referee here. Therefore, tense has been changed from present to past for the entire Conclusions section, with the exception of the opening sentence of the section (Pg 7317, lines 5-14).

P7317, L13-14: not sure I agree. How would this help? Suggest remove sentence.

This statement was meant to provide an example application of the improved precision of light models described in Table 4. However, we agree that this sentence is a bit of an overstatement. Therefore, it has been removed, as suggested. Between this and the previous comment, the Conclusions section has been edited to read as follows:

"The results of this study show that the fDOM-CDOM absorption relationship is variable both between and within West Falmouth Harbor, Barnegat Bay, and Chincoteague Bay, and depends upon DOM source. DOM that was 13C-enriched (higher δ13C values) also had a higher absorption coefficient per unit fluorescence. Additionally, fDOM-salinity relationship was variable between and within these estuaries. The exception here was the lack of variability in these relationships within Chincoteague Bay. Future work in relation to this study might involve a stable carbon isotope analysis at Chincoteague Bay similar to the analysis carried out here for West Falmouth Harbor and Barnegat Bay. Results of such an analysis could further elucidate the effects of DOM source on the fDOM-CDOM

absorption ratio. Finally, spectral slopes for use in light models were consistent between and within Barnegat and Chincoteague Bays, with more variability observed at West Falmouth Harbor."

Figures:

Almost all figures are not developed well enough:

The comments provided were very constructive in improving the quality of the figures, and therefore the communication of results. We are confident that the figure edits outlined below, along with those suggested by Anonymous Referee #1, have raised the figures to publication quality.

Fig1: text too small, even if there is enough space. Also please use more contrast/a line for the shore.

These suggested edits have been incorporated to make Figure 1 more readable.

Fig2+3: see earlier comment. Also, what is the purpose of many similar looking dashed lines?

The dashed lines represent the best linear fits to the data, as determined by linear regression analyses for the presented data. While this may not have been clear in the original Figures 2 and 3, this should be much more apparent in the updated figures and their associated captions.

Fig 4+5: can't see colors, increase dot size.

Figures 4 and 5 have been edited to be more readable.

Tables: See earlier comment

As discussed in response to the earlier comment referenced here, the tables containing information already presented in the figures have been moved to a supplementary information section.

Further Revisions from Additional Review

We received an additional non-journal referee review, which presented some constructive comments that we have chosen to include. The minor changes made in response to this third review are included below:

Pg. 7302, Lines 9-11 now read, "We quantified the variability in this relationship within three estuaries along the mid-Atlantic margin of the eastern United States: West Falmouth Harbor (MA), Barnegat Bay (NJ), and Chincoteague Bay (MD/VA)."

To include the possibility of DOC contributions from seagrass and macroalgae in the introduction, the following text has been added: "It is worth noting that both seagrass and macroalgae can contribute DOC in these systems as well (Barron et al., 2014; Pregnall, 1983)." (Pg 7304, line 5)

Pg 7304, lines 10-13 now read, "The goal of this study is to improve understanding of light attenuation in the estuarine water column by characterizing the optical properties and sources of CDOM in three diverse estuaries located along the mid-Atlantic US margin: West Falmouth Harbor (MA), Barnegat Bay (NJ), and Chincoteague Bay (MD, VA)."

The following sentence has been added at the very end of Section 2.1 (Pg 7305, line 4): "Zostera spp. eelgrass is also present in the harbor (Del Barrio et al., 2014)."

A slight correction was made to the explanation for Equation 1. The sentence that originally read, "Absorbance measurements at each wavelength were converted to absorption coefficients as follows," now reads, "Absorbance measurements were converted to absorption coefficients as follows." (Pg 7307, lines 8-9)

Pg 7314, lines 6-8 now read, "Previous studies of DOC in eastern US estuaries have suggested a marine end-member δ13C value of -24‰ to -22‰, and a freshwater end-member δ13C of -28‰ to -26‰ (Peterson et al., 1994)."

As a caveat to the geographic comparisons made in Section 5.3, the following text has been added: "Considering the movement of water and potential for mixing during residence in the estuary, this geographic analysis is by no means definitive, but does provide some insights." (Pg 7315, line 8)

To clarify the "exception" described in the conclusions section, the text has been edited to read, "The exception to this variability is the relatively uniform relationships observed at Chincoteague Bay." (Pg 7317, line 6)

The revisions listed here also include two additional references, listed here:

Barron, C., Apostolaki, E. T., and Duarte, C. M.: Dissolved organic carbon fluxes by seagrass meadows and macroalgal beds, Front Mar Sci, 1, doi: 10.3389/fmars.2014.00042, 2014.

Pregnall, A. M.: Release of dissolved organic carbon from the estuarine intertidal macroalga Enteromorpha prolifera, Marine Biology, 73, 37-42, 1983.

Figures for Response to Referee Comments

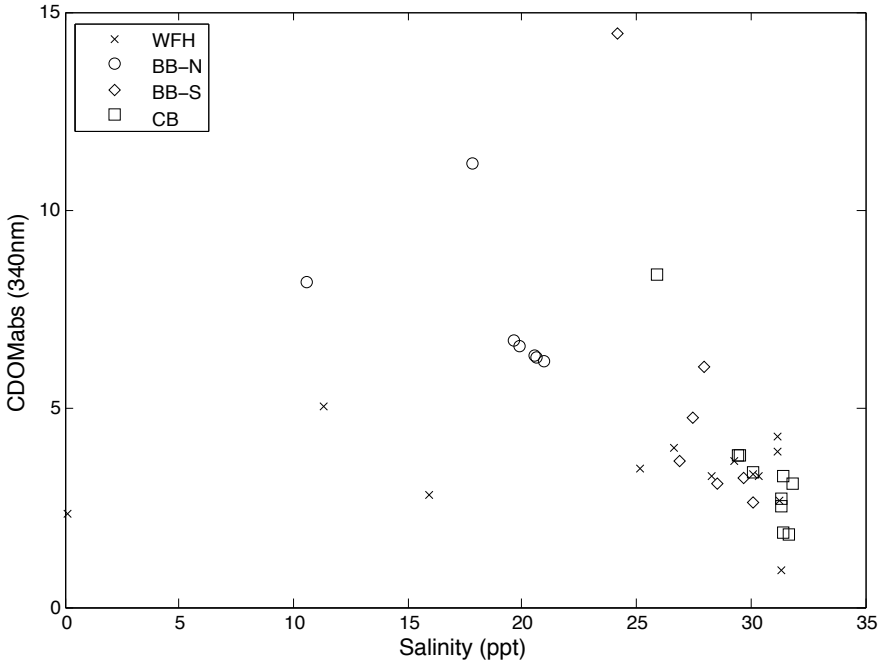

Figure R1. CDOM absorbance coefficient at 340 nm versus salinity for all sample sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), South Barnegat Bay (BB-S), and Chincoteague Bay (CB).

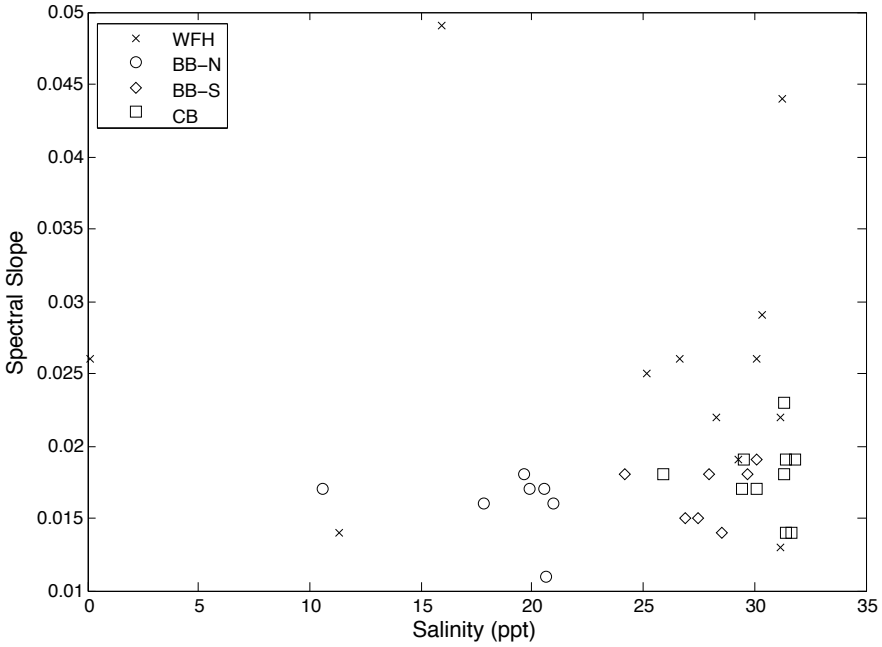

Figure R2. Spectral slope versus salinity for all sample sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), South Barnegat Bay (BB-S), and Chincoteague Bay (CB).

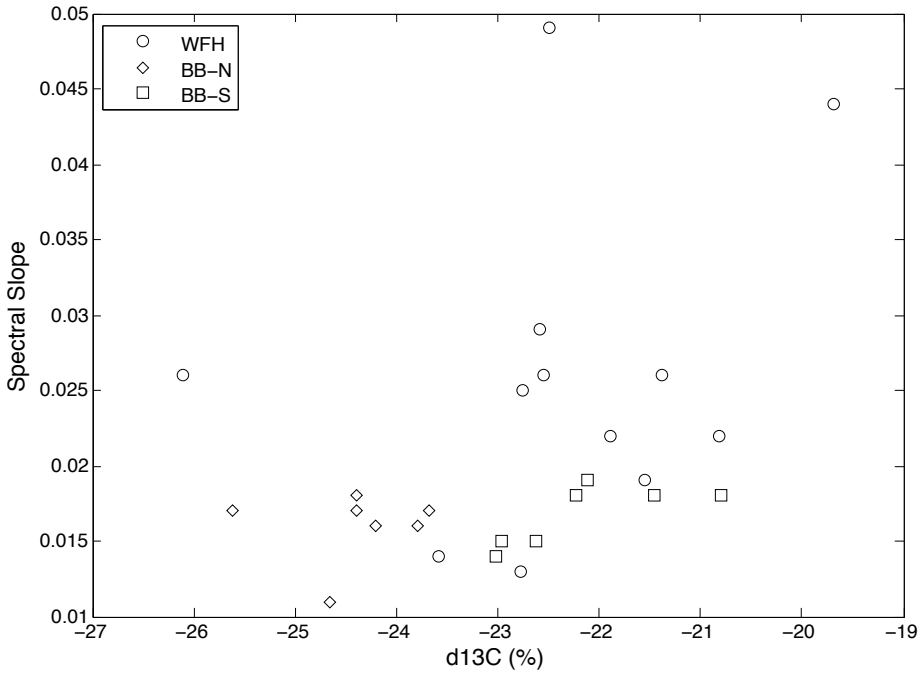

Figure R3. Spectral slope versus isotopic signature for all sample sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), and South Barnegat Bay (BB-S).

Colored dissolved organic matter in shallow estuaries: relationships between carbon sources and light attenuation

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Abstract

Light availability is of primary importance to the ecological function of shallow estuaries. For example, benthic primary production by submerged aquatic vegetation is contingent upon light penetration to the seabed. A major component that attenuates light in estuaries is colored dissolved organic matter (CDOM). CDOM is often measured via a proxy, fluorescing dissolved organic matter (fDOM), due to the ease of *in situ* fDOM measurements. Fluorescence must be converted to CDOM absorbance for use in light attenuation calculations. However, this fDOM-CDOM relationship varies among and within estuaries. We quantified the variability in this relationship within three estuaries along the mid-Atlantic margin of the eastern United States: West Falmouth Harbor (MA), Barnegat Bay (NJ), and Chincoteague Bay (MD/VA). Land use surrounding these estuaries ranges from urban to developed, with varying sources of nutrients and organic matter. Measurements of fDOM (excitation and emission wavelengths of 365nm (±5nm) and 460nm (±40nm), respectively) and CDOM absorbance were taken along a terrestrial-to-marine gradient in all three estuaries. The ratio of the absorption coefficient at 340nm (m^{-1}) to fDOM (QSU) was higher in West Falmouth Harbor (1.22) than in Barnegat Bay (0.22) and Chincoteague Bay (0.17). The fDOM-CDOM absorption ratio was variable between sites within West Falmouth Harbor and Barnegat Bay, but consistent between sites within Chincoteague Bay. Stable carbon isotope analysis for constraining the source of dissolved organic matter in West Falmouth Harbor and Barnegat Bay yielded $\delta^{13}C$ values ranging from -

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19.7‰ to -26.1‰ and -20.8‰ to -26.7‰, respectively. Stable carbon isotope mixing models of DOC in the estuaries indicate contributions from marine plankton, terrestrial plants, and a source from within the marsh that is relatively ^{13}C-enriched (e.g. *Spartina* cordgrass or *Zostera* eelgrass). Comparison of DOC source to fDOM-CDOM absorption ratio at each site demonstrates the influence of source on optical properties. Samples with a greater contribution from marsh organic material had higher fDOM-CDOM absorption ratios than samples with greater contribution from terrestrial organic material. Applying a uniform fDOM-CDOM absorption ratio and spectral slope within a given estuary yields errors in modeled light attenuation ranging from 11-33% depending on estuary. The application of a uniform absorption ratio across all estuaries doubles this error. These results demonstrate that continuous monitoring of light attenuation in estuaries requires some quantification of CDOM absorption and source to refine light models.

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1 Introduction

Benthic primary production in estuaries, including those along the Atlantic coast of the United States, is typically dominated by seagrass (Heck et al., 1995). Furthermore, seagrass acts as an ecosystem engineer in temperate coastal ecosystems via habitat provision and nutrient cycling (Ehlers et al. 2008). Recent anthropogenic nutrient loading to these ecosystems due to industrial and agricultural development has caused a loss of seagrass density. This occurs as eutrophication creates water column algal blooms and increases benthic algae populations (Burkholder et al., 2007; Hauxwell et al., 2003). These algal processes reduce penetration of the light necessary for survival of seagrasses. As anthropogenic impacts on coastal ecosystems compound with increasing urbanization of coastal zones (McGranahan et al., 2007), it is important to understand the factors controlling light attenuation in the estuarine water column.

Four main factors attenuate light in the water column: water itself, non-algal particulate material, phytoplankton, and colored dissolved organic matter (CDOM) (Kirk, 1994). Proxies are typically used to quantify these factors *in situ*: depth, turbidity, chlorophyll-a fluorescence, and fluorescing dissolved organic matter (fDOM), respectively (Ganju et al. 2014). The use of fDOM as a proxy for the CDOM component is widespread due to the ease of measuring *in situ* fluorescence, and the relationship between fDOM and CDOM absorbance. However,

considerable variability in the fDOM-CDOM absorption ratios has been observed both between and within numerous aquatic systems (Hoge et al. 1993; Del Castillo et al. 1999; Clark et al., 2004). Quantifying and understanding the variability in this relationship is required to accurately model light attenuation and seagrass viability in estuaries.

Estuaries are transition zones between freshwater and marine systems where dissolved organic carbon from a variety of sources mixes (Raymond and Bauer, 2001). The major sources of DOC to estuaries are typically terrestrial DOC from riverine inputs, oceanic DOC from phytoplankton, and tidal marsh DOC from emergent and submergent marsh vegetation (Peterson et al., 1994). It is worth noting that both seagrass and macroalgae can contribute DOC in these systems as well (Barron et al., 2014; Pregnall, 1983). Marine and terrestrial DOM exhibit different structural characteristics (Harvey et al., 1983) that are reflected in the optical properties of CDOM (Helms et al., 2008; De Souza Sierra et al., 1994). Due to its role in attenuating light in the water column, measurement of CDOM and enhanced understanding of its source-dependent optical properties is important for modeling light availability in estuaries.

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The goal of this study is to improve understanding of light attenuation in the estuarine water column by characterizing the optical properties and sources of CDOM in three diverse estuaries located along the mid-Atlantic US margin: West Falmouth Harbor (MA), Barnegat Bay (NJ), and Chincoteague Bay (MD, VA). Our objectives are to quantify the fDOM-CDOM absorption ratio, establish absorption spectral slopes for use in light models (Gallegos et al., 2011), determine the sources of CDOM in these estuaries, and identify variation in the fDOM-CDOM absorption ratio as a function of source.

2 Site Descriptions

2.1 West Falmouth Harbor

West Falmouth Harbor is a small (0.7 km^2), groundwater-fed estuary on the western shore of Cape Cod, Massachusetts (Fig. 1b). The harbor has a mean depth of approximately 1 m, and is connected to Buzzard's Bay (and ultimately the Atlantic Ocean) by a 3-m deep, 150-m wide channel. Residence time in the harbor is approximately one day (Hayn et al., 2014). Tide range is 1.9 m during spring tides and 0.7 m during neap tides, with tidal currents at the mouth

approaching 0.5 m/s. The dominant source of freshwater and nutrients is groundwater. Land use surrounding the harbor is largely residential, with influence from a legacy wastewater plume within the aquifer (Ganju et al., 2012). Plant coverage in surrounding wetlands is variable, but *Spartina alterniflora* and *Spartina patens* tend to dominate, with some lesser coverage by *Juncus gerardi* and forbs such as *Salicornia* spp., *Limonium carolinianum,* and *Solidago sempervirens* (Buchsbaum and Valiela, 1987). *Zostera* spp. eelgrass is also present in the harbor (Del Barrio et al., 2014).

2.2 Barnegat Bay

The Barnegat Bay-Little Egg Harbor estuary is a back-barrier system along the New Jersey Atlantic coast (Fig. 1c). The estuary is approximately 70 km long, 2-6 km wide, and 1.5 m deep. Bay and ocean water exchange occurs at three inlets: the Point Pleasant Canal at the northern limit, Barnegat Inlet in the middle of the barrier island, and Little Egg Inlet at the southern limit. Limited exchange through these inlets leads to a spatially variable residence time exceeding 30 d in some locations (Defne and Ganju, 2014). For the purpose of this study, sites north of Barnegat Inlet are referred to as "North Barnegat Bay," while sites parallel to and south of Barnegat Inlet are referred to as "South Barnegat Bay." Tides are semidiurnal and range from <0.1-1.5 m, and current velocities range from <0.5-1.5 m/s (Kennish et al., 2013; Ganju et al., 2014); there is also a pronounced south-to-north gradient in tidal range and flushing (Defne and Ganju, 2014). While the land surrounding the northern portion of the bay is developed with mixed urban-residential land use, the area south of Barnegat Inlet is less developed and retains much of the original marsh (Wieben and Baker, 2009). The salt marshes south of Barnegat Inlet are dominated by *Spartina alterniflora* (Olsen and Mahoney, 2001). Freshwater inputs are largest at the northern end of the bay due to the Toms River, Metedeconk River, and Cedar Creek (U.S. EPA, 2007).

2.3 Chincoteague Bay

Chincoteague Bay is along the Atlantic coast of the Delmarva Peninsula (Fig. 1d). This estuary has an area of 355 km^2 and an average depth of 2 m. The watershed surrounding Chincoteague Bay is 487 km^2, and consists of 36% forest, 31% agricultural development, 25% wetlands, and 8% urban development (Bricker et al., 1999). Vegetation in the wetland portion is dominated by *Spartina alterniflora*, much like South Barnegat Bay (Keefe and Boynton, 1973). Tide range averages 0.5 m, and residence time has been estimated at 8 days (Bricker et al., 1999). The Bay

is connected to the ocean via two inlets: Ocean City Inlet in the north and Chincoteague Inlet in the south (Allen et al., 2007). Historically, Chincoteague Bay has been marked by extensive seagrass coverage and higher water quality, especially compared to other more developed and less well-flushed bays on the Atlantic coast (Wazniak et al., 2004).

3 Methods

3.1 Fluorescence measurements

Sampling sites were approached by both land (WF01-WF13, BB01-BB07) and sea (BB08-BB16, CB01-CB10). Sampling occurred from June 25, 2014 to July 17, 2014 (Table 1). Either a bucket (sites approached on foot) or one-liter Nalgene sampling bottle (sites approached by boat) was rinsed with native water and then used to collect a surface water sample. A pre-calibrated YSI EXO 2 multisonde, measuring fDOM, temperature, salinity, pH, turbidity, chlorophyll-a fluorescence, blue-green algae fluorescence, and dissolved oxygen concentration was placed in each sample. Excitation and emission wavelengths for the fluorescing dissolved organic matter sensor were 365nm (±5nm) and 460nm (±40nm), respectively. Measurements of each parameter were collected at 1 s intervals for approximately 60 s and averaged. For sites approached on foot, the YSI EXO was deployed immediately; for sites approached by boat, the YSI EXO was deployed later on land (in concurrence with absorbance measurements, as described below).

Temperature, turbidity, and inner filter effects (IFE) have been shown to alter fluorescence measurements (Baker, 2005; Downing et al., 2012). For this reason, we corrected fluorescence measurements to account for temperature, turbidity, and IFE, according to Downing et al. (2012).

3.2 Absorbance measurements

A 60-ml syringe was used to draw a water sample from these buckets for absorbance measurements. Fifteen ml of this sample was filtered through a 0.2-µm inorganic membrane filter into a 5-cm path length cuvette. Absorbance measurements were recorded in 20-nm increments over the range of 340-440 nm (West Falmouth Harbor) or 340-720 nm (Barnegat Bay and Chincoteague Bay). Although the range of measurements differed for West Falmouth Harbor, spectral slope was calculated over both the entire 340-720 nm range and the 340-440 nm range for Barnegat Bay and Chincoteague Bay to allow for direct comparison to West Falmouth Harbor. The estimated photometric accuracy of the spectrophotometer was 0.003 absorbance

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units. Offsets from zero were determined for the WFH CDOM spectra by running a blank sample (Milli-Q water) at 440nm (the high end of the recorded spectrum). For BB and CB, offsets from zero were determined by running a blank sample before measurement at each wavelength (340-720nm). Absorbance measurements were converted to absorption coefficients as follows:

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$$a(\lambda) = 2.303A(\lambda)/l \quad (1)$$

where $A(\lambda)$ is the absorbance at 340 nm, l is the cell length in meters (0.05 m for this study), and $a(\lambda)$ is the absorption coefficient (Green and Blough, 1994). 340 nm had the highest absorbance values across the range scanned and therefore was chosen as the absorbance wavelength for calculating the absorbance coefficient. Spectral slopes were calculated by plotting the natural log of absorption coefficient against wavelength. Due to use of the natural log, non-positive absorption coefficients were discarded to calculate spectral slope, as described in Equation 2 (Bricaud et al., 1981):

$$S = ln(a(\lambda)/a(r))(r - \lambda) \quad (2)$$

where λ is wavelength, r is a reference wavelength, $a(\lambda)$ is absorption coefficient at a given wavelength, $a(r)$ is absorption coefficient at the reference wavelength, and S is the spectral slope. The value of S shows the rate at which absorption decreases with increasing wavelength (Green and Blough, 1994). This parameter can be used to predict absorption coefficients across the spectrum based on absorption at one reference wavelength (Bricaud et al., 1981).

3.3 Isotope Analysis

At each site in West Falmouth Harbor and Barnegat Bay, water samples were collected for stable carbon isotope analysis of DOC (Chincoteague Bay was excluded due to logistical limitations). Following absorbance measurements, 30 ml of the collected sample was filtered through a 0.2-µm inorganic membrane filter, collected in a 40-ml glass autosampler vial that had been baked at 450 °C for 4 hours and sealed with caps and Teflon-faced silicon septa that had been soaked and rinsed with 10% (by volume) HCl. Additionally, trace metal grade 12N HCl (Sigma Aldrich) was added to each isotope water sample to achieve pH<2. The vials were then stored at 4 °C. Samples were analyzed by High Temperature Combustion - Isotope Ratio Mass Spectrometry (HTC-IRMS) at the USGS-WHOI Dissolved Carbon Isotope Lab (DCIL), as described by Lalonde et al. (2014). The stable carbon isotope ratios are reported in the standard δ-notation

relative to Vienna Pee Dee Belemnite (VPDB) and are corrected by mass balance to account for the analytical blank, which was less than the equivalent of 15 µM DOC in the sample. By comparison, the sample DOC concentrations ranged from 60.7 to 581 µM. Thus the blank correction was always less than 25% of the sample concentration. The analytical precision of the $\delta^{13}C$ analysis was less than 0.3‰.

Salinity and $\delta^{13}C$ values for freshwater and marine endmembers from West Falmouth Harbor and Barnegat Bay were used to construct isotope mixing models for the estuaries (Kaldy et al., 2005). Marine and freshwater end-members are defined as the most and least saline samples collected at each estuary. Because of the number of samples clustered near the highest salinity for each estuary, marine end-members were checked with geographic location. For West Falmouth Harbor, the site chosen as marine end-member (WF01) was taken from the mouth of the harbor where the estuary connects to Buzzard's Bay. For Barnegat Bay, the site of highest salinity (BB13) was taken from the middle of Little Egg Harbor in South Barnegat Bay. However, a more geographically intuitive marine end-member would be site BB16, near Little Egg Inlet. The only slightly lower salinity at this site (29.69 psu) as compared to BB13 (30.08 psu), along with the geographic location of BB16 at an oceanic inlet, makes BB16 a more appropriate marine end-member. Therefore, end-members used in the conservative mixing models were as follows: WF06 (freshwater), WF01 (marine), BB01 (freshwater), and BB16 (marine). The conservative mixing models (Kaldy et al., 2005) were constructed as:

$$C_{mix} = fC_R + (1 - f)C_O \quad (3)$$

where C_{mix} is the calculated concentration for use in the mixing model, C_R and C_O are freshwater and marine end-member DOC concentrations, respectively, and f is the fraction of freshwater calculated from salinity:

$$f = (S_O - S_M)/(S_O - S_R) \quad (4)$$

where S_M is measured salinity at a specific site, and S_R and S_O are freshwater and marine end-member salinities, respectively. These calculations lead to the modeled isotope ratio of each sample as:

$$\delta_{mix} = [fC_R\,\delta_R + (1 - f)C_O\,\delta_O]/C_{mix} \quad (5)$$

where all subscripts and variables are the same as described for Eq. 3 and 4.

4 Results

4.1 Spectral slopes

The estuary-wide average spectral slope (over the range 340-440 nm) for West Falmouth was higher than for Barnegat and Chincoteague, with S_{avg} equal to 0.021, 0.016, and 0.018, respectively (Table S1). At West Falmouth Harbor, spectral slope ranged from 0.013 – 0.044, with a standard deviation of 0.010. At Barnegat Bay, S ranged from 0.011 – 0.019, with a standard deviation of 0.002. At Chincoteague Bay, S ranged from 0.014 – 0.023, with a standard deviation of 0.003. Spectral slope values for Barnegat and Chincoteague were slightly higher over the range 340-440 nm as compared to S calculated over the range 340-720 nm (Table S1).

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4.2 Fluorescence measurements (fDOM)

At West Falmouth, fDOM ranged from 0.63 – 10.21 QSU, with a standard deviation of 2.57 QSU. At Barnegat Bay, fDOM ranged from 12.06 – 84.40 QSU, with a standard deviation of 20.82 QSU. At Chincoteague Bay, fDOM ranged from 11.15 – 49.49 QSU, with a standard deviation of 10.95 QSU. Values observed for fDOM were within ranges reported for similar estuaries and coastal waters (Callahan et al., 2004; Clark et al., 2002; Green and Blough, 1994). Sites at West Falmouth and Barnegat Bay represented a freshwater to seawater gradient, with salinity ranging from 0.13 – 31.28 psu at West Falmouth and 3.41 – 30.08 psu at Barnegat. At Chincoteague Bay, salinity ranged from 25.88 – 31.85 psu. A complete salinity gradient was not sampled at Chincoteague due to the relatively high salinity found throughout the main basin of the bay, and low freshwater input. fDOM correlated inversely with salinity (Fig. 2), as expected because riverine input is typically the main external source of DOM. However, the slope and strength of the fDOM-salinity relationship differed both between and within estuaries. The steepest relationship (most rapidly decreasing fDOM signal with increasing salinity) was observed at Chincoteague Bay and in South Barnegat Bay. These two areas displayed a similar fDOM-salinity relationship, fDOM and salinity showed a slightly less negative relationship at South Barnegat Bay, and even less negative at West Falmouth Harbor.

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4.3 CDOM absorption and fDOM-CDOM ratios

At West Falmouth, $a(340)$ ranged from 0.92 – 5.07 m^{-1}, with a standard deviation of 1.02 m^{-1}. At Barnegat Bay, $a(340)$ ranged from 0.97 – 14.97 m^{-1}, with a standard deviation of 3.99 m^{-1}. At Chincoteague Bay, $a(340)$ ranged from 1.84 – 8.38 m^{-1}, with a standard deviation of 1.86 m^{-1} (Table 2). Absorption coefficients for West Falmouth and Chincoteague were comparable to those reported for similar estuaries and coastal waters (Chen et al., 2003; Green and Blough, 1994); absorption coefficients for Barnegat Bay were somewhat higher, but within the range reported by Green and Blough (1994). The ratio between $a(340)$ and fDOM differed both between and within estuaries, as expected (Table S1; Fig. 3). The mean ratio of $a(340)$ to fDOM was relatively higher in West Falmouth Harbor (1.22) than in Barnegat Bay (0.22) and Chincoteague Bay (0.17). There were two significant outliers at Barnegat Bay: BB01, which had a lower absorption coefficient (0.97 m^{-1}) than expected based on its higher fDOM value (69.92 QSU); and BB15, which showed a much higher absorption coefficient (14.97 m^{-1}) than expected based on its lower fDOM value (16.50 QSU). West Falmouth also demonstrated substantial variability in $a(340)$/fDOM ratio between sites. Chincoteague Bay however, showed a highly consistent ratio.

4.4 Stable carbon isotope analysis

The observed isotope-salinity relationship at West Falmouth Harbor and Barnegat Bay had numerous $\delta^{13}C$ values well outside the range predicted by the conservative mixing models (Table S2; Figs. 4a and 5a), which suggests an additional DOM source from within the estuaries (discussed further in Section 5.3). For West Falmouth Harbor, end-members of the conservative mixing model had $\delta^{13}C$ values of -23.0‰ and -26.1‰. The observed $\delta^{13}C$ data however, ranged from -19.7‰ to -26.1‰, six of which were more ^{13}C-enriched samples than the modeled range. For Barnegat Bay, end-members of the conservative mixing model had $\delta^{13}C$ values of -22.1‰ and -26.7‰. The observed $\delta^{13}C$ data ranged from -20.8‰ to -26.7‰, four of which were more ^{13}C-enriched than the modeled range. The two points from North Barnegat Bay falling well above the model (Fig. 5a) correspond to sites BB04 and BB09. The two points from South Barnegat Bay falling well above the model correspond to sites BB12 and BB14. These ^{13}C-enriched samples from Barnegat were all taken from areas near significant stretches of marsh along the western edge of Barnegat Bay. Spatial representation of $\delta^{13}C$ values at Barnegat Bay

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Moved down [1]: 4b) show ^{13}C-depleted samples in the northeastern corner of the harbor, the location of a freshwater culvert discharging groundwater (Ganju, 2011).

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(Fig. 5b) shows significantly less negative $\delta^{13}C$ values in South Barnegat Bay compared to North Barnegat Bay. This indicates more ^{13}C-enriched samples from South Barnegat Bay.

4.5 Comparison of isotopic signature and fDOM-CDOM absorption ratio

Comparison of the isotopic and optical analyses suggests a relationship between $\delta^{13}C$ signature and fDOM-CDOM absorption ratio (Fig. 6). For both West Falmouth Harbor and Barnegat Bay, the more ^{13}C-enriched samples also had a higher absorption coefficient per unit fluorescence. This trend is highlighted by the extremes of the dataset, with the most ^{13}C-enriched sample (WF02) displaying the highest fDOM-CDOM absorption ratio, and the least ^{13}C-enriched sample (BB01) displaying the lowest fDOM-CDOM absorption ratio. Furthermore, West Falmouth Harbor samples had both higher fDOM-CDOM absorption ratios (-0.032, natural log scale, average) and ^{13}C enrichment ($\delta^{13}C$ average of -22.4‰) as compared to Barnegat Bay (-1.75 and -23.4‰, respectively).

5 Discussion

5.1 Spectral slope ranges

All values observed for spectral slope were within ranges reported for similar estuaries and coastal waters (Keith et al., 2002; Green and Blough, 1994). At Barnegat Bay and Chincoteague Bay, the range of calculated spectral slopes was quite small (Table S1). At West Falmouth Harbor, however, there was significantly more variability in spectral slope. This is likely due to a combination of at least two factors. For one, the relatively low DOC concentrations from West Falmouth Harbor contributed to more instrumental variability in spectral slope values at this estuary. Significantly lower fDOM and absorbance measurements were recorded at West Falmouth Harbor compared to Barnegat Bay and Chincoteague Bay (Table S1). Secondly, West Falmouth Harbor is a relatively dynamic system with multiple freshwater point sources and unique mixing characteristics (Ganju et al., 2012). Considering that DOM source is known to affect its optical properties (Helms et al., 2008; De Souza Sierra et al., 1994) some of the variability in spectral slopes observed at West Falmouth Harbor may be attributable to the physical complexity and short residence time of the estuary. More specifically, previous studies have shown that DOM comprised of primarily fulvic acids has steeper spectral slopes than DOM

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comprised of primarily humic acids (Carder et al., 1989). Considering the complexity of point sources at West Falmouth Harbor, variable organic matter composition and spectral slope is not surprising.

5.2 Variability in fDOM-salinity relationship

The inverse relationship between fDOM and salinity observed for these three estuaries is consistent with previous studies of similar waters (Clark et al., 2002; Green and Blough, 1994). The slope of this inverse relationship varied between and within estuaries. This is due to differences in organic matter composition and fluorescence between the freshwater sources (Stedmon et al., 2003; Parlanti et al., 2000). It is noteworthy that South Barnegat Bay and Chincoteague Bay display a very similar fDOM-salinity relationship, while South Barnegat Bay and North Barnegat Bay show a divergent relationship. South Barnegat Bay and Chincoteague Bay also have geographic and land use similarities with less development and extensive *Spartina alterniflora*-dominated marshes (Wieben and Baker, 2009; Olsen and Mahoney, 2001; Keefe and Boynton, 1973), whereas North Barnegat Bay is much more developed (Wieben and Baker, 2009). Furthermore, North and South Barnegat Bay appear to have different organic matter sources (determined via isotope analysis; see Section 5.3). This information considered together supports the idea of differing organic matter sources due to various inputs affecting fluorescence properties. As for the variability seen within West Falmouth Harbor, this is again likely attributable to the relatively low fluorescence signals observed throughout the estuary, along with the variety of freshwater inputs to this complex system.

5.3 Role of additional end-member in isotope mixing

The disparity between observed $\delta^{13}C$ values and those predicted by conservative mixing models (Figs. 4a and 5a) suggest an additional DOM source within the estuaries. Previous studies of DOC in eastern US estuaries have suggested a marine end-member $\delta^{13}C$ value of -24‰ to -22‰, and a freshwater end-member $\delta^{13}C$ of -28‰ to -26‰ (Peterson et al., 1994). Observed values falling above the mixing model and approaching much more ^{13}C-enriched values than the defined marine end-member is likely due to the influence of DOC from *Spartina* spp. cordgrass in nearby salt marshes. Analysis of DOC *Spartina* spp. by past studies has indicated a $\delta^{13}C$ signature of about -16.4‰ to -11.7‰ (Komada et al., 2012; Chmura and Aharon, 1995). The tendency of values from this study towards this ^{13}C-enriched signature, in combination with

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knowledge of *Spartina* coverage around the sites differing from conservative mixing models, suggests a DOM source derived from *Spartina* cordgrass. The influence of this end-member is particularly notable in South Barnegat Bay (specifically sites BB12 and BB14), where *Spartina* coverage is extensive (Olsen and Mahoney 2001), and the $\delta^{13}C$ of the DOC is -21.6‰ and -20.9‰ for BB12 and BB14, respectively. Although *Spartina* coverage in North Barnegat Bay is not as extensive as in South Barnegat Bay, the sites with DOC $\delta^{13}C$ values that are more enriched than the conservative mixing model for North Barnegat Bay (BB04 and BB09) were taken from inland sampling locations, specifically the north bank of the lower Toms River and Reedy Creek, where stands of *Spartina* are present.

However, the observed ^{13}C-enrichment could also be attributed to *Zostera* eelgrass, which has been shown to exhibit a ^{13}C-enriched signature (Hemminga and Mateo, 1996). For this reason, the aforementioned samples falling well above the conservative mixing models cannot necessarily be considered a result of *Spartina* influence. However, a comparison of site locations to known seagrass and *Spartina* wetland coverage can yield some indication of the most likely source of ^{13}C-enriched DOC. Seagrass coverage maps (Lathrop and Haag, 2011) and maps of estuarine intertidal wetland coverage (U.S. Fish & Wildlife Service, 2015) for Barnegat Bay show intertidal wetland coverage and no seagrass coverage for sites BB09, BB12, and BB14. Site BB04 is characterized by neither coverage, but its inland location places it much closer to known intertidal wetland coverage (U.S. Fish & Wildlife Service, 2015). This geographic comparison indicates *Spartina* as the more likely additional end-member at Barnegat Bay, though *Zostera* influence is still possible. Considering the movement of water and potential for mixing during residence in the estuary, this geographic analysis is by no means definitive, but does provide some insights.

For West Falmouth Harbor, sites falling well above the conservative mixing model (WF02, WF03, WF04, WF05, WF07, WF11) were compared to known seagrass (Del Barrio et al., 2014) and intertidal wetland (U.S. Fish & Wildlife Service, 2015) coverage for West Falmouth Harbor. For sites WF03, WF05, WF07, and WF11, there is known intertidal wetland coverage and no known *Zostera* coverage. For site WF02, there is both intertidal wetland coverage and *Zostera* coverage, whereas WF04 corresponds to neither *Spartina* nor *Zostera*. This comparison yields a less clear picture of DOC sources, but this is to be expected considering the aforementioned

complexity of surrounding land uses, potential DOC inputs, and limited mixing at West Falmouth Harbor. Furthermore, spatial representation of $\delta^{13}C$ values at West Falmouth Harbor (Fig. 4b) show ^{13}C-depleted samples in the northeastern corner of the harbor, the location of a freshwater culvert discharging groundwater (Ganju, 2011).

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5.4 Variability in fDOM-CDOM absorption relationship

The significant variability within a somewhat consistent overall trend between fDOM and absorption by CDOM in these estuaries was expected based on the results of previous studies (Hoge et al., 1993; Del Castillo et al., 1999; Clark et al., 2004). West Falmouth Harbor in particular showed a different absorption coefficient to fDOM ratio as compared to the general trend for Barnegat and Chincoteague Bays (Fig. 3). It should be noted that the CDOM absorbance signal was generally low for all WFH samples, meaning analytical noise in the data could affect this ratio. Furthermore, the fact that WFH samples were zeroed at 440 nm only for absorbance measurements could enhance such noise. However, the low signals observed for WFH inspire confidence in the data, considering that West Falmouth Harbor is marked by strong groundwater influence (Ganju, 2011). In studies of both estuarine and other systems, CDOM levels have been measured at low levels in groundwater as compared to other sources (Shen et al., 2015; Chen et al., 2010; Huang and Chen, 2009).

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Even with these caveats taken into consideration, the variability in this study can be explained in part by the differing DOC sources within the estuaries. In this study, ^{13}C-enriched DOC sources correspond to a higher absorption coefficient per unit fluorescence (Fig. 6). While the relatively uniform CDOM-fDOM relationship for Barnegat Bay results in clustering of Barnegat Bay points in the center of Figure 6, this relationship is highlighted by both the Barnegat Bay outliers and the higher CDOMabs/fDOM observed for the more ^{13}C-enriched samples at West Falmouth Harbor. Points such as the outliers at Barnegat Bay are indicative of how the fDOM-CDOM relationship can be altered in an estuary with such diverse sources and transport mechanisms. This assertion of variable fDOM-CDOM relationship depending on source is supported by the findings of Tzortziou et al., 2008, which suggested that marsh-exported DOC has a lower fluorescence per unit absorbance as compared to humic DOC (associated with a freshwater source). For our study, ^{13}C-enriched DOC (likely *Spartina* source) was associated with a lower fluorescence per unit absorbance. ^{13}C-depleted DOC (terrestrial source) was associated with a

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higher fluorescence per unit absorbance. While other studies have focused on differences in the fluorescence-absorbance relationship as a function of molecular weight (Belzile and Guo, 2006; Stewart and Wetzel, 1980), the combination of CDOM optical and isotopic analyses presented here provide a connection between CDOM source and optical characteristics, as suggested by Tzortziou et al., 2008.

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5.5 Ramifications for light attenuation modeling

The variability of fDOM optical properties between and within estuaries has important consequences for light attenuation models. Continuous estimates of light attenuation are possible with continuous proxy measurements of turbidity (for sediment), chlorophyll-a fluorescence, and fDOM (Gallegos et al., 2011), but Ganju et al. (2014) found that light models can be highly sensitive to the fDOM/CDOM relationship, specifically in Barnegat Bay. We applied the light model of Gallegos et al. (2011) to the individual measurements of turbidity, chlorophyll-a fluorescence, and fDOM collected in this study. We explored two cases to calculate light attenuation: 1) use of the individual point fDOM/CDOM ratio and spectral slope from measurements; and 2) use of an estuary-wide average fDOM/CDOM ratio and spectral slope (model parameters related to sediment particles and chlorophyll were held constant to values reported in Ganju et al., 2014). Variation in the DOM properties led to average light attenuation errors ranging from 11 to 33% (Table 2), with individual site errors over 200% at sites with the highest deviation from the estuary mean (site BB01, at the landward end of Barnegat Bay). This suggests that constraining optical properties of the DOM pool is critical for light modeling, and that high variability within an estuary may confound use of spatially constant parameters.

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6 Conclusions

The results of this study show that the fDOM-CDOM absorption relationship is variable both between and within West Falmouth Harbor, Barnegat Bay, and Chincoteague Bay, and depends upon DOM source. DOM that was ^{13}C-enriched (higher $\delta^{13}C$ values) also had a higher absorption coefficient per unit fluorescence. Additionally, fDOM-salinity relationship was variable between and within these estuaries. The exception here was the lack of variability in these relationships within Chincoteague Bay. Future work in relation to this study might involve

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a stable carbon isotope analysis at Chincoteague Bay similar to the analysis carried out here for West Falmouth Harbor and Barnegat Bay. Results of such an analysis could further elucidate the effects of DOM source on the fDOM-CDOM absorption ratio. Finally, spectral slopes for use in light models were consistent between and within Barnegat and Chincoteague Bays, with more variability observed at West Falmouth Harbor.

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Acknowledgements

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Author Contributions

W.K.O. executed the sampling strategy and analyzed data. N.K.G. and J.W.P. designed the experiment and assisted in data interpretation. S.E.S. assisted in designing and executing the sampling strategy. All authors contributed to the drafting of the manuscript.

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Table 1. Sampling sites and procedures.

Estuary	No. of sites	Site ID's	Isotope Analysis (Y/N)	Date
West Falmouth Harbor, MA	13	WF01-WF13	Yes	June 25, 2014
Barnegat Bay, NJ	16	BB01-BB16	Yes	July 14-15, 2014
North Barnegat Bay (BB-N)	8	BB01-BB04; BB08-BB11	Yes	July 14-15, 2014
South Barnegat Bay (BB-S)	8	BB05-BB07; BB12-BB16	Yes	July 14-15, 2014
Chincoteague Bay, MD/VA	10	CB01-CB10	No	July 17, 2014

Table 2.

Light attenuation model parameters and ensuing errors arising from usage of estuary-wide mean values. Note reduced number of significant figures for reporting of spectral slope as compared to Table S1.

Estuary	Mean fDOM/CDOM ratio (range)	Mean spectral slope (range)	Mean light attenuation error (range)
West Falmouth Harbor, MA	1.2 (0.50-4.3)	0.03 (0.01-0.05)	15% (0-52%)
Barnegat Bay, NJ	0.23 (0.01-0.96)	0.01 (0.01-0.02)	33% (0-220%)
Chincoteague Bay, MD/VA	0.17 (0.16-0.19)	0.01 (0.01-0.02)	11% (0.01-28%)

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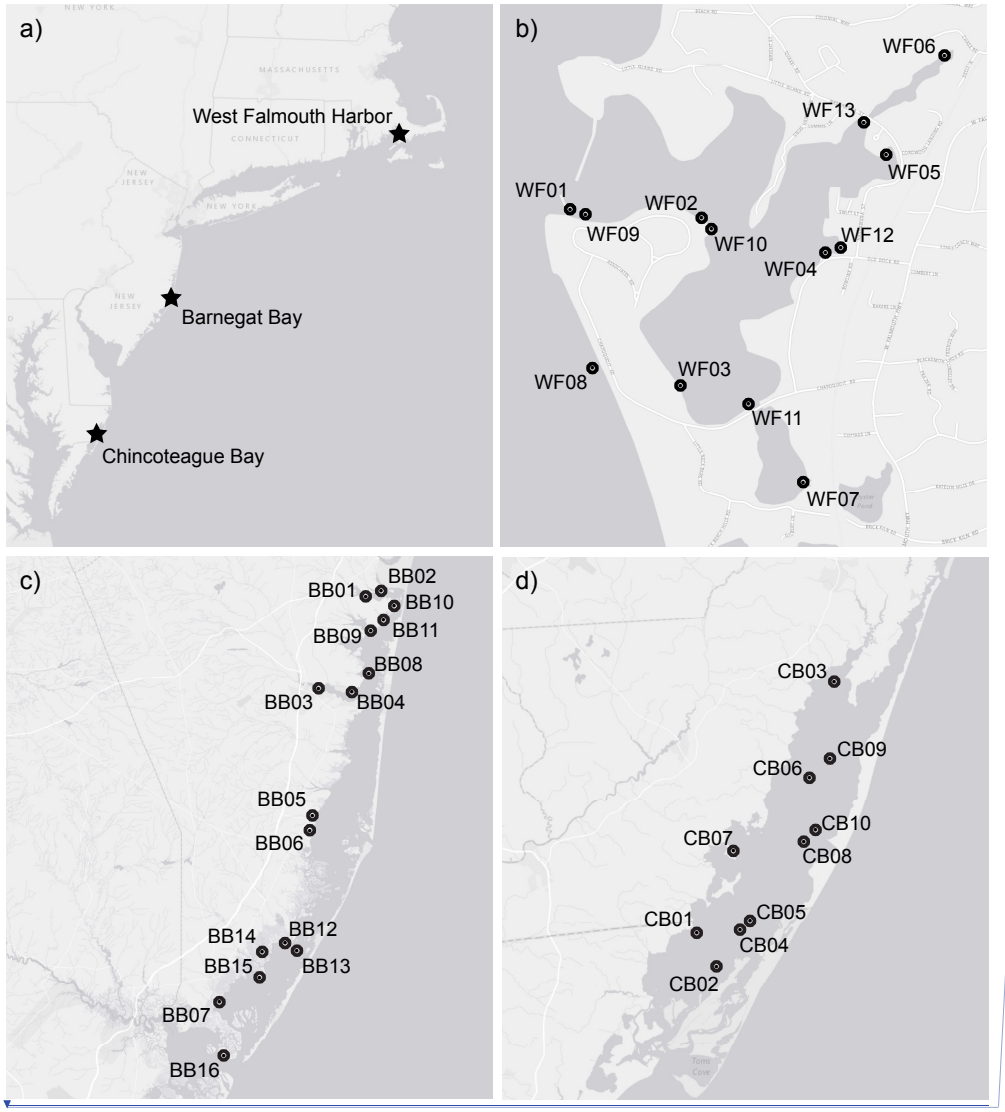

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2 Figure 1. (a) Location of estuaries on the U.S. Atlantic Coast. Study sites within (b) West
3 Falmouth Harbor; (c) Barnegat Bay; (d) Chincoteague Bay.

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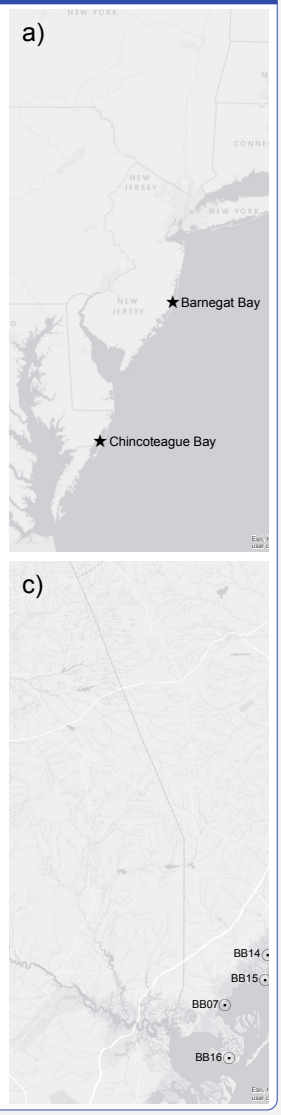

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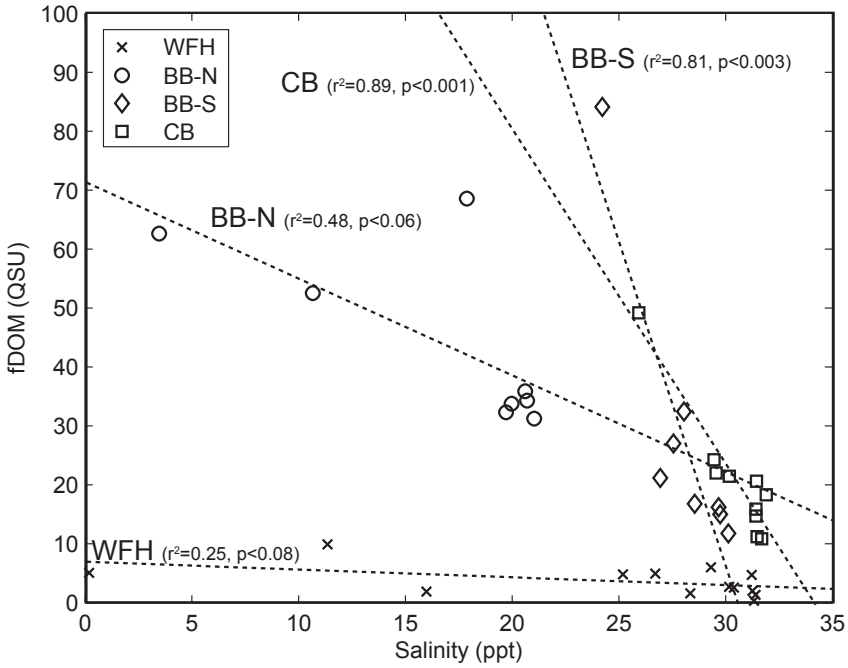

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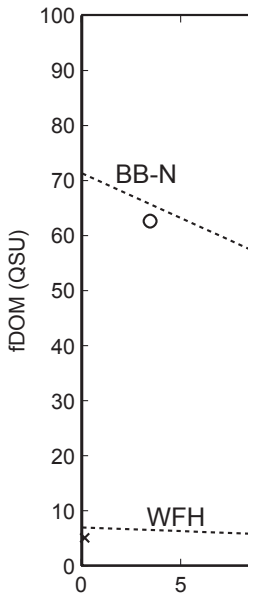

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Figure 2. Fluorescence measurement versus salinity for all sample sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), South Barnegat Bay (BB-S), and Chincoteague Bay (CB). Dashed lines indicate the best linear fits to the data, with associated R^2 and p-value.

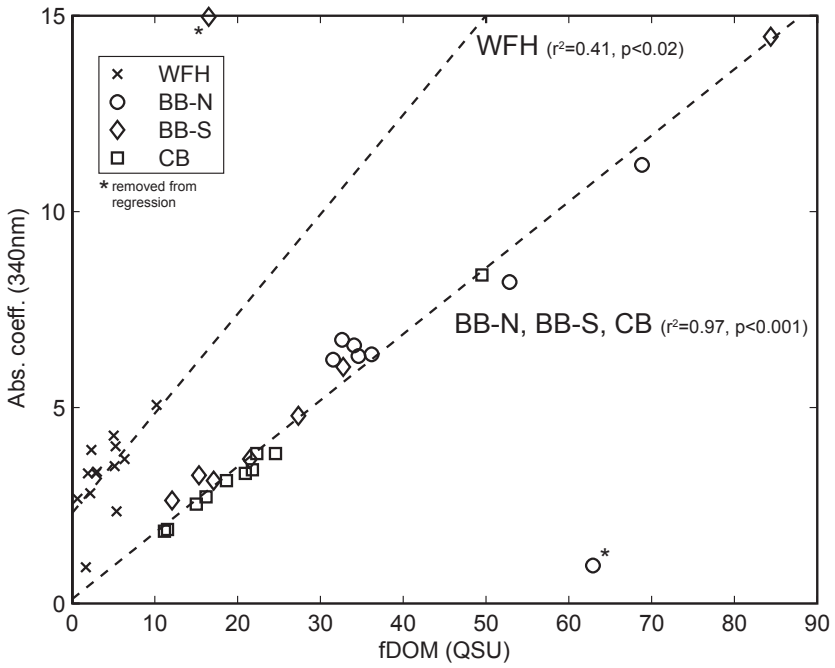

Figure 3. Absorption coefficient at 340nm versus fluorescence measurement for all sampling sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), South Barnegat Bay (BB-S), and Chincoteague Bay (CB). Dashed lines indicate the best linear fit to the data, with associated R^2 and p-value. Two outliers (indicated by "*") removed from the regressions for Barnegat Bay.

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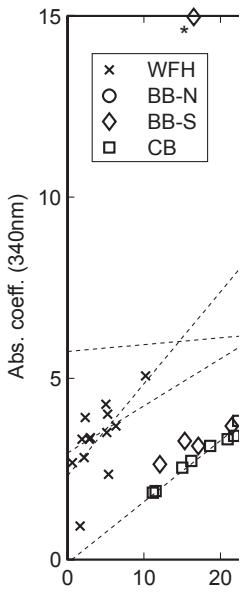

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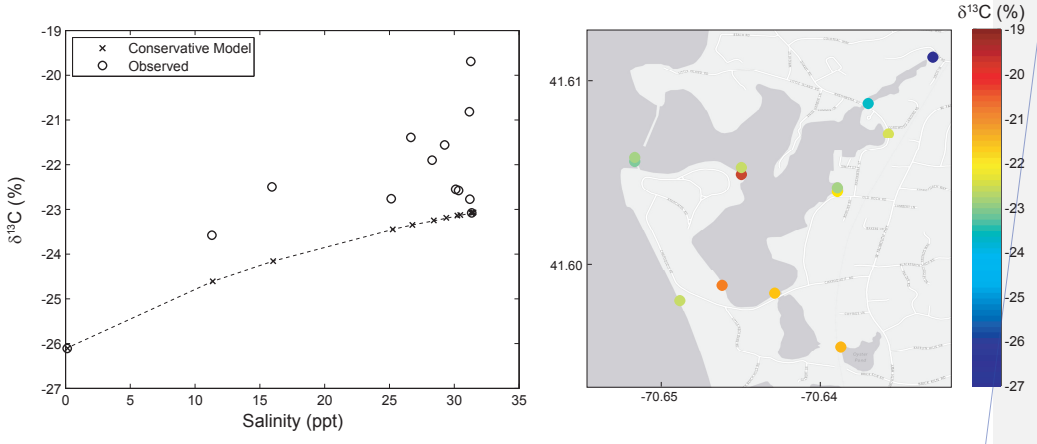

Figure 4. (a) Measured $\delta^{13}C$-DOC values and salinity for West Falmouth Harbor are plotted against an isotopic conservative mixing model for location. Deviations from the model suggest contributions of DOC that is distinct from the assumed end-members. (b) Spatial plot of isotopic signatures measured at West Falmouth Harbor.

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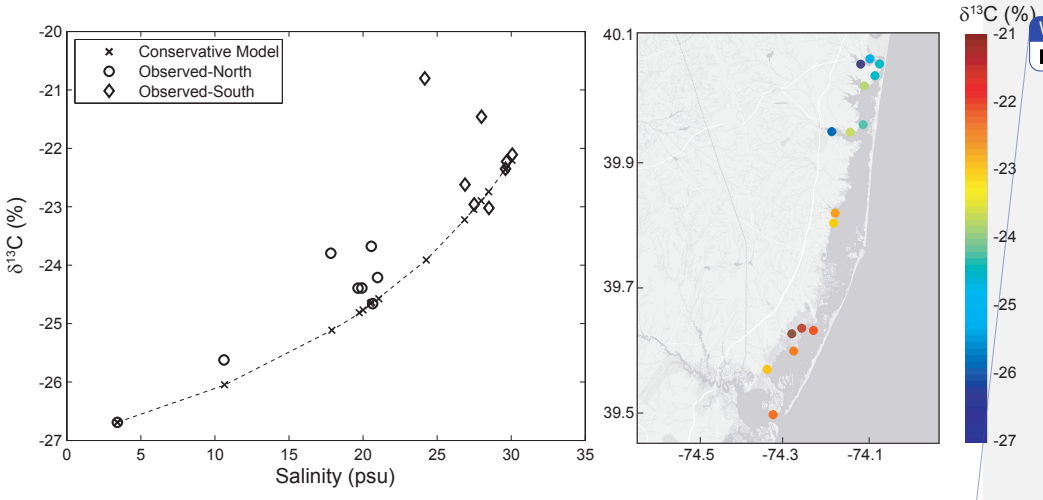

Figure 5. (a) Measured $\delta^{13}C$-DOC values and salinity for both North and South Barnegat Bay are plotted against an isotopic conservative mixing model for location. Deviations from the model suggest contributions of DOC that is distinct from the assumed end-members. (b) Spatial plot of isotopic signatures measured at Barnegat Bay.

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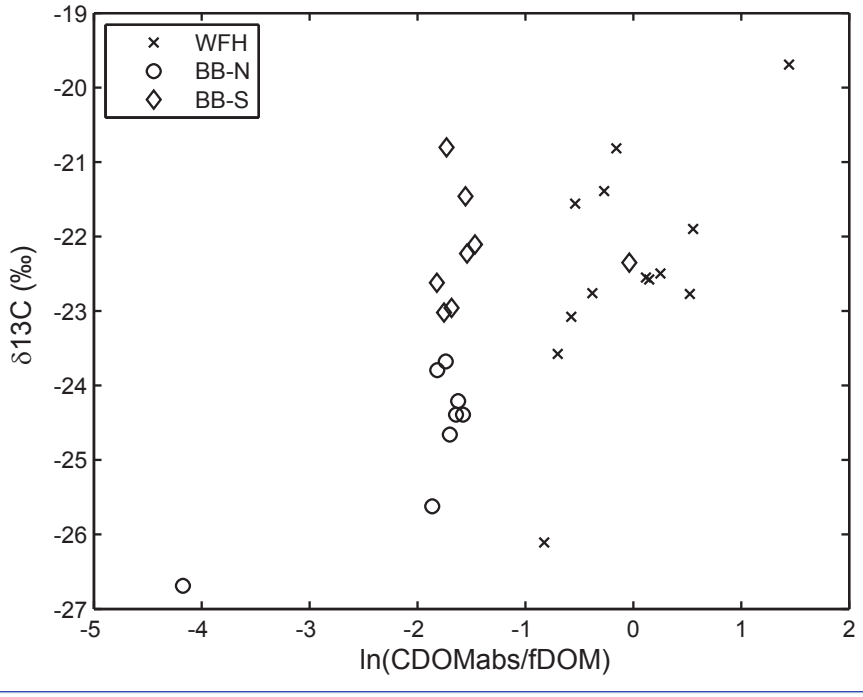

Figure 6. Isotopic signature versus CDOM absorption coefficient (340nm) divided by fluorescence for all sites at West Falmouth Harbor (WFH), North Barnegat Bay (BB-N), and South Barnegat Bay (BB-S). CDOM absorption coefficient per unit fluorescence presented on natural log scale.

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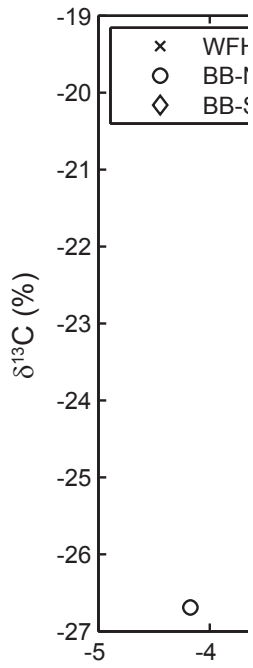

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