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Evaluating the Distribution of Terrestrial Dissolved Organic Matter in a Complex Coastal Ecosystem Using Fluorescence Spectroscopy

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1	Evaluating the distribution of terrestrial dissolved organic matter in a complex coastal
2	ecosystem using fluorescence spectroscopy
3	
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1 Abstract

 $\mathbf{2}$ The coastal zone of the Florida Keys features the only living coral reef in the continental 3 United States and as such represents a unique regional environmental resource. 4 Anthropogenic pressures combined with climate disturbances such as hurricanes can affect $\mathbf{5}$ the biogeochemistry of the region and threaten the health of this unique ecosystem. As such, 6 water quality monitoring has historically been implemented in the Florida Keys, and six 7 spatially distinct zones have been identified. In these studies however, dissolved organic 8 matter (DOM) has only been studied as a quantitative parameter, and DOM composition 9 can be a valuable biogeochemical parameter in assessing environmental change in coastal 10 regions. Here we report the first data of its kind on the application of optical properties of 11 DOM, in particular excitation emission matrix fluorescence with parallel factor analysis 12 (EEM-PARAFAC), throughout these six Florida Keys regions in an attempt to assess 13 spatial differences in DOM sources. Our data suggests that while DOM in the Florida Keys 14can be influenced by distant terrestrial environments such as the Everglades, spatial 15differences in DOM distribution were also controlled in part by local surface runoff/fringe 16 mangroves, contributions from seasgrass communities, as well as the reefs and waters from 17the Florida Current. Application of principal component analysis (PCA) of the relative 18 abundance of EEM-PARAFAC components allowed for a clear distinction between the 19 sources of DOM (allochthonous vs. autochthonous), between different autochthonous 20sources and/or the diagenetic status of DOM, and further clarified contribution of terrestrial 21DOM in zones where levels of DOM were low in abundance. The combination between 22EEM-PARAFAC and PCA proved to be ideally suited to discern DOM composition and 23source differences in coastal zones with complex hydrology and multiple DOM sources.

2	Highlights
3	Spatial distribution of optical properties of DOM was reported for the Florida Keys
4	Regional autochthonous and allochthonous DOM sources were determined
5	• Terrestrial DOM inputs ranged from far locations of the Everglades to local runoff
6	• PCA of EEM-PARAFAC can be useful for monitoring DOM quality in coastal
7	environments
8	
9	Keywords
10	Dissolved organic matter (DOM), Fluorescent dissolved organic matter (FDOM), excitation
11	emission matrix (EEM), Parallel factor analysis (PARAFAC), Environmental monitoring,
12	Florida Keys
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1 **1. Introduction**

 $\mathbf{2}$ In coastal environments, a significant fraction of dissolved organic matter (DOM) is of 3 terrestrial origin. The importance of terrestrial DOM on the coastal ecosystem function has 4 been widely recognized. For example, colored fraction of DOM namely chromophoric $\mathbf{5}$ DOM (CDOM) absorbs and controls the penetration of UV light into the water column 6 (Nelson and Siegel, 2013), protecting aquatic organisms (e.g. corals) from photo-inhibition 7 (Baker et al., 2008). Also, terrestrial DOM may affect phytoplankton dynamics (Glibert et 8 al. 2004; Maie et al., 2012) by providing limiting nutrients in the form of dissolved organic 9 nitrogen (DON) or phosphorus (DOP). As such, variations in DOM dynamics in coastal 10 areas, in particular coral reefs, may have an important impact on these light sensitive, 11 oligotrophic ecosystems. Since coral reefs have been identified as ecosystems particularly 12 sensitive to climate change (Baker et al., 2008) and DOM export from terrestrial 13environments is expected to respond environmental changes (Monteith et al., 2007; Moore 14et al., 2013), detailed studies for evaluating spatial and temporal variations of terrestrial 15DOM in such coastal environments is important. 16 While riverine influences to the nearshore zone can be monitored by the distribution of

salinity, DOC concentrations have been shown to negatively relate to salinity (Cauwet,
2002), indicating that combination of salinity and DOC can be useful to monitor the
transport and fate of terrestrial DOM in estuaries. However, DOC in coastal environments
is a mixture of allochthonous and autochthonous components (Cauwet, 2002; Yamashita et
al., 2008), and thus, the terrestrial DOM component cannot easily be separated from in situ
produced DOM based only on DOC-salinity relationships. In addition, sometimes salinity

evaporation may be important in controlling salinity, or where long residence times obscure
 the salinity effect (Milbrandt et al., 2010; Maie et al., 2012; Cawley et al., 2012). Therefore,
 other monitoring techniques are needed for evaluating the distribution of terrestrial DOM in
 such coastal environments.

 $\mathbf{5}$ Excitation emission matrix fluorescence coupled with parallel factor analysis 6 (EEM-PARAFAC) has been applied to evaluating the dynamics of fluorescent groups of 7 DOM (FDOM) in coastal environments (Stedmon and Markager, 2005; Yamashita et al., 8 2008; Fellman et al., 2010). The advantage of this technique is its high sensitivity and 9 power to successfully differentiate between autochthonous and allochthonous fluorescent 10 components of DOM. In addition, efforts to develop in-situ fluorescence sensors based on 11 EEM-PARAFAC data have shown much promise thus far (Guéguen et al., 2012). Thus, 12 EEM-PARAFAC seems ideally suited for monitoring the distribution of autochthonous as 13well as allochthonous DOM in coastal environments. Most of the coastal zone 14EEM-PARAFAC applications have focused on evaluating the sources and environmental 15dynamics of individual fluorescent groups in river-dominated estuaries based on their 16 fluorescent distribution patterns and relationship with salinity (e.g. Yamashita et al., 2008; 17Kowalczuk et al., 2009; Fellman et al., 2010; Osburn et al., 2012). However, applications in 18 coastal areas where DOM dynamics are not dominated by river discharge have been scarce 19 (Maie et al., 2012; Cawley et al., 2012). 20In the present study, we report the first EEM-PARAFAC data to assess the distribution 21of FDOM in the Florida Keys where salinity cannot be used as a freshwater tracer and

22 multiple sources of DOM prevail. We hypothesized that EEM-PARAFAC can aid in the

23 assessment of DOM sources, and thus, can be used as a monitoring tool for terrestrial DOM

1 at complex coastal environments where salinity cannot be used as a freshwater tracer.

2 Long-term water quality monitoring programs (including total organic carbon; TOC) have

3 been conducted continuously throughout the Florida Keys and Florida Bay for many years,

4 and successfully classified the Florida Keys coastal zone into 6 areas in terms of differences

5 in water quality (Boyer et al., 1997; Boyer and Briceño, 2010). Thus, the Florida Keys,

6 home of the only coral reef track in the continental US, is an area ideally suited to test this7 approach.

8

9 **2.** Site description

10 The Florida Keys region is an archipelago of sub-tropical islands of Pleistocene origin, 11 which extend in a northeast to southwest direction from Miami to Key West and out to the 12 Dry Tortugas (Fig. 1). The waters of the Florida Keys are characterized by complex water 13circulation patterns and are directly influenced by the Florida Current, the Gulf of Mexico 14Loop Current, inshore currents of the southwest Florida Shelf, freshwater discharge from 15mangrove rivers from the south western and southern areas of the Everglades, and by tidal 16 exchange with both Florida Bay and Biscayne Bay (Lee et al., 1994; Lee et al., 2002). 17Seagrass communities are common throughout Florida Bay and along the Florida Keys, 18 while coral reef development occurs mostly offshore on the Atlantic side of the Florida 19 Keys archipelago (Porter and Porter, 2002). Thus, water quality of the Florida Keys may be 20directly affected both by external nutrient and DOM transport and internal loadings through 21natural runoff and anthropogenic activities (Gibson et al., 2008). Previous reports using 22satellite imagery suggest that terrestrial CDOM exported through river drainage of the 23southwest region of the Everglades (Jaffé et al., 2004; Bergamaschi et al., 2011; Cawley et

al., 2013) can reach the Florida Keys (Hu et al., 2003, 2004). On the other hand, seagrasses,
mangrove patches surrounding the Keys and scattered throughout Florida Bay, and
photo-dissolution of re-suspended sediments were suggested to be other contributors to the
CDOM pool in this region (Stabenau et al., 2004; Zepp et al., 2008; Shank et al., 2010a;
Shank et al., 2011; Maie et al., 2012).

6 Based on water quality parameters collected over 15 years

7 (http://serc.fiu.edu/wgmnetwork/), the Florida Keys coastal environment was statistically 8 divided into six zones (Fig. 1; Boyer and Briceño, 2010). The BACK zone was composed 9 primarily of stations located inside and north of the Lower Keys (Fig. 1). This zone was 10 characterized by the highest nutrient and TOC levels among the six zones. The BACK zone 11 is believed to be influenced by solutes transported from the southwest Florida Shelf and/or 12 derived from benthic sources. The BAY zone included sites most influenced by Florida 13Bay and waters moving in a southerly direction from the southwest Florida Shelf. This zone 14is characterized by highest in SiO₂ and elevated TOC levels, but was relatively low in 15inorganic nutrients and chlorophyll a (Boyer and Briceño, 2010). The water quality of the 16 INSHORE, MARQ, REEF, and TORT zones was most similar to each other but 17statistically distinct. The INSHORE included the innermost sites of the Keys, which are 18 shallow, and closest to any possible anthropogenic sources. The REEF was made up of all 19 Hawk Channel and reef tract sites off the mainland Keys. The INSHORE was slightly 20elevated in inorganic nitrogen and TOC relative to the REEF. The MARQ is a zone of 21relatively shallow water which separates the southwest Florida Shelf from the Atlantic 22Ocean. The MARQ had higher total phosphorus (TP) and chlorophyll a (Chla) than TORT 23and REEF but was comparable in nitrogen. The TORT was composed of sites located

1	western part of the study region, including those in Dry Tortugas National Park. The
2	distinction between the REEF and TORT was driven by the slightly higher TOC
3	concentrations and lower TP found in the REEF zone (Boyer and Briceño, 2010).
4	Here we report EEM-PARAFAC data on FDOM from the six abovementioned water
5	quality zones, based on one intensive sampling exercise, in an attempt to assess spatial
6	DOM source distributions and to test the applicability of this technique to spatial
7	monitoring in complex coastal regions.
8	
9	3. Materials and methods
10	Surface water samples were collected from 155 sites located in the Florida Keys during
11	8 January – 15 February 2008 (Fig. 1). These stations represented the same sampling sites
12	originally established for the long-term water quality monitoring program in the Florida
13	Keys (Boyer and Briceño, 2010). Samples for optical characterization of DOM were
14	collected in pre-washed (soaked in 0.1 M HCl and 0.1 M NaOH for 24 h each), brown
15	polyethylene bottles and were stored on ice, returned to the laboratory, and filtered through
16	pre-combusted (450°C, 3h) GF/F filters prior to analysis. The sample collection, field
17	measurements and analytical methods for water quality parameter reported here, i.e.,
18	temperature, salinity, NO_x , NH_4^+ , soluble reactive phosphorus (SRP), SiO ₂ , Chl <i>a</i> , and TOC
19	were described in detailed by Boyer and Briceño (2010).
20	EEM spectra were obtained using a Horiba Jovin Yvon SPEX Fluoromax-3 fluorometer
21	using the method of Maie et al. (2006) and Santín et al. (2009). All fluorescence spectra
22	were acquired in S/R mode, and several post-acquisition steps were involved in the
23	correction of the fluorescence spectra, including inner-filter correction (McKnight et al.,

2001), instrumental bias correction, subtraction of EEM of Milli-Q water, and unit
 conversion to quinine sulfate units (QSU) according to Santín et al. (2009). For inner-filter
 correction, absorbance spectra were measured with a Varian Cary-50 Bio
 spectrophotometer between 250 and 800 nm in a 1-cm quartz cuvette using Milli-Q water
 as the blank.

6 PARAFAC statistically enables characterization and identification of individual 7 fluorescent groups in the EEMs (Stedmon et al., 2003). The approach of PARAFAC 8 modeling to EEMs has been described in detail elsewhere (Stedmon et al., 2003; Ohno and 9 Bro, 2006). The PARAFAC modeling was conducted in MATLAB (Mathworks, Natick, 10 MA) with the DOMFluor toolbox (Stedmon and Bro, 2008). The wavelength range used for 11 this purpose was 260-455 nm and 290-500 nm for excitation and emission, respectively. 12 The validation of the PARAFAC model was conducted according to Stedmon and Bro 13(2008).14A PARAFAC model for evaluating the EEMs obtained at sites in Everglades National 15Park (ENP) and its surrounding area has been established and successfully used for 16 evaluating environmental dynamics of DOM (Chen et al., 2010; Yamashita et al., 2010; 17Pisani et al., 2011; Maie et al., 2012; Cawley et al., 2013). Thus, we expected to apply this 18 ENP-PARAFAC model for comparison of DOM in ENP and Florida Keys. However, the 19 majority of EEMs obtained from Florida Keys were considerably different from those from 20ENP most likely due to the great contribution of subtropical marine waters that are known 21to feature among the lowest CDOM contenting water in the global ocean (Swan et al.,

22 2009; Yamashita and Tanoue, 2008; 2009). Therefore, it was not appropriate to apply the

1 ENP-PARAFAC model for the Florida Keys study, and a new PARAFAC model was $\mathbf{2}$ established.

3 Principal component analysis using relative abundance (%) of PARAFAC components 4 was conducted using KyPlot (v2.0, KyensLab Inc., Japan). Differences in DOM $\mathbf{5}$ characteristics among zones were assessed by non-parametric Mann–Whitney U test 6 (KyPlot, v2.0, KyensLab Inc., Japan).

7

8 4. Results and discussion

9 4.1 Water quality parameters

10 Figure 2 shows the spatial distributions of water quality parameters for the six zones in 11 the Florida Keys for the sample set discussed herein. Salinity ranged from 35.7 to 37.6 (Fig. 12 2a). Lowest salinity was found at BAY and the northern part of REEF where higher 13temperature values were evident (Fig. 2b). The highest salinity values were evident in the 14northern part of BACK and MARQ. The hypersalinity present in this area during this 15sampling event was most probably due to advection of hypersaline surface waters from the 16 southwest Florida Shelf into the Florida Keys (Fig. 2a) as a result of low freshwater input 17from the Everglades (Boyer & Briceño, 2009). The marked difference in water temperature 18 also supports this inference (Fig. 2b). Thus, freshwater contributions could not be 19 determined during the study period solely based on salinity distributions. 20 NO_x , NH_4^+ , and SRP concentrations were higher at TORT and MARQ compared to the 21other zones (Fig. 2c-e). The highest levels of these nutrients are usually reported for the 22BACK zone due to the influence of benthic flux contributions (Boyer and Briceño, 2010). 23

10

The unusual distribution patterns of these inorganic nutrients suggest anomalous conditions

1	in the TORT and MARQ regions probably from advection of waters from the Gulf of
2	Mexico. In addition, levels of NO_x and NH_4^+ were relatively higher at northern part of the
3	INSHORE compared to the adjacent REEF (Fig. 2c, d), suggesting anthropogenic inputs of
4	inorganic nitrogen at the northern part of INSHORE (Boyer and Briceño, 2010).
5	Interestingly, the distribution patterns of SiO ₂ were largely independent from other
6	inorganic nutrients as the highest level of SiO ₂ is found at BAY (Fig. 2f). This is in
7	agreement with data from over 15 years of monitoring efforts, as Boyer and Briceño (2010)
8	reported the same general distribution pattern of SiO_2 and suggested that terrestrial SiO_2
9	from the Everglades or the Florida Shelf reaches the BAY region through water moving
10	southward from the southwest Florida Shelf and/or from Florida Bay.
11	Levels of Chla were less than 0.5 μ g L ⁻¹ at most sites (>0.5 μ g L ⁻¹ at only 9 sites) and
12	did not show any spatial trend (Fig. 2g). TOC concentration was highest at BAY and its
13	distribution was similar to that of SiO ₂ (Fig. 2h), implying that substantial amount of TOC
14	at BAY might be derived from the Florida Shelf. Boyer and Briceño (2010) reported that
15	levels of TOC at BACK are usually higher or similar to those at BAY, however, TOC
16	concentrations at BACK were significantly lower than those at BAY (p <0.01; 180±38 µM
17	vs 287±120 μ M) for the current dataset. Such low levels of TOC at BACK observed in this
18	study were due to advection of hypersalinine waters from the SW Florida Shelf to this
19	region. TOC concentrations at INSHORE were significantly higher than those at REEF
20	(p <0.01; 116±32 µM vs 84±10 µM). Distributions of water quality parameters presented
21	above were generally in agreement with long-term data for the region (Boyer and Briceño,
22	2010), therefore validating this extensive sample set as a representative for this region.
23	

4.2 Distributional characteristics of DOM determined by EEM-PARAFAC

2	Based on the PARAFAC modeling of EEMs, a five-component model was validated
3	(Fig. 3). Spectral characteristics of component 1 (C1; excitation/emission = <260, 305/412)
4	are similar with marine humic-like fluorophore (peak M; Coble et al., 1996). In terrestrial
5	aquatic environments, this component is also known to be produced during microbial
6	degradation of organic matter and is often defined as microbial humic-like fluorophore
7	(Cory and McKnight, 2005; Yamashita et al., 2010). This component is also similar to C4
8	established for the ENP-PARAFAC model ($R = 0.99$ and 0.65 for excitation and emission,
9	respectively) that showed less seasonal variability at the Florida Bay due to relatively
10	higher autochthonous contribution (Maie et al., 2012). Components 2 (C2;
11	excitation/emission = 360 , <260 , 455) and 4 (C4; excitation/emission = <260 , $405/497$)
12	could be categorized as humic-like fluorophore (mixture of peaks A and C; Coble et al.,
13	1996). Similar PARAFAC components with these 3 humic-like components were also
14	reported in other coastal environments (Yamashita et al., 2008; Kowalczuk et al., 2009;
15	Fellman et al., 2010). In these reports, components similar to C1 are usually suggested to be
16	in situ produced in the water column, while components similar to C2 and C4 are
17	considered to be of terrestrial origin. It should be noted C2 and C4 found in this study were
18	not present in the ENP-PARAFAC model (Maie et al., 2012). Such differences between
19	ENP-PARAFAC and Florida Keys-PARAFAC models might be result of greater
20	contributions of wetlands derived DOM and subtropical marine-derived DOM at ENP and
21	Florida Keys respectively. Two other components, i.e., components 3 (C3;
22	excitation/emission = $275/308$) and 5 (C5; excitation/emission = $290/338$), are

23 characterized as protein-like components, namely tyrosine-like and tryptophan-like,

1	respectively (Yamashita and Tanoue, 2003). In coastal environments, a major source of
2	protein-like components is thought to be autochthonous production by phytoplankton
3	(Yamashita et al., 2008; Kowalczak et al., 2009; Mendoza et al., 2011) or from seagrass
4	communities (Maie et al., 2012; Cawley et al., 2012).
5	Figure 4 shows the spatial distribution of fluorescence intensities of individual
6	PARAFAC components along the Florida Keys. Distributional patterns of humic-like C1,
7	C2, and C4 were almost identical (Fig. 4a, b, d), indicating that marine (microbial)
8	humic-like C1 might be allochthonous in origin for this study area. The highest
9	fluorescence intensity of the three humic-like components was evident at BAY and
10	gradually decreased westward toward TORT. At the Atlantic side, levels of three
11	humic-like components were relatively high along the Keys and decreased offshore. These
12	distributional patterns were similar to that of TOC, and TOC concentrations were
13	significantly correlated with the fluorescence intensities of the three humic-like components
14	$([TOC]=15.8\times[C1]+61.3, R^2=0.91, p<0.01, n=138; [TOC]=35.5\times[C2]+57.6, R^2=0.88,$
15	<i>p</i> <0.01, <i>n</i> =138; [TOC]=34.1×[C4]+49.5, <i>R</i> ² =0.88, <i>p</i> <0.01, <i>n</i> =138), implying that a major
16	fraction of TOC (as well as DOC) in this region might consist of terrestrial humic-like
17	components.
18	Fluorescence intensities of protein-like components C3 and C5 (especially the latter),
19	were also similarly distributed with TOC (Fig. 4c, 4e), but were less correlated with TOC
20	compared with humic-like components ([TOC]= $31.7 \times [C3]$ -39.8, R^2 =0.55, p <0.01, n =138;
21	$[TOC] = 48.0 \times [C5] + 11.0, R^2 = 0.79, p < 0.01, n = 138$). The decreases in the levels of
22	protein-like components from BAY to TORT were relatively small compared with those of
23	TOC and humic-like components. Also, relatively high levels of protein-like components

1	were found at REEF. These distributional patterns indicate the autochthonous character of
2	protein-like components in combination with allochthonous (terrestrial) sources. However,
3	distributional patterns of protein-like components also did not correlate with Chla,
4	suggesting that phytoplankton might not be the dominant source of protein-like components.
5	Similar results were also observed other coastal environments (Yamashita et al., 2011;
6	Maie et al., 2012), even though linear relationship between protein-like component and
7	density of the toxic dinoflagellate Karenia brevis has been reported (Mendoza et al., 2011).
8	Thus, bacterial communities (Romera-Castillo et al. 2011; Shimonotori et al., 2011;
9	Guillemette and del Giorgio, 2012), seagrass communities (Maie et al., 2012; Cawley et al.,
10	2012) and coral reefs (Tedetti et al., 2011) might also be important sources for protein-like
11	components. Interestingly, distribution patterns were different between tyrosine-like C3 and
12	tryptophan-like C5, suggesting that environmental dynamics, i.e., sources and sinks, are
13	different between them.
14	At the Atlantic side, TOC concentrations were slightly but significantly higher at
15	INSHORE compared to REEF, as mentioned above (Fig. 2h). There are several possible
16	sources that could explain these higher levels of TOC at INSHORE, including
17	anthropogenic sources, autochthonous production, and/or contribution of terrestrial runoff
18	(including mangroves). Relatively higher levels of inorganic nitrogen, especially NO_x , at
19	INSHORE compared to REEF, indicative of anthropogenic nutrients (Boyer and Briceño,
20	2010), were evident (Fig. 2c, 2d). Since the majority of homes in the Florida Keys have
21	only recently been converted from on-site septic systems to centralized sewers (Briceño and
22	Boyer, 2012), sewage may be an important nearshore source of DOM. It is well known that
23	protein-like fluorophores dominated in waters impacted by domestic sewage and farm

1	wastes (Baker, 2001, 2002). However, in this study, clear spatial gradients of protein-like
2	components from INSHORE to REEF were not evident (Fig. 4c, 4e), suggesting that
3	contribution of anthropogenic DOM and DOM production induced by anthropogenic
4	inorganic nitrogen are minor in this region or are not easily visualized due to interferences
5	from autochthonous protein-like fluorescence from seagrass and reef communities. The
6	relatively high levels of protein-like component fluorescence observed at REEF, MARQ,
7	and TORT, also imply that there are substantial contributions of autochthonous DOM in
8	these zones.
9	The distributional patterns of TOC and humic-like components were correlated based
10	on the observed concentration gradients from nearshore to offshore sites on the Atlantic
11	side of the Keys (Figs. 2h, 4a, 4b, 4d). Fluorescence intensities of humic-like components
12	normalized to TOC were greater at INSHORE compared with REEF (p <0.01; 0.034±0.012
13	vs 0.016±0.007 for C1/TOC, 0.018±0.004 vs 0.009±0.004 for C2/TOC, 0.020±0.005 vs
14	0.012±0.004 for C3/TOC). Such differences in distributional patterns indicate that
15	terrestrial DOM rich in humic-like components from terrestrial runoff (including
16	mangroves) or groundwater inputs is transported from INSHORE to REEF even though
17	salinity did not differ considerably (Fig. 2a).
18	While high salinity groundwater samples off the Florida Keys have been reported to
19	feature DOM enriched in humic-like fluorescence, the general flow of groundwater is
20	believed to be from the Atlantic side of the Keys to Florida Bay (Chen et al., 2010 and
21	references therein). Thus, groundwater is a less likely DOM source to the INSHORE than
22	fringe mangroves, a common habitat along the Florida Keys, and a well known source of
23	DOC as well as CDOM (Jaffé et al., 2004; Dittmar et al., 2006). In addition, Shank et al.

(2011) and Pisani et al. (2011) reported the generation of CDOM rich in terrestrial
 humic-like components during sunlight exposure of organic rich sediments and detrital
 materials. Consequently, the DOM rich in humic-like components at INSHORE is likely
 derived from mangrove and other coastal habitats, with potential contributions of sediment
 photo-dissolution, and transported to REEF with dilution.

6

7 4.3 Statistical analysis of EEM-PARAFAC data

8 To further assess the spatial DOM distribution we applied principal component analysis 9 (PCA) using the relative abundance (%) of individual PARAFAC components from the 10 entire dataset. Figure 5 shows the cross plot of PC1 and PC2 loadings, where the first and 11 second principal component (PC1 and PC2) explained 76% and 19% of the variability, 12 respectively. The three humic-like components clustered close together with positive PC1 13loadings and near 0 for PC2 loadings. In contrast, the two protein-like components showed 14negative values for PC1 loadings, and tyrosine-like C3 and tryptophan-like C5 showed 15significant negative and positive values for their PC2 loadings, respectively. Based on this 16 distribution, PC1 clearly distinguishes samples based on sources (i.e., allochthonous 17humic-like vs. autochthonous protein-like components), while PC2 may be indicative of 18 differences in autochthonous sources and/or the state of degradation of the FDOM. 19 Various sources of DOM have been suggested for the Florida Keys, namely, terrestrial 20DOM from the Everglades (Hu et al., 2003, 2004), seagrasses (Stabenau et al., 2004), 21mangrove patches surrounding the Keys (Shank et al., 2010a), and photo-dissolution from 22sediments (Shank et al., 2011; Pisani et al., 2011). It is also well known that 23photo-bleaching (Nieto-Cid et al., 2006; Shank et al., 2010b) and bio-degradation

1	(Romera-Castillo et al., 2011) are major sinks for CDOM in coastal environments. The
2	statistical clustering of the three humic-like components (Fig. 5) seems to imply some
3	degree of coupling between these parameters, and suggests that different spatial zones share
4	a similar source for the humic-like components. The humic-like composition does not seem
5	to change significantly during transport from the source to the Florida Keys. As mentioned
6	above, the distributional patterns of TOC and humic-like components (Fig. 2h, 4a, 4b, 4d)
7	suggest that humic-like components are primarily derived from DOC exported from
8	mangrove and other vegetated habitats from land to the INSHORE and REEF zones.
9	It is also likely that major fractions of humic-like components at BAY and BACK (Fig.
10	4a, 4b, 4d) are derived from terrestrial sources. In fact, this has been suggested for DOM
11	sources in Florida Bay (Maie et al., 2012), where much of the CDOM and FDOM during
12	the wet season seem derived from Everglades vegetation, include mangroves. Fringe
13	mangrove forests have been suggested to be major contributor of CDOM to the southwest
14	Florida Shelf (Jaffé et al., 2004; Bergamashi et al., 2011; Cawley et al., 2013). Satellite
15	imagery and filed observations suggest a significant contribution of terrestrial DOM from
16	the southwest Florida Shelf to the Florida Keys as well as Florida Bay (Hu et al., 2003
17	2004; Milbrandt et al., 2011; Maie et al., 2012). These reports support our suggestion that
18	an important fraction of terrestrial humic-like components on the Bay side of the Florida
19	Keys (BAY and BACK) are indeed mangrove and Everglades derived.
20	The spatial distribution of PC1 scores, i.e., indicators of sources of FDOM, showed
21	clear spatial distribution (Fig. 6a). The highest PC1 was found at BAY where TOC
22	concentration was highest, and the distributional patterns of PC1 were similar to those for
23	TOC. However, the PC1 scores were not linearly related to TOC for the whole region of the

1	Florida Keys (Fig. 7). The PC1 values were fairly narrowly distributed for the BAY
2	although a large variability of TOC concentration was evident in that zone. The patterns
3	observed for BAY indicate that the FDOM composition (i.e., terrestrial character) did not
4	change noticeably with significant variations in TOC concentrations, implying that
5	terrestrially-derived DOM originates from one main source (in this case the Everglades)
6	while the loadings are highly variable. On the other hand, TOC concentrations and PC1
7	values were low and were within the narrow range at TORT, implying minor contribution
8	of terrestrial DOM at TORT. Decreases in PC1 values with decreases in TOC
9	concentrations were evident at BACK, MARQ, and INSHORE. Such relationships suggest
10	that distribution of DOM is basically controlled by mixing of higher levels of allochthonous
11	DOM and lower levels of autochthonous DOM at these zones. PC1 showed wide variability,
12	while TOC values were within the narrow range at REEF, suggesting significant variations
13	in DOM contributions between allochthonous and autochthonous sources occur in this zone,
14	even though variations in TOC concentration are relatively small. It is interesting to note
15	that relatively high values of PC1 expanded from INSHORE to REEF at middle Keys
16	region (Fig. 6a), suggesting that terrestrial DOM found at BAY might be transported to the
17	INSHORE through cuts and channels connecting the northern and southern waters in the
18	Keys (see arrow Fig. 6a), allowing DOM from the Everglades and Florida Bay to reach the
19	REEF zone. It was reported that atmospheric cold fronts from the north supplement tidal
20	flow from Florida Bay to the Atlantic side of the Florida Keys through channels along the
21	Keys (Smith and Lee, 2003). The transport of CDOM from Florida Bay to Keys through
22	this tidal flow was also suggested (Shank et al., 2010a; Milbrandt et al., 2011). In this study,
23	contribution of TOC and CDOM from BAY to INSHORE and REEF was not evident based

 $\mathbf{2}$ components (Fig. 4a, 4b, 4d). Thus, combining EEM-PARAFAC and PCA might be a 3 suitable approach for monitoring the distribution of terrestrial DOM in coastal 4 environments. $\mathbf{5}$ With regards to PC2, this parameter only explained 19% of the variability in the dataset, 6 and may be significantly less valuable as an assessment proxy compared to PC1. PC2 7 values were near 0 for the three humic-like components which were clustered along a very 8 narrow range on the PC2 axis. In contrast, large difference in PC2 was observed for the 9 protein-like components, C3 and C5 respectively (Fig. 5). If PC2 is indeed controlled by 10 the differences in the distribution of the protein-like components, PC2 might reflect 11 differences in autochthonous sources and/or diagenetic state of DOM. The distribution of 12 PC2 scores showed the spatial variations among zones (Fig. 6b). At BAY and BACK, 13where contribution of terrestrial DOM was greatest (Fig. 6a), the PC2 scores were near 0. 14Positive PC2 scores were found at MARQ and TORT, and negative PC2 scores were found 15at the mid to upper part of REEF (Fig. 6b). PC2 distributions indicate that the protein-like 16 C3 (tyrosine-like) and C5 (tryptophan-like) predominate at REEF and TORT/MARQ, 17respectively. Interestingly, inorganic nutrients at MARQ and TORT were higher compared 18 to REEF (Fig. 2c, 2d, 2e). Previous studies have reported on the dominance of 19 tryptophan-like fluorophore during phytoplankton blooms in coastal environments as well 20as reef waters (Para et al., 2010; Tedetti et al., 2011). On the other hand, Determann et al 21(1998) reported that only tryptophan fluorescence and both tyrosine and tryptophan 22fluorescence were found for bacteria and phytoplankton cultures, respectively. In addition, 23the shallow waters of TORT and MARQ are known to be populated by seagrass

on the distribution of TOC concentrations alone (Fig. 2h) or even from those of humic-like

1

1	communities, while the deeper waters of REEF are influenced by coral reef communities
2	and the Florida Current; characteristics that all might influence the protein-like distribution.
3	The degradability of DOM also might affect the composition of protein-like components.
4	Based on the fluorescence characteristics of peptides/proteins, the dominance of
5	tryptophan-like (C5) fluorophores is suggested to be an indicator of freshly produced DOM,
6	while tyrosine-like (C3) is suggested as degradation products of peptides/proteins
7	(Yamashita and Tanoue, 2003). On the other hand, Cory and Kaplan (2012) indicated that
8	tyrosine-like component can easily to be consumed by bacteria compared to tryptophan-like
9	component for riverine ecosystems. Little is known about how environmental conditions
10	and different coastal habitats influence the relative abundance of tryptophan-like and
11	tyrosine-like fluorescence, and additional studies are needed to determine such relationship.
12	
13	5. Conclusion
14	In the present study, we hypothesized that EEM-PARAFAC can aid in the assessment
15	of multiple source contributions to the coastal DOM pool, and can be used as a monitoring
16	tool for terrestrial DOM at complex coastal environments where salinity cannot be used as
17	a freshwater tracer. The distribution patterns of humic-like components along the Florida
18	Keys obtained by EEM-PARAFAC showed clear spatial trends and were similar but not
19	consistently equal to those of TOC. These results indicate that regionally, TOC
20	concentration enrichments might be influenced by inputs from terrestrial environments,
21	such as the Everglades. EEM-PARAFAC data revealed that spatial differences in DOM
22	distribution along the Florida Keys were also controlled in part by surface runoff and/or
23	fringe mangrove DOM inputs (particularly in the BAY), and autochthonous production

1 (particularly for the REEF, TORT and MARQ zones). PCA using relative abundance of $\mathbf{2}$ EEM-PARAFAC components clearly distinguished the sources of DOM as allochthonous 3 humic-like components and autochthonous protein-like components, and between different 4 autochthonous sources and/or the diagenetic status of DOM, and further clarified $\mathbf{5}$ contributions of terrestrial DOM in zones where levels of TOC and humic-like components 6 were low. Linear correlations between TOC values and PC1 suggest that potential changes 7 in DOM sources throughout the region would most likely have the greatest impact in the 8 REEF zones where a high range in terrestrial contributions to the FDOM pool were 9 observed over a very narrow TOC range. In addition to diluted terrestrial inputs transported 10 from the INSHORE, combined with channel water exchange with the BAY, DOM derived 11 from coral reefs and possible contributions from the Florida Current in the REEF zone were 12 observed. In particular, the REEF zone may be particularly sensitive to TOC changes as 13any increment in terrestrial runoff or anthropogenic outfalls may enhance its humic-like 14component contribution to a degree that it may affect the health of the coral reef 15communities. The results of the present study clearly prove that EEM-PARAFAC 16 combined with PCA is useful tool for monitoring the terrestrial DOM and autochthonous 17sources/diagenetic state of DOM in complex coastal environments.

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- $\mathbf{2}$ $\mathbf{5}$ $\overline{7}$
- 23 Figure captions

1	Figure 1: Map of sampling locations and six zones of the Florida Keys determined by water
2	quality parameters (Boyer and Briceño, 2010). Schematic representations of the average
3	flow patterns are modified from Hunt and Nuttle (2007).
4	
5	Figure 2: Distributions of water quality parameters: a) salinity; b) temperature (°C); c) NO_x
6	(μ M); d) NH ₄ ⁺ (μ M); e) soluble reactive phosphorus, SRP (μ M); f) SiO ₂ (μ M); g)
7	chlorophyll a, chla (µg/L); h) total organic carbon, TOC (µM)
8	
9	Figure 3: Spectral characteristics of five PARAFAC components: a) component 1 (C1); b)
10	component 2 (C2); c) component 3 (C3); d) component 4 (C4); e) component 5 (C5)
11	
12	Figure 4: Distributions of fluorescence intensity (QSU) of PARAFAC components: a)
13	microbial humic-like C1; b) humic-like C2; c) tyrosine-like C3; d) humic-like C4; e)
14	tryptophan-like C5
15	
16	Figure 5. Cross plots of 1 st and 2 nd principal component loadings: closed circle, closed
17	triangle, and closed square indicates microbial humic-like C1, humic-like C2, and
18	humic-like C4 respectively, and open triangle and open circle indicates tyrosine-like C3
19	and tryptophan-like C5 respectively.
20	
21	Figure 6. Distribution of 1^{st} (a) and 2^{nd} (b) principal component scores
22	
23	Figure 7. Relationships between 1 st principal component scores and total organic carbon

1 (TOC)



Figure 1. Yamashita et al.



Figure 2. Yamashita et al.



Figure 3. Yamashita et al.



Figure 4. Yamashita et al.



Figure 5. Yamashita et al.



Figure 6. Yamashita et al.



Figure 7. Yamashita et al.