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Author(s)	Yamashita, Youhei; Boyer, Joseph N.; Jaffe, Rudolf
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Evaluating the distribution of terrestrial dissolved organic matter in a complex coastal ecosystem using fluorescence spectroscopy

Youhei Yamashita[1*], Joseph N. Boyer[2#], Rudolf Jaffé[3]

[1]Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido, 060-0810, JAPAN

[2]Southeast Environmental Research Center and Department of Earth and Environment, Florida International University, Miami, Florida 33199, USA

[3]Southeast Environmental Research Center and Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, USA

[#] Center for the Environment and Depertment of Environmental Science & Policy, Plymouth State University, 17 High Street, MSC #63, Plymouth, NH 03264, USA

***corresponding author:**

Phone number: +81-11-706-2349

E-mail address: yamashiy@ees.hokudai.ac.jp

Postal address: Faculty of Environmental Earth Science, Hokkaido University, N10, W5, Kita-ku, Sapporo, Hokkaido, 060-0810, JAPAN

Abstract

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

Highlights

- Spatial distribution of optical properties of DOM was reported for the Florida Keys
- Regional autochthonous and allochthonous DOM sources were determined
- Terrestrial DOM inputs ranged from far locations of the Everglades to local runoff
- PCA of EEM-PARAFAC can be useful for monitoring DOM quality in coastal environments

Keywords

Dissolved organic matter (DOM), Fluorescent dissolved organic matter (FDOM), excitation emission matrix (EEM), Parallel factor analysis (PARAFAC), Environmental monitoring, Florida Keys

1. Introduction

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

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 cannot be used alone as a simple freshwater tracer in complex coastal systems, where 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.

Excitation emission matrix fluorescence coupled with parallel factor analysis (EEM-PARAFAC) has been applied to evaluating the dynamics of fluorescent groups of DOM (FDOM) in coastal environments (Stedmon and Markager, 2005; Yamashita et al., 2008; Fellman et al., 2010). The advantage of this technique is its high sensitivity and power to successfully differentiate between autochthonous and allochthonous fluorescent components of DOM. In addition, efforts to develop in-situ fluorescence sensors based on EEM-PARAFAC data have shown much promise thus far (Guéguen et al., 2012). Thus, EEM-PARAFAC seems ideally suited for monitoring the distribution of autochthonous as well as allochthonous DOM in coastal environments. Most of the coastal zone EEM-PARAFAC applications have focused on evaluating the sources and environmental dynamics of individual fluorescent groups in river-dominated estuaries based on their fluorescent distribution patterns and relationship with salinity (e.g. Yamashita et al., 2008; Kowalczuk et al., 2009; Fellman et al., 2010; Osburn et al., 2012). However, applications in coastal areas where DOM dynamics are not dominated by river discharge have been scarce (Maie et al., 2012; Cawley et al., 2012).

In the present study, we report the first EEM-PARAFAC data to assess the distribution of FDOM in the Florida Keys where salinity cannot be used as a freshwater tracer and multiple sources of DOM prevail. We hypothesized that EEM-PARAFAC can aid in the assessment of DOM sources, and thus, can be used as a monitoring tool for terrestrial DOM at complex coastal environments where salinity cannot be used as a freshwater tracer. Long-term water quality monitoring programs (including total organic carbon; TOC) have been conducted continuously throughout the Florida Keys and Florida Bay for many years, and successfully classified the Florida Keys coastal zone into 6 areas in terms of differences in water quality (Boyer et al., 1997; Boyer and Briceño, 2010). Thus, the Florida Keys, home of the only coral reef track in the continental US, is an area ideally suited to test this approach.

2. Site description

The Florida Keys region is an archipelago of sub-tropical islands of Pleistocene origin, which extend in a northeast to southwest direction from Miami to Key West and out to the Dry Tortugas (Fig. 1). The waters of the Florida Keys are characterized by complex water circulation patterns and are directly influenced by the Florida Current, the Gulf of Mexico Loop Current, inshore currents of the southwest Florida Shelf, freshwater discharge from mangrove rivers from the south western and southern areas of the Everglades, and by tidal exchange with both Florida Bay and Biscayne Bay (Lee et al., 1994; Lee et al., 2002).

Seagrass communities are common throughout Florida Bay and along the Florida Keys, while coral reef development occurs mostly offshore on the Atlantic side of the Florida Keys archipelago (Porter and Porter, 2002). Thus, water quality of the Florida Keys may be directly affected both by external nutrient and DOM transport and internal loadings through natural runoff and anthropogenic activities (Gibson et al., 2008). Previous reports using satellite imagery suggest that terrestrial CDOM exported through river drainage of the southwest 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).

Based on water quality parameters collected over 15 years (http://serc.fiu.edu/wqmnetwork/), the Florida Keys coastal environment was statistically divided into six zones (Fig. 1; Boyer and Briceño, 2010). The BACK zone was composed primarily of stations located inside and north of the Lower Keys (Fig. 1). This zone was characterized by the highest nutrient and TOC levels among the six zones. The BACK zone is believed to be influenced by solutes transported from the southwest Florida Shelf and/or derived from benthic sources. The BAY zone included sites most influenced by Florida Bay and waters moving in a southerly direction from the southwest Florida Shelf. This zone is characterized by highest in SiO_2 and elevated TOC levels, but was relatively low in inorganic nutrients and chlorophyll *a* (Boyer and Briceño, 2010). The water quality of the INSHORE, MARQ, REEF, and TORT zones was most similar to each other but statistically distinct. The INSHORE included the innermost sites of the Keys, which are shallow, and closest to any possible anthropogenic sources. The REEF was made up of all Hawk Channel and reef tract sites off the mainland Keys. The INSHORE was slightly elevated in inorganic nitrogen and TOC relative to the REEF. The MARQ is a zone of relatively shallow water which separates the southwest Florida Shelf from the Atlantic Ocean. The MARQ had higher total phosphorus (TP) and chlorophyll *a* (Chl*a*) than TORT and REEF but was comparable in nitrogen. The TORT was composed of sites located western part of the study region, including those in Dry Tortugas National Park. The distinction between the REEF and TORT was driven by the slightly higher TOC concentrations and lower TP found in the REEF zone (Boyer and Briceño, 2010).

Here we report EEM-PARAFAC data on FDOM from the six abovementioned water quality zones, based on one intensive sampling exercise, in an attempt to assess spatial DOM source distributions and to test the applicability of this technique to spatial monitoring in complex coastal regions.

3. Materials and methods

Surface water samples were collected from 155 sites located in the Florida Keys during 8 January – 15 February 2008 (Fig. 1). These stations represented the same sampling sites originally established for the long-term water quality monitoring program in the Florida Keys (Boyer and Briceño, 2010). Samples for optical characterization of DOM were collected in pre-washed (soaked in 0.1 M HCl and 0.1 M NaOH for 24 h each), brown polyethylene bottles and were stored on ice, returned to the laboratory, and filtered through pre-combusted (450ºC, 3h) GF/F filters prior to analysis. The sample collection, field measurements and analytical methods for water quality parameter reported here, i.e., temperature, salinity, NO_x, NH_4^+, soluble reactive phosphorus (SRP), SiO_2, Chl*a*, and TOC were described in detailed by Boyer and Briceño (2010).

EEM spectra were obtained using a Horiba Jovin Yvon SPEX Fluoromax-3 fluorometer using the method of Maie et al. (2006) and Santín et al. (2009). All fluorescence spectra were acquired in S/R mode, and several post-acquisition steps were involved in the 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.

PARAFAC statistically enables characterization and identification of individual fluorescent groups in the EEMs (Stedmon et al., 2003). The approach of PARAFAC modeling to EEMs has been described in detail elsewhere (Stedmon et al., 2003; Ohno and Bro, 2006). The PARAFAC modeling was conducted in MATLAB (Mathworks, Natick, MA) with the DOMFluor toolbox (Stedmon and Bro, 2008). The wavelength range used for this purpose was 260-455 nm and 290-500 nm for excitation and emission, respectively. The validation of the PARAFAC model was conducted according to Stedmon and Bro (2008).

A PARAFAC model for evaluating the EEMs obtained at sites in Everglades National Park (ENP) and its surrounding area has been established and successfully used for evaluating environmental dynamics of DOM (Chen et al., 2010; Yamashita et al., 2010; Pisani et al., 2011; Maie et al., 2012; Cawley et al., 2013). Thus, we expected to apply this ENP-PARAFAC model for comparison of DOM in ENP and Florida Keys. However, the majority of EEMs obtained from Florida Keys were considerably different from those from ENP most likely due to the great contribution of subtropical marine waters that are known to feature among the lowest CDOM contenting water in the global ocean (Swan et al., 2009; Yamashita and Tanoue, 2008; 2009). Therefore, it was not appropriate to apply the

ENP-PARAFAC model for the Florida Keys study, and a new PARAFAC model was established.

Principal component analysis using relative abundance (%) of PARAFAC components was conducted using KyPlot (v2.0, KyensLab Inc., Japan). Differences in DOM characteristics among zones were assessed by non-parametric Mann–Whitney *U* test (KyPlot, v2.0, KyensLab Inc., Japan).

4. Results and discussion

4.1 Water quality parameters

Figure 2 shows the spatial distributions of water quality parameters for the six zones in the Florida Keys for the sample set discussed herein. Salinity ranged from 35.7 to 37.6 (Fig. 2a). Lowest salinity was found at BAY and the northern part of REEF where higher temperature values were evident (Fig. 2b). The highest salinity values were evident in the northern part of BACK and MARQ. The hypersalinity present in this area during this sampling event was most probably due to advection of hypersaline surface waters from the southwest Florida Shelf into the Florida Keys (Fig. 2a) as a result of low freshwater input from the Everglades (Boyer & Briceño, 2009). The marked difference in water temperature also supports this inference (Fig. 2b). Thus, freshwater contributions could not be determined during the study period solely based on salinity distributions.

NO_x, NH_4^+, and SRP concentrations were higher at TORT and MARQ compared to the other zones (Fig. 2c-e). The highest levels of these nutrients are usually reported for the BACK zone due to the influence of benthic flux contributions (Boyer and Briceño, 2010). The unusual distribution patterns of these inorganic nutrients suggest anomalous conditions in the TORT and MARQ regions probably from advection of waters from the Gulf of Mexico. In addition, levels of NO_x and NH_4^+ were relatively higher at northern part of the INSHORE compared to the adjacent REEF (Fig. 2c, d), suggesting anthropogenic inputs of inorganic nitrogen at the northern part of INSHORE (Boyer and Briceño, 2010).

Interestingly, the distribution patterns of SiO_2 were largely independent from other inorganic nutrients as the highest level of SiO_2 is found at BAY (Fig. 2f). This is in agreement with data from over 15 years of monitoring efforts, as Boyer and Briceño (2010) reported the same general distribution pattern of SiO_2 and suggested that terrestrial SiO_2 from the Everglades or the Florida Shelf reaches the BAY region through water moving southward from the southwest Florida Shelf and/or from Florida Bay.

Levels of Chl*a* were less than 0.5 μg L^{-1} at most sites (>0.5 μg L^{-1} at only 9 sites) and did not show any spatial trend (Fig. 2g). TOC concentration was highest at BAY and its distribution was similar to that of SiO_2 (Fig. 2h), implying that substantial amount of TOC at BAY might be derived from the Florida Shelf. Boyer and Briceño (2010) reported that levels of TOC at BACK are usually higher or similar to those at BAY, however, TOC concentrations at BACK were significantly lower than those at BAY ($p<0.01$; 180±38 μM *vs* 287±120 μM) for the current dataset. Such low levels of TOC at BACK observed in this study were due to advection of hypersalinine waters from the SW Florida Shelf to this region. TOC concentrations at INSHORE were significantly higher than those at REEF ($p<0.01$; 116±32 μM *vs* 84±10 μM). Distributions of water quality parameters presented above were generally in agreement with long-term data for the region (Boyer and Briceño, 2010), therefore validating this extensive sample set as a representative for this region.

4.2 Distributional characteristics of DOM determined by EEM-PARAFAC

Based on the PARAFAC modeling of EEMs, a five-component model was validated (Fig. 3). Spectral characteristics of component 1 (C1; excitation/emission = <260, 305/412) are similar with marine humic-like fluorophore (peak M; Coble et al., 1996). In terrestrial aquatic environments, this component is also known to be produced during microbial degradation of organic matter and is often defined as microbial humic-like fluorophore (Cory and McKnight, 2005; Yamashita et al., 2010). This component is also similar to C4 established for the ENP-PARAFAC model (R = 0.99 and 0.65 for excitation and emission, respectively) that showed less seasonal variability at the Florida Bay due to relatively higher autochthonous contribution (Maie et al., 2012). Components 2 (C2; excitation/emission = 360, <260, 455) and 4 (C4; excitation/emission = <260, 405/497) could be categorized as humic-like fluorophore (mixture of peaks A and C; Coble et al., 1996). Similar PARAFAC components with these 3 humic-like components were also reported in other coastal environments (Yamashita et al., 2008; Kowalczuk et al., 2009; Fellman et al., 2010). In these reports, components similar to C1 are usually suggested to be in situ produced in the water column, while components similar to C2 and C4 are considered to be of terrestrial origin. It should be noted C2 and C4 found in this study were not present in the ENP-PARAFAC model (Maie et al., 2012). Such differences between ENP-PARAFAC and Florida Keys-PARAFAC models might be result of greater contributions of wetlands derived DOM and subtropical marine-derived DOM at ENP and Florida Keys respectively. Two other components, i.e., components 3 (C3; excitation/emission = 275/308) and 5 (C5; excitation/emission = 290/338), are characterized as protein-like components, namely tyrosine-like and tryptophan-like, respectively (Yamashita and Tanoue, 2003). In coastal environments, a major source of protein-like components is thought to be autochthonous production by phytoplankton (Yamashita et al., 2008; Kowalczak et al., 2009; Mendoza et al., 2011) or from seagrass communities (Maie et al., 2012; Cawley et al., 2012).

Figure 4 shows the spatial distribution of fluorescence intensities of individual PARAFAC components along the Florida Keys. Distributional patterns of humic-like C1, C2, and C4 were almost identical (Fig. 4a, b, d), indicating that marine (microbial) humic-like C1 might be allochthonous in origin for this study area. The highest fluorescence intensity of the three humic-like components was evident at BAY and gradually decreased westward toward TORT. At the Atlantic side, levels of three humic-like components were relatively high along the Keys and decreased offshore. These distributional patterns were similar to that of TOC, and TOC concentrations were significantly correlated with the fluorescence intensities of the three humic-like components ([TOC]=15.8×[C1]+61.3, R^2=0.91, p<0.01, n=138; [TOC]=35.5×[C2]+57.6, R^2=0.88, p<0.01, n=138; [TOC]=34.1×[C4]+49.5, R^2=0.88, p<0.01, n=138), implying that a major fraction of TOC (as well as DOC) in this region might consist of terrestrial humic-like components.

Fluorescence intensities of protein-like components C3 and C5 (especially the latter), were also similarly distributed with TOC (Fig. 4c, 4e), but were less correlated with TOC compared with humic-like components ([TOC]= 31.7×[C3]-39.8, R^2=0.55, p<0.01, n=138; [TOC]= 48.0×[C5]+11.0, R^2=0.79, p<0.01, n=138). The decreases in the levels of protein-like components from BAY to TORT were relatively small compared with those of TOC and humic-like components. Also, relatively high levels of protein-like components were found at REEF. These distributional patterns indicate the autochthonous character of protein-like components in combination with allochthonous (terrestrial) sources. However, distributional patterns of protein-like components also did not correlate with Chl*a*, suggesting that phytoplankton might not be the dominant source of protein-like components. Similar results were also observed other coastal environments (Yamashita et al., 2011; Maie et al., 2012), even though linear relationship between protein-like component and density of the toxic dinoflagellate *Karenia brevis* has been reported (Mendoza et al., 2011). Thus, bacterial communities (Romera-Castillo et al. 2011; Shimonotori et al., 2011; Guillemette and del Giorgio, 2012), seagrass communities (Maie et al., 2012; Cawley et al., 2012) and coral reefs (Tedetti et al., 2011) might also be important sources for protein-like components. Interestingly, distribution patterns were different between tyrosine-like C3 and tryptophan-like C5, suggesting that environmental dynamics, i.e., sources and sinks, are different between them.

At the Atlantic side, TOC concentrations were slightly but significantly higher at INSHORE compared to REEF, as mentioned above (Fig. 2h). There are several possible sources that could explain these higher levels of TOC at INSHORE, including anthropogenic sources, autochthonous production, and/or contribution of terrestrial runoff (including mangroves). Relatively higher levels of inorganic nitrogen, especially NO_x, at INSHORE compared to REEF, indicative of anthropogenic nutrients (Boyer and Briceño, 2010), were evident (Fig. 2c, 2d). Since the majority of homes in the Florida Keys have only recently been converted from on-site septic systems to centralized sewers (Briceño and Boyer, 2012), sewage may be an important nearshore source of DOM. It is well known that protein-like fluorophores dominated in waters impacted by domestic sewage and farm wastes (Baker, 2001, 2002). However, in this study, clear spatial gradients of protein-like components from INSHORE to REEF were not evident (Fig. 4c, 4e), suggesting that contribution of anthropogenic DOM and DOM production induced by anthropogenic inorganic nitrogen are minor in this region or are not easily visualized due to interferences from autochthonous protein-like fluorescence from seagrass and reef communities. The relatively high levels of protein-like component fluorescence observed at REEF, MARQ, and TORT, also imply that there are substantial contributions of autochthonous DOM in these zones.

The distributional patterns of TOC and humic-like components were correlated based on the observed concentration gradients from nearshore to offshore sites on the Atlantic side of the Keys (Figs. 2h, 4a, 4b, 4d). Fluorescence intensities of humic-like components normalized to TOC were greater at INSHORE compared with REEF ($p<0.01$; 0.034±0.012 *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* 0.012±0.004 for C3/TOC). Such differences in distributional patterns indicate that terrestrial DOM rich in humic-like components from terrestrial runoff (including mangroves) or groundwater inputs is transported from INSHORE to REEF even though salinity did not differ considerably (Fig. 2a).

While high salinity groundwater samples off the Florida Keys have been reported to feature DOM enriched in humic-like fluorescence, the general flow of groundwater is believed to be from the Atlantic side of the Keys to Florida Bay (Chen et al., 2010 and references therein). Thus, groundwater is a less likely DOM source to the INSHORE than fringe mangroves, a common habitat along the Florida Keys, and a well known source of 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.

4.3 Statistical analysis of EEM-PARAFAC data

To further assess the spatial DOM distribution we applied principal component analysis (PCA) using the relative abundance (%) of individual PARAFAC components from the entire dataset. Figure 5 shows the cross plot of PC1 and PC2 loadings, where the first and second principal component (PC1 and PC2) explained 76% and 19% of the variability, respectively. The three humic-like components clustered close together with positive PC1 loadings and near 0 for PC2 loadings. In contrast, the two protein-like components showed negative values for PC1 loadings, and tyrosine-like C3 and tryptophan-like C5 showed significant negative and positive values for their PC2 loadings, respectively. Based on this distribution, PC1 clearly distinguishes samples based on sources (i.e., allochthonous humic-like vs. autochthonous protein-like components), while PC2 may be indicative of differences in autochthonous sources and/or the state of degradation of the FDOM.

Various sources of DOM have been suggested for the Florida Keys, namely, terrestrial DOM from the Everglades (Hu et al., 2003, 2004), seagrasses (Stabenau et al., 2004), mangrove patches surrounding the Keys (Shank et al., 2010a), and photo-dissolution from sediments (Shank et al., 2011; Pisani et al., 2011). It is also well known that photo-bleaching (Nieto-Cid et al., 2006; Shank et al., 2010b) and bio-degradation

(Romera-Castillo et al., 2011) are major sinks for CDOM in coastal environments. The statistical clustering of the three humic-like components (Fig. 5) seems to imply some degree of coupling between these parameters, and suggests that different spatial zones share a similar source for the humic-like components. The humic-like composition does not seem to change significantly during transport from the source to the Florida Keys. As mentioned above, the distributional patterns of TOC and humic-like components (Fig. 2h, 4a, 4b, 4d) suggest that humic-like components are primarily derived from DOC exported from mangrove and other vegetated habitats from land to the INSHORE and REEF zones.

It is also likely that major fractions of humic-like components at BAY and BACK (Fig. 4a, 4b, 4d) are derived from terrestrial sources. In fact, this has been suggested for DOM sources in Florida Bay (Maie et al., 2012), where much of the CDOM and FDOM during the wet season seem derived from Everglades vegetation, include mangroves. Fringe mangrove forests have been suggested to be major contributor of CDOM to the southwest Florida Shelf (Jaffé et al., 2004; Bergamashi et al., 2011; Cawley et al., 2013). Satellite imagery and filed observations suggest a significant contribution of terrestrial DOM from the southwest Florida Shelf to the Florida Keys as well as Florida Bay (Hu et al., 2003 2004; Milbrandt et al., 2011; Maie et al., 2012). These reports support our suggestion that an important fraction of terrestrial humic-like components on the Bay side of the Florida Keys (BAY and BACK) are indeed mangrove and Everglades derived.

The spatial distribution of PC1 scores, i.e., indicators of sources of FDOM, showed clear spatial distribution (Fig. 6a). The highest PC1 was found at BAY where TOC concentration was highest, and the distributional patterns of PC1 were similar to those for TOC. However, the PC1 scores were not linearly related to TOC for the whole region of the

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

With regards to PC2, this parameter only explained 19% of the variability in the dataset, and may be significantly less valuable as an assessment proxy compared to PC1. PC2 values were near 0 for the three humic-like components which were clustered along a very narrow range on the PC2 axis. In contrast, large difference in PC2 was observed for the protein-like components, C3 and C5 respectively (Fig. 5). If PC2 is indeed controlled by the differences in the distribution of the protein-like components, PC2 might reflect differences in autochthonous sources and/or diagenetic state of DOM. The distribution of PC2 scores showed the spatial variations among zones (Fig. 6b). At BAY and BACK, where contribution of terrestrial DOM was greatest (Fig. 6a), the PC2 scores were near 0. Positive PC2 scores were found at MARQ and TORT, and negative PC2 scores were found at the mid to upper part of REEF (Fig. 6b). PC2 distributions indicate that the protein-like C3 (tyrosine-like) and C5 (tryptophan-like) predominate at REEF and TORT/MARQ, respectively. Interestingly, inorganic nutrients at MARQ and TORT were higher compared to REEF (Fig. 2c, 2d, 2e). Previous studies have reported on the dominance of tryptophan-like fluorophore during phytoplankton blooms in coastal environments as well as reef waters (Para et al., 2010; Tedetti et al., 2011). On the other hand, Determann et al (1998) reported that only tryptophan fluorescence and both tyrosine and tryptophan fluorescence were found for bacteria and phytoplankton cultures, respectively. In addition, the shallow waters of TORT and MARQ are known to be populated by seagrass communities, while the deeper waters of REEF are influenced by coral reef communities and the Florida Current; characteristics that all might influence the protein-like distribution. The degradability of DOM also might affect the composition of protein-like components. Based on the fluorescence characteristics of peptides/proteins, the dominance of tryptophan-like (C5) fluorophores is suggested to be an indicator of freshly produced DOM, while tyrosine-like (C3) is suggested as degradation products of peptides/proteins (Yamashita and Tanoue, 2003). On the other hand, Cory and Kaplan (2012) indicated that tyrosine-like component can easily to be consumed by bacteria compared to tryptophan-like component for riverine ecosystems. Little is known about how environmental conditions and different coastal habitats influence the relative abundance of tryptophan-like and tyrosine-like fluorescence, and additional studies are needed to determine such relationship.

5. Conclusion

In the present study, we hypothesized that EEM-PARAFAC can aid in the assessment of multiple source contributions to the coastal DOM pool, and can be used as a monitoring tool for terrestrial DOM at complex coastal environments where salinity cannot be used as a freshwater tracer. The distribution patterns of humic-like components along the Florida Keys obtained by EEM-PARAFAC showed clear spatial trends and were similar but not consistently equal to those of TOC. These results indicate that regionally, TOC concentration enrichments might be influenced by inputs from terrestrial environments, such as the Everglades. EEM-PARAFAC data revealed that spatial differences in DOM distribution along the Florida Keys were also controlled in part by surface runoff and/or fringe mangrove DOM inputs (particularly in the BAY), and autochthonous production (particularly for the REEF, TORT and MARQ zones). PCA using relative abundance of EEM-PARAFAC components clearly distinguished the sources of DOM as allochthonous humic-like components and autochthonous protein-like components, and between different autochthonous sources and/or the diagenetic status of DOM, and further clarified contributions of terrestrial DOM in zones where levels of TOC and humic-like components were low. Linear correlations between TOC values and PC1 suggest that potential changes in DOM sources throughout the region would most likely have the greatest impact in the REEF zones where a high range in terrestrial contributions to the FDOM pool were observed over a very narrow TOC range. In addition to diluted terrestrial inputs transported from the INSHORE, combined with channel water exchange with the BAY, DOM derived from coral reefs and possible contributions from the Florida Current in the REEF zone were observed. In particular, the REEF zone may be particularly sensitive to TOC changes as any increment in terrestrial runoff or anthropogenic outfalls may enhance its humic-like component contribution to a degree that it may affect the health of the coral reef communities. The results of the present study clearly prove that EEM-PARAFAC combined with PCA is useful tool for monitoring the terrestrial DOM and autochthonous sources/diagenetic state of DOM in complex coastal environments.

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Figure captions

Figure 1: Map of sampling locations and six zones of the Florida Keys determined by water quality parameters (Boyer and Briceño, 2010). Schematic representations of the average flow patterns are modified from Hunt and Nuttle (2007).

Figure 2: Distributions of water quality parameters: a) salinity; b) temperature (°C); c) NO_x (μM); d) NH_4^+ (μM); e) soluble reactive phosphorus, SRP (μM); f) SiO_2 (μM); g) chlorophyll *a*, chl*a* (μg/L); h) total organic carbon, TOC (μM)

Figure 3: Spectral characteristics of five PARAFAC components: a) component 1 (C1); b) component 2 (C2); c) component 3 (C3); d) component 4 (C4); e) component 5 (C5)

Figure 4: Distributions of fluorescence intensity (QSU) of PARAFAC components: a) microbial humic-like C1; b) humic-like C2; c) tyrosine-like C3; d) humic-like C4; e) tryptophan-like C5

Figure 5. Cross plots of 1st and 2nd principal component loadings: closed circle, closed triangle, and closed square indicates microbial humic-like C1, humic-like C2, and humic-like C4 respectively, and open triangle and open circle indicates tyrosine-like C3 and tryptophan-like C5 respectively.

Figure 6. Distribution of 1st (a) and 2nd (b) principal component scores

Figure 7. Relationships between 1st principal component scores and total organic carbon

1 (TOC)

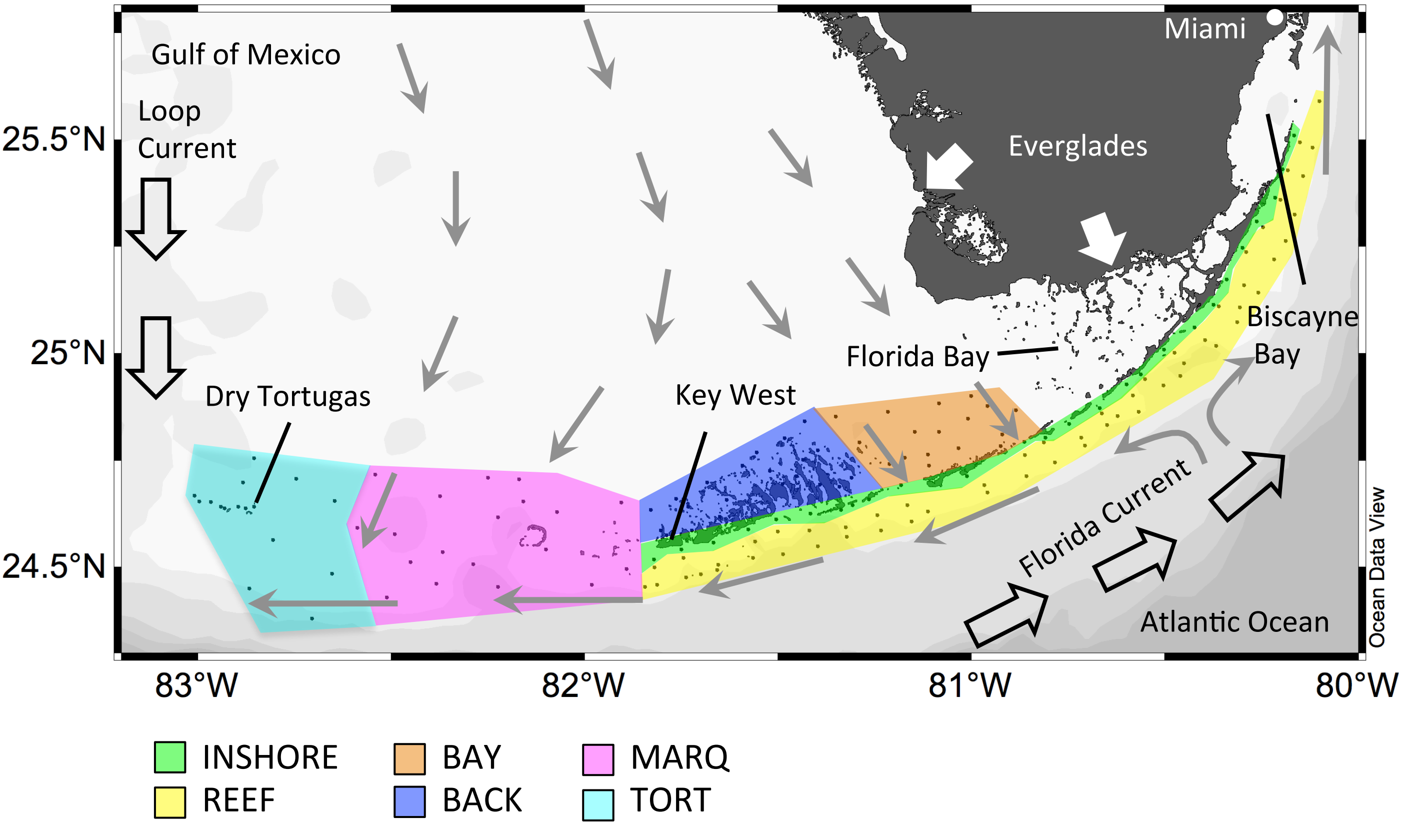

Figure 1. Yamashita et al.

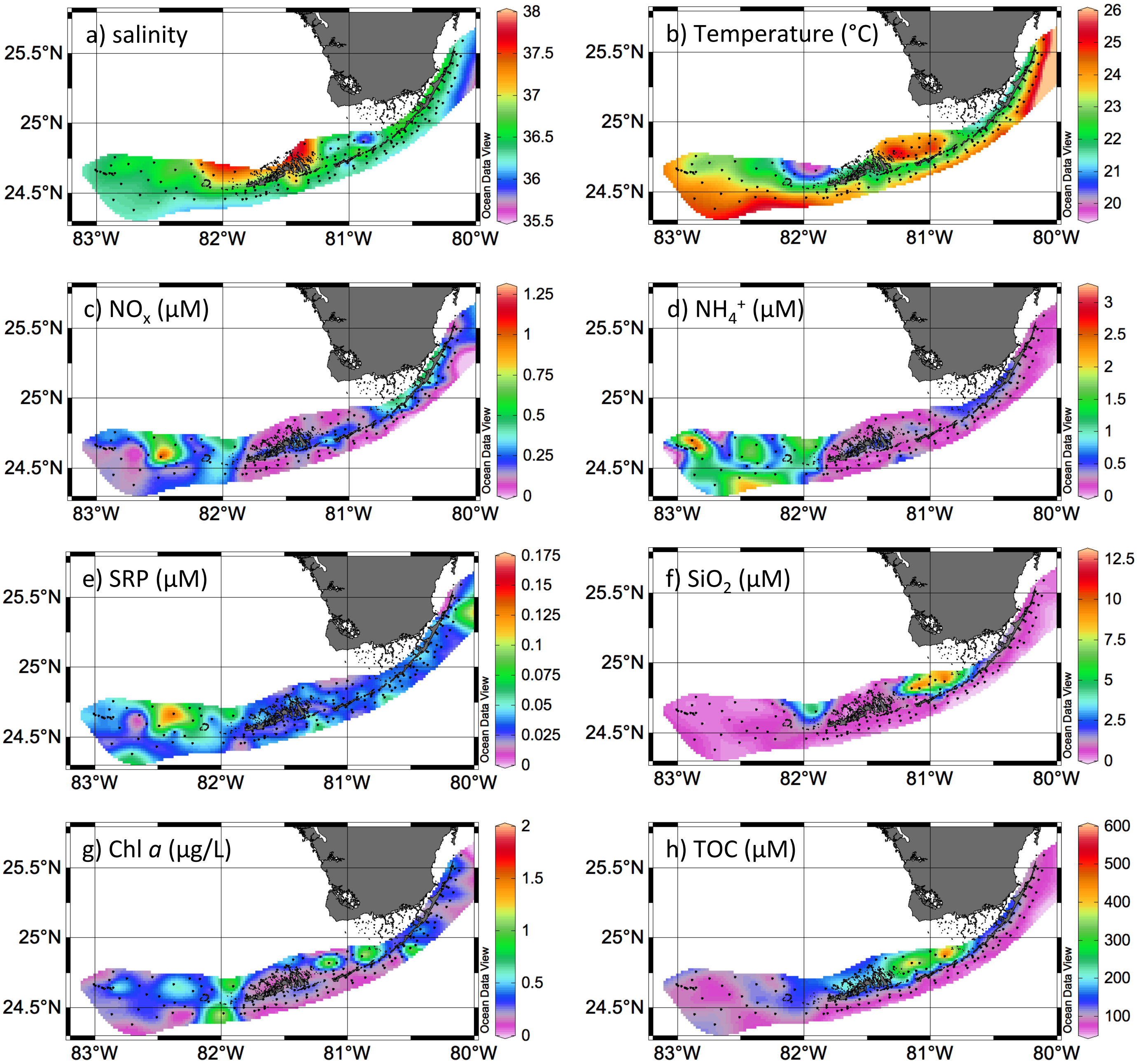

Figure 2. Yamashita et al.

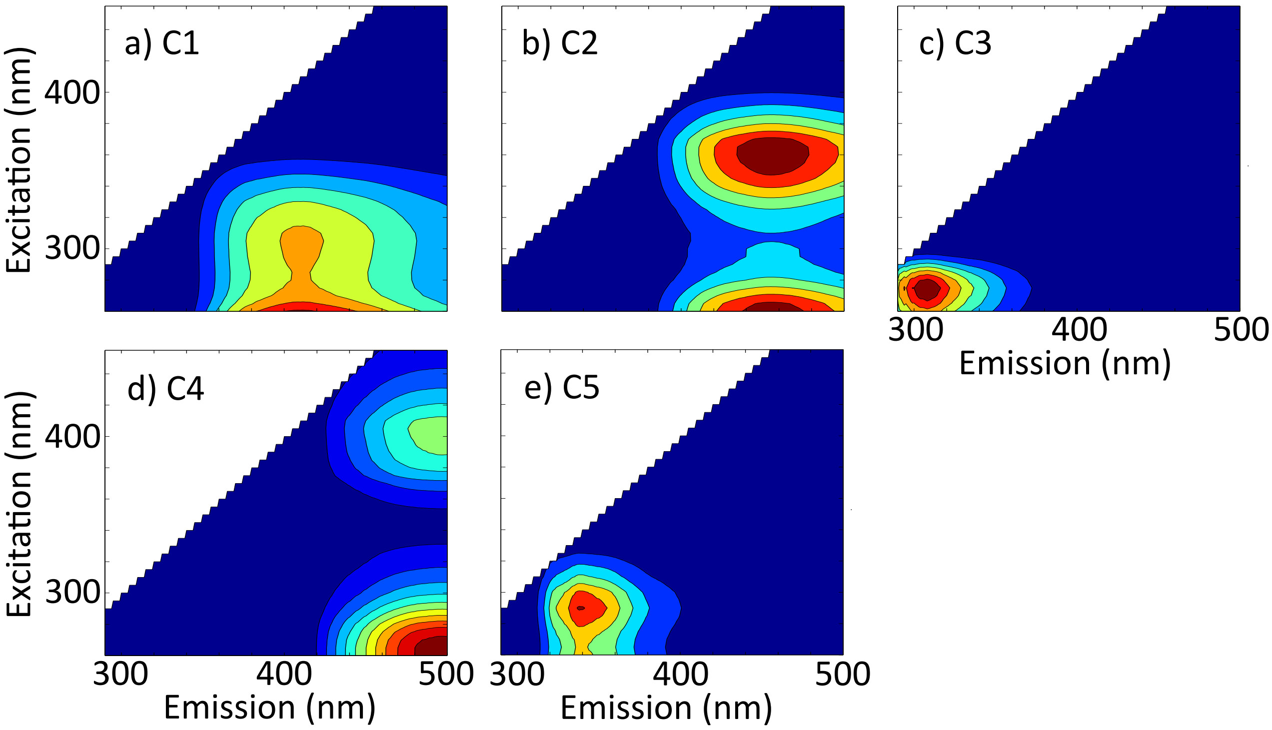

Figure 3. Yamashita et al.

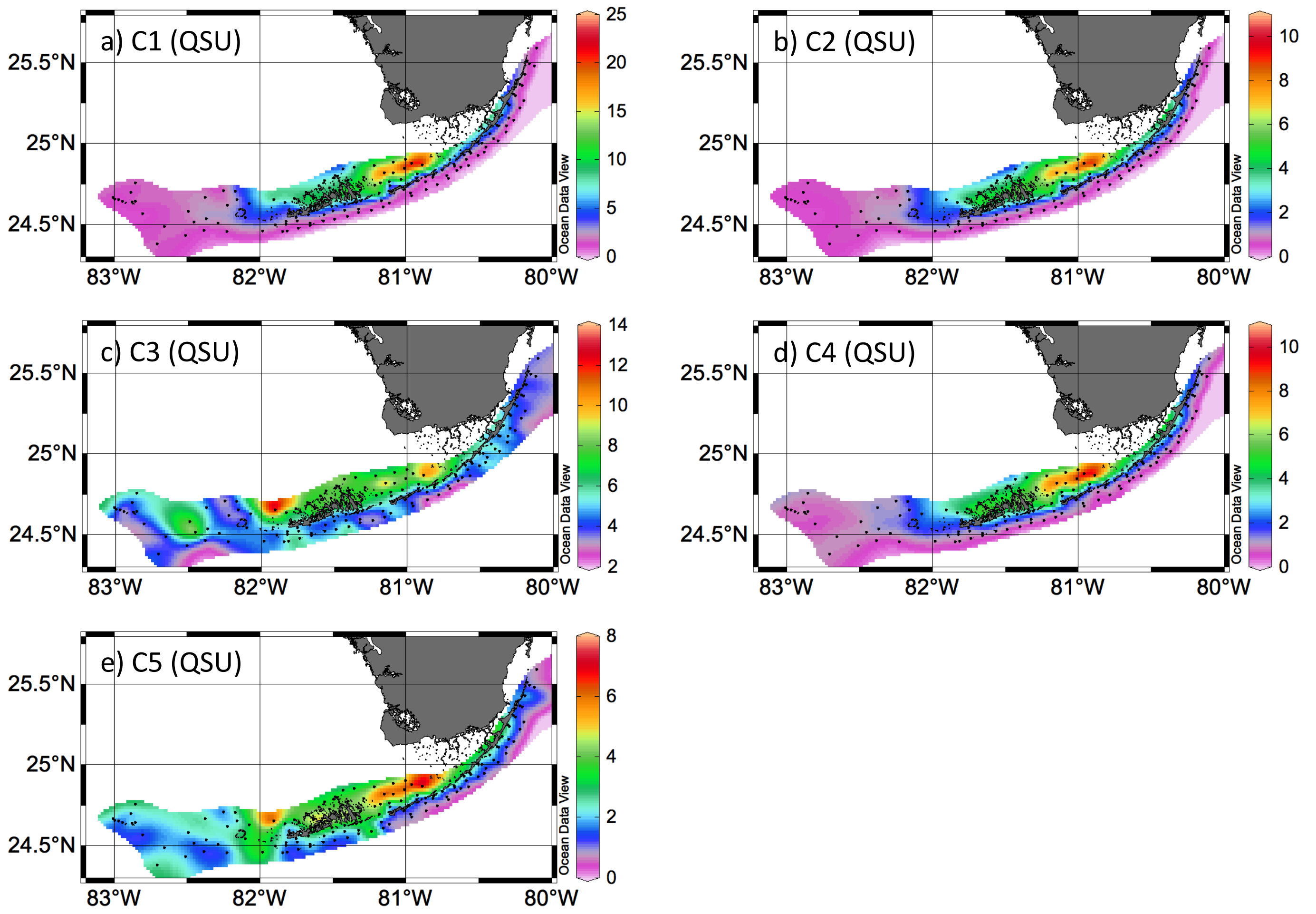

Figure 4. Yamashita et al.

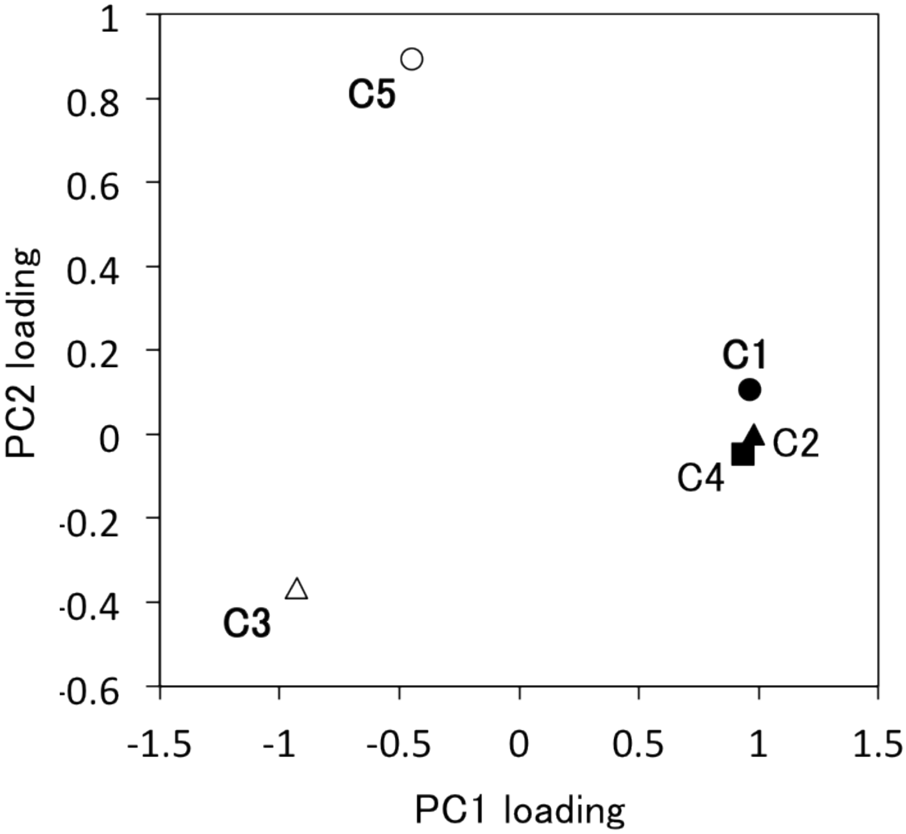

Figure 5. Yamashita et al.

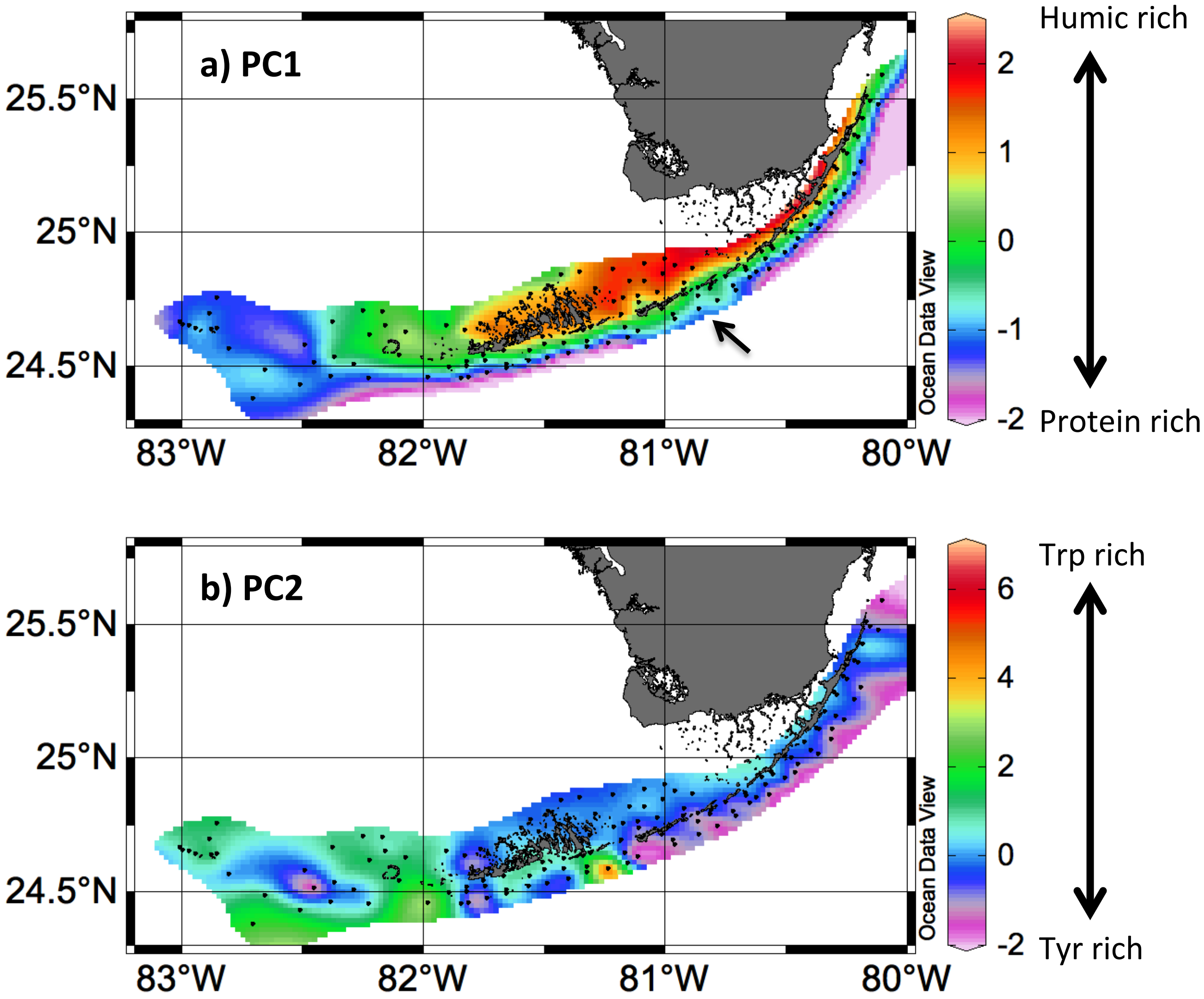

Figure 6. Yamashita et al.

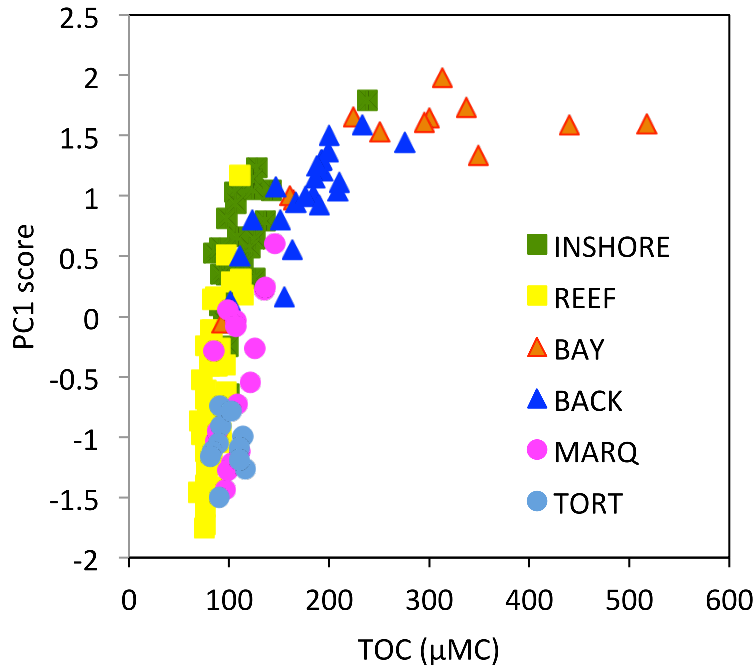

Figure 7. Yamashita et al.