

Characterising the sources and fate of dissolved organic matter in Shark Bay, Australia: a preliminary study using optical properties and stable carbon isotopes

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Abstract. Low latitude, seagrass-dominated coastal bays, such as Shark Bay, Australia, are potential sources of chromophoric dissolved organic matter (CDOM) to coastal regions. Dissolved organic matter (DOM) is known to influence aquatic nutrient dynamics, microbial community structure, and depth of light penetration in estuarine systems. Shark Bay is a sub-tropical ecosystem with limited freshwater inputs and restricted tidal flushing. As such, much of the DOM is expected to be seagrass-derived. However, combining excitation/emission fluorescence spectroscopy and parallel factor analysis (EEM-PARAFAC) with ¹³C stable isotope analysis of DOM, we found evidence for DOM inputs from terrestrial (riverine and possibly groundwater), autochthonous plankton, macroalgae, and seagrass sources. Isotopic analysis of ¹³C in DOM supports the idea that seagrass inputs contribute substantially to the DOM pool in Shark Bay, whereas, EEM-PARAFAC data suggests that much of this input is derived from decomposing seagrass detritus and to a lesser extent due to exudation during primary production. We also report increases in DOM concentrations and changes in DOM characteristics with increasing salinity in surface water samples, indicating that evaporation is an important control on DOM concentration and photo-degradation may play a critical role in transforming DOM within the system.

Additional keywords: EEM-PARAFAC, fluorescence, seagrass, stable isotopes.

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Introduction

The dissolved organic matter (DOM) found in all natural waters is derived from autochthonous sources such as microorganisms and aquatic plants, and from allochthonous sources such as riverine inflow and atmospheric deposition. As such, the characteristics and distribution of DOM in a coastal environment can be important indicators of ecosystem processes (Maie *et al.* 2005; Jaffé *et al.* 2008; Chen *et al.* 2010; Yamashita *et al.* 2010; Maie *et al.* 2012). Seagrasses are well known primary producers in coastal regions (Duarte 1999 and references therein) and because of their large biomass have been suggested as important autochthonous contributors of DOM in coastal bays (Ziegler and Benner 2000; Stabenau *et al.* 2004; Maie *et al.* 2005; Maie *et al.* 2012). The character of the DOM in a coastal water body is a function not just of the relative importance of autochthonous and allochthonous source inputs, but also of internal processing of the DOM. DOM produced through photo-dissolution of particulate organic matter (POM) has been found to be dominated by

humic-like substances, but also showing characteristics of protein-like material (Pisani *et al.* 2011; Shank *et al.* 2011). Algae, diatoms, bacteria, and other microorganisms produce DOM that tends to have lower UV absorbance per unit carbon, higher nitrogen content and less aromatic carbon than DOM from terrestrial/higher plant sources (McKnight *et al.* 1994). Similarly, seagrasses produce DOM during primary productivity and detrital degradation, which has been reported to be enriched in bio-labile compounds such as carbohydrates and proteins (Ziegler and Benner 2000; Maie *et al.* 2005; Barrón and Duarte 2009; Apostolaki *et al.* 2010; Maie *et al.* 2012). The distinctive chemical characteristics of DOM imparted by source material leads to variations in its environmental fate, such as differences in bioavailability (Ziegler and Benner 2000; McCallister *et al.* 2006a; Khodse and Bhosle 2011) and photo-reactivity (Shank and Evans, 2011).

DOM is an important, though not well studied, component of seagrass ecosystems, which are found in shallow coastal areas

and estuaries across the globe. In such ecosystems, DOM has been reported to influence aquatic nutrient dynamics, phytoplankton community structure, and microbial loop dynamics by providing bio-available DOM (Ziegler and Benner 2000; Childers 2006; Devlin and Schaffelke 2009; Fellman *et al.* 2011). In particular, dissolved organic nitrogen (DON), often assumed to be dominated by protein-like structures (Maie *et al.* 2006b), is an ecologically important component of DOM since many marine ecosystems are nitrogen limited. DON from algal sources has been reported to be bioavailable to seagrass and macroalgae in laboratory experiments and had similar uptake rates as inorganic nitrogen species (Van Engeland *et al.* 2011). DOM can also serve the function of light attenuator and seagrasses collected from areas of an estuary dominated by two different DOM sources, terrestrial (high UV absorbance) and microbial (low UV absorbance), showed different pigment concentrations and types indicating shade adaptation in the riverine seagrass population (Kahn and Durako 2009). Seagrass-derived chromophoric DOM (CDOM) has also been suggested to fill this role in coastal areas (Stabenau *et al.* 2004). Since climate change is predicted to increase DOM export from the terrestrial environments to the coastal ocean (Whitehead *et al.* 2009), understanding DOM source, fate, and transport is crucial for the management and protection of critical marine ecosystems, such as the sub-tropical seagrass meadows of Shark Bay and Florida Bay.

In coastal areas where DOM loadings are not determined by river discharges, the primary DOM source is often the ocean or autochthonous production by seagrass, algae, and mangroves (McCallister *et al.* 2006b; Shank *et al.* 2010). Florida Bay, an embayment dominated by autochthonous DOM (Stabenau *et al.* 2004; Maie *et al.* 2005), is located at the southernmost end of the Everglades ecosystem and is characterised by benthic seagrass meadows and long water residence times of at least 6–12 months (Lee *et al.* 2006). Spatial and temporal changes in DOM concentration and composition were determined to be controlled by primary productivity changes and seasonal variations in freshwater inflow (Maie *et al.* 2006a; Maie *et al.* 2012). Increased DOM concentrations, along with increased turbidity and phytoplankton blooms, were linked to seagrass losses in Florida (Carlson *et al.* 2010). The consequences of such losses in seagrass are not well understood, but seagrass detritus can generate significant amounts of DOM and CDOM with optical characteristics similar to those of terrestrial DOM (Stabenau *et al.* 2004), potentially resulting in further shading of the water column. Much is known about temporal and spatial DOM characteristics within Florida Bay, however, making quantitative estimates of DOM source material is still difficult. For Shark Bay, another ecologically important, seagrass-dominated ecosystem, no information is yet available on DOM dynamics and distribution.

Characterisation of DOM by traditional chemical techniques, ^{13}C -NMR, py-GC/MS, mass spectrometry, and others, can be laborious and expensive (Stabenau *et al.* 2004; Maie *et al.* 2005; Maie *et al.* 2006b, 2006c). In lieu of such exhaustive characterisation, optical methods, such as UV-visible and fluorescence spectroscopy, have been used successfully to elucidate DOM source, fate, and transport in sub-tropical estuaries and many other types of freshwater and saline ecosystems

(McKnight *et al.* 2001; Maie *et al.* 2006a; Jaffé *et al.* 2008; Yamashita *et al.* 2008; Chen *et al.* 2010; Costa *et al.* 2011; Fellman *et al.* 2011). Optical properties have been widely used in characterising DOM, however, the application of stable carbon isotopes of DOM has been less frequently reported (Maie *et al.* 2006b; Osburn and Stedmon 2011; Osburn *et al.* 2011) mainly because of analytical limitations (Osburn and St-Jean 2007). In this study we used optical properties, statistical analysis using parallel factor analysis (PARAFAC) and principal component analysis (PCA), and $\delta^{13}\text{C}$ values of DOM to assess DOM dynamics in Shark Bay, Australia. The goal of this study was to generate preliminary information on DOM characteristics in Shark Bay, determine the likely sources and dominant transformation processes, and compare the DOM dynamics in Shark Bay to those in Florida Bay, USA. To our best knowledge, this is the first study to characterise DOM in Shark Bay.

Materials and methods

Shark Bay is a shallow subtropical bay located on the western coast of Australia ($\sim 25^{\circ}45'\text{S}$, $113^{\circ}44'\text{E}$; Fig. 1). Shark Bay is open on its northern end and mixes tidally with the ocean, but is inhibited somewhat by Faure Sill, a sandy bank in the bay's eastern lobe (Fig. 1). In general, there is little precipitation in Shark Bay during the austral summer except for storms, which cause heavy precipitation over a short time period, and most of the annual precipitation falls between May and August during the austral winter. The Wooramel River discharges intermittently and had recently flooded as a result of a monsoonal low in the second half of December 2010 (Australian Bureau of Meteorology). Geologically, Shark Bay is underlain by sandstone and Tamala limestone bedrock (Hearty and O'Leary 2008).

Surface water samples were collected from the Shark Bay ecosystem from the 4 to 18 March 2011. The salinity of water samples was measured by YSI Model 30 SCT meter at the time of collection. Samples of 60 mL were filtered immediately after collection through pre-combusted glass fibre filters (GF/F, $0.7\ \mu\text{m}$) into pre-acid and base cleaned amber polycarbonate Nalgene bottles (Thermo Fisher Scientific, Rochester, NY, USA) and kept refrigerated until processing and analysis. Sample locations are denoted on Fig. 1. Dissolved organic carbon (DOC) concentrations were measured using high temperature combustion on a Shimadzu TOC-5000 (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a platinum catalyst and an infrared detector. Samples were acidified to $\text{pH} < 2$ and sparged with carbon-free compressed air to remove inorganic carbon before analysis.

Samples for stable isotope analysis were extracted using a reversed-phase solid phase extraction (SPE) method (Dittmar, 2008). Briefly, samples ($\sim 1\ \text{L}$) were acidified to $\text{pH} = 2$ using concentrated HCl (32%, Fisher Scientific) and gravity-fed onto a PPL cartridge (1 g, Varian Bond Elute), which had been pre-conditioned with 20 mL of MeOH immediately before use. Salts were rinsed off the cartridges with 0.01 N HCl (40 mL) immediately after applying the sample. Cartridges were then dried using high purity N_2 gas and eluted with 10 or 20 mL MeOH ensuring that the eluent became colourless. The eluates were stored in

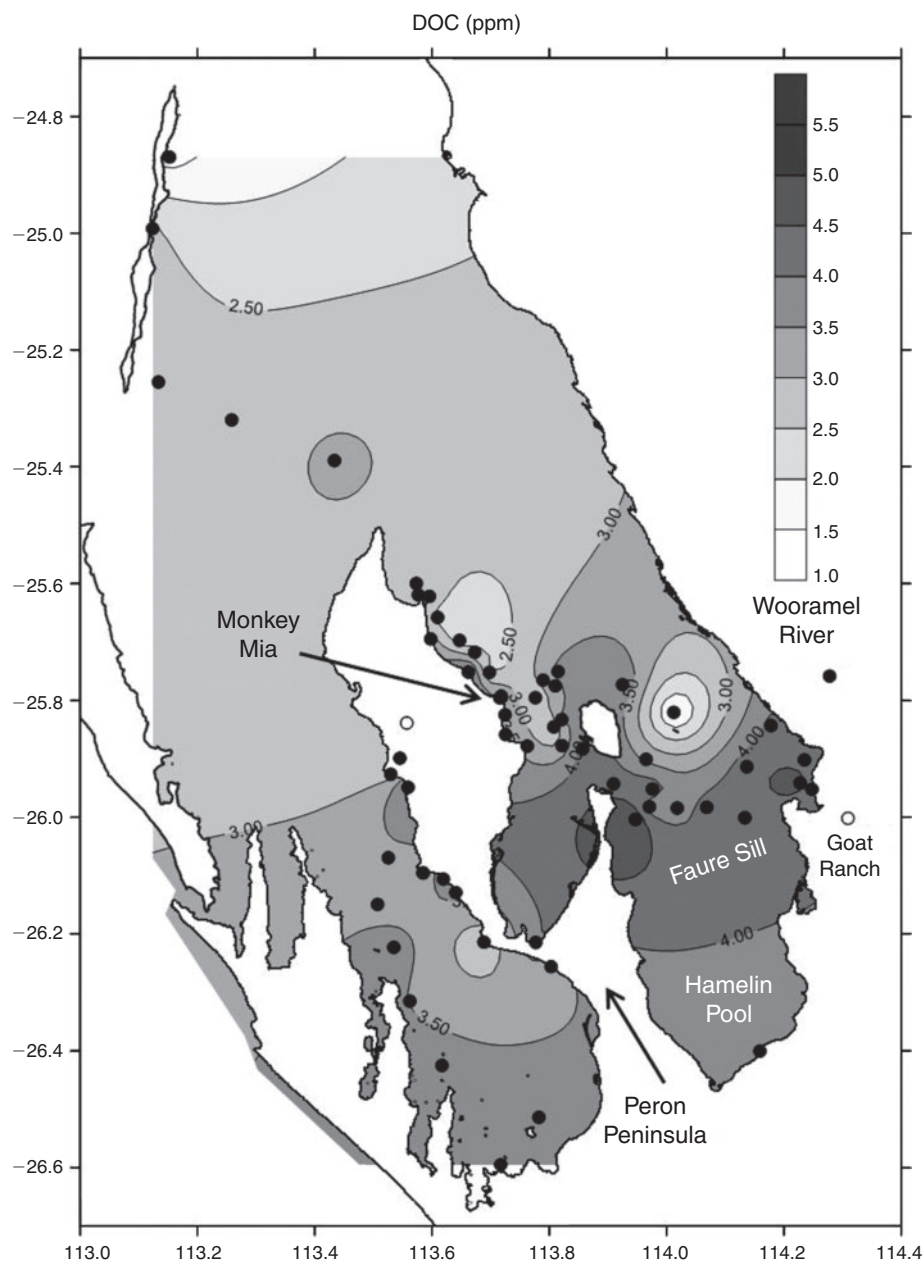


Fig. 1. Contour plots of dissolved organic carbon (DOC) concentration and notable geographic features. The open circles denote groundwater sites that were sampled. White area indicates land.

MeOH at -18°C until further analysis (Dittmar, 2008). The methanol solution was pipetted into a tin capsule and dried in an oven at 65°C until it contained $\sim 100\ \mu\text{g}$ of dried sample. Stable ^{13}C isotope analyses were performed at the FIU SERC Stable Isotope Laboratory using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) procedures (Anderson and Fourqorean, 2003). The EA was used to combust organic material to CO_2 , which was measured on a Finnigan MAT Delta C IRMS (Thermo Electron Corporation, West Palm Beach, FL, USA) in a continuous flow mode. The samples' isotopic ratios (R) are reported in the standard delta notation (‰): $\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000$. The results are presented with respect to the international standards of Vienna Pee Dee

belemnite (VPDB), which is a standard reference (Coplen *et al.* 2006). Analytical reproducibility of this study on the basis of sample replicates is better than $\pm 0.15\text{‰}$ for $\delta^{13}\text{C}$.

UV-visible absorbance was measured on a Varian spectrophotometer using a 1-cm quartz cuvette. Slope ratios (S_R) were calculated as the ratio of the slopes of the absorbance between 275–295 nm and 350–400 nm, where a higher S_R is an indicator of lower molecular weight (Helms *et al.* 2008). The specific UV absorbance at 254 nm (SUVA_{254}), which is the ratio of the absorbance of the sample at 254 nm to the DOC concentration, was also determined. SUVA_{254} is positively correlated with aromaticity and molecular weight (Chin *et al.* 1994; Weishaar *et al.* 2003).

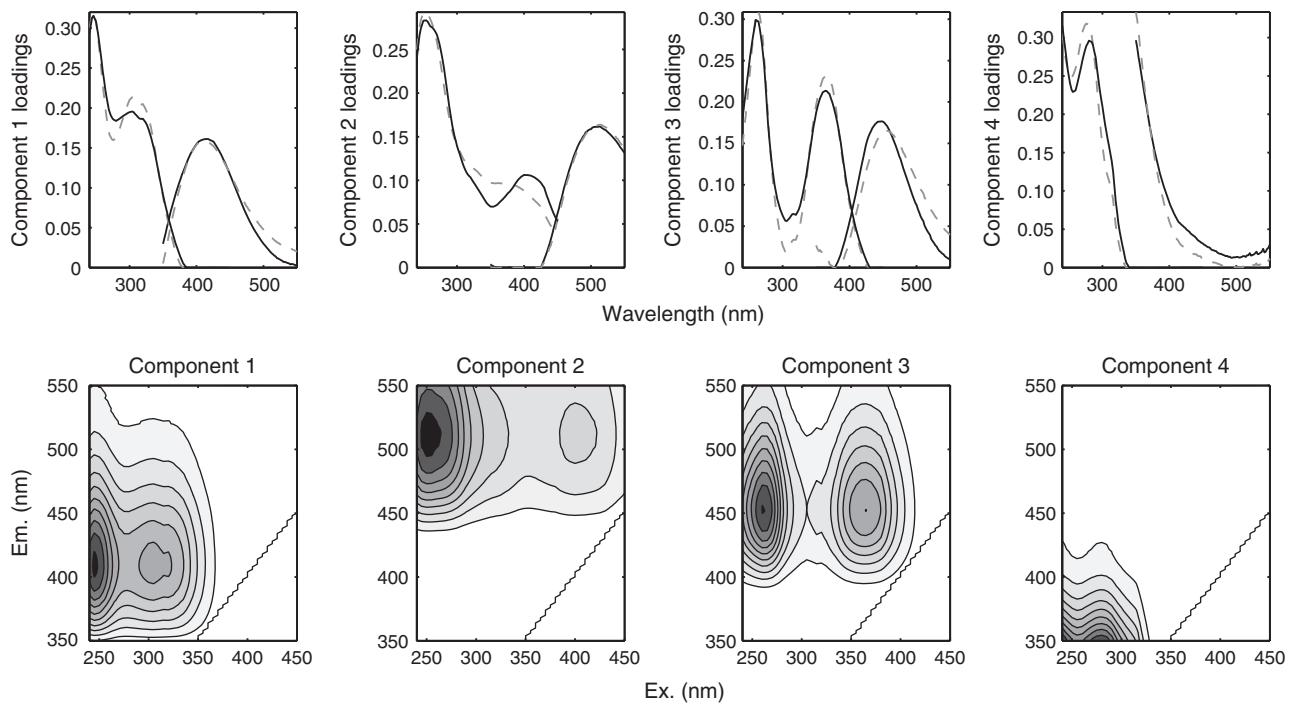


Fig. 2. Contour plots of parallel factor analysis (PARAFAC) components' spectral characteristics and 2-dimensional plots of split-half validation results.

Fluorescence excitation emission matrices (EEMs) were collected on a Horiba Jobin Yvon SPEX Fluoromax-3 spectrofluorometer (Horiba Scientific, Edison, NJ, USA). EEMs were collected over an excitation wavelength range of 240–450 nm with an increment of 5 nm and an emission range of 300–550 nm with an increment of 2 nm in a 1-cm quartz cuvette. The excitation and emission slit widths were both set to 5 nm. Fluorescence scans were collected in signal/reference ratio mode with an integration time of 0.25 s and reported in quinine sulfate units (QSU). EEMs were corrected for instruments optics and inner-filter effects according to Ohno (2002) and Raman normalised and blank subtracted using Matlab v2009a software (Mathworks, Natick, MA, USA). A parallel factor analysis (PARAFAC) model was built using 83 EEMs from Shark Bay and the N-way matlab toolbox (Stedmon and Bro 2008). Model components were split-half validated (Fig. 2). Model fit was considered suitable if the signal in the residual EEM (modelled EEM subtracted from measured EEM) was less than 10% of the measured EEM. The Fluorescence Index (FI), which indicates DOM source (McKnight *et al.* 2001), was calculated as the ratio of the emission intensities at 470 nm and 520 nm at an excitation wavelength of 370 nm (Jaffé *et al.* 2008). A higher FI value, e.g. 1.8, indicates a microbial DOM source, whereas, a lower value, e.g. 1.2, indicates a terrestrial source; intermediate values are indicative of a mixed DOM source.

Statistical analyses, including principal component analysis (PCA), Pearson correlations, and *t*-tests were determined using SPSS statistics 17.0 software (IBM, Armonk, NY, USA). The PCA analysis was run on the correlation matrix with a Varimax rotation and eigenvalues greater than one were retained. An average linkage cluster analysis was performed using R

software (R Foundation for Statistical Computing, Vienna, Austria), the vegan toolbox, and the Bray–Curtis distance matrix.

Results

Salinity values for surface water samples collected in Shark Bay ranged from 35 (regular seawater) to 60 along the coast in Hamelin Pool. Samples collected from freshwater, brackish water, and groundwater wells near Shark Bay had salinities less than 10 (see Table 1). DOM concentrations (DOC) vary substantially throughout Shark Bay (Fig. 1). For the saline samples (salinity > 10) DOC and salinity were linearly correlated ($P < 0.05$, $r = 0.626$) indicating that DOC may be concentrated by evaporation, along with inorganic salts, in some areas of Shark Bay. A general dilution of the DOC along a South to North transect was observed and likely related to prevailing water mass mixing as induced by wind, tidal exchange and gravity currents resulting from high salinity waters in Hamelin Pool. Interestingly, the highest (20 ppm) and lowest (1.2 ppm) DOC values were measured in brackish groundwater samples near Monkey Mia resort, which is a shallow site potentially impacted by anthropogenic surface activities, and a deep, old groundwater well, respectively. The hydrologic factors controlling groundwater flow may be important to fully understanding the drivers for DOM concentration in groundwater surrounding Shark Bay.

SUVA₂₅₄ negatively correlated with DOC in the saline samples ($P = 0.004$, $r = -0.376$). The S_R values were not significantly correlated with salinity or any other water quality parameters. However, the S_R for groundwater samples (mean $S_R = 0.92$) was significantly lower (i.e. of higher molecular

Table 1. DOC concentrations and relative abundance of parallel factor analysis (PARAFAC) components for groundwater and non-saline surface water samples collected near Shark Bay, Australia

Well	Type	SUVA ₂₅₄	Salinity	DOC (ppm)	% C1	% C2	% C3	% C4
Goat Ranch	Deep	3.12	3.2	1.23	42%	19%	23%	15%
Hot Tub	Deep	1.85	1.4	1.82	42%	18%	21%	19%
T-3	Surficial	2.41	5	5.62	42%	28%	28%	2%
RM1	Surficial	3.31	4.5	4.05	40%	29%	27%	3%
RM2	Surficial	2.68	8	4.06	42%	28%	28%	2%
RM3	Surficial	3.49	6	4.77	40%	28%	27%	5%
BW6	Surficial	4.02	3.5	1.71	35%	26%	23%	15%
BW7	Surficial	2.01	5	5.44	39%	24%	22%	15%
BW8	Surficial	3.16	8.5	20.03	40%	31%	29%	0%

Table 2. Characteristics of the four parallel factor analysis (PARAFAC) components developed from Shark Bay, Australia
Wavelengths in parenthesis are secondary, lower intensity excitation or emission maxima

Component	Excitation maximum	Emission maximum	Yamashita <i>et al.</i> (2010)	Excitation maximum	Emission maximum	Assignment
C1	245 (305)	410	C3	<260 (305)	416	Humic-like
C2	250 (400)	512	C5	275 (405)	>500	Humic-like
C3	260 (365)	452	C1	<260 (345)	462	Humic-like
C4	240 (280)	<350	C7/C8	275/300	326/342	Protein-like

weight) than for surface water samples (mean $S_R = 1.3$; $P = 0.002$). The fluorescence index (FI) in Shark Bay was found to range from 1.29 for a near-shore saline surface water sample (collected near the beach) to 1.63 for the marine end-member. The FI values varied throughout Shark Bay and did not show a clear trend with salinity or geography. The total fluorescence intensity, calculated by summing the fluorescence intensity over the whole EEM, correlated significantly with SUVA₂₅₄ ($P < 0.05$, $r = 0.611$) for the saline surface water samples.

We were able to validate a PARAFAC model with four components. Components 1, 2, and 3 (C1, C2, and C3) were classified as humic-like and component 4 (C4) was classified as protein-like on the basis of spectral characteristics (Fig. 2, Table 2). Humic-like C1 (maximum ex = 245(305) nm, em = 410 nm) shows spectral similarities to Everglades (including Florida Bay) component 3 and Everglades component 6, which were identified as terrestrial humic-like and ubiquitous humic-like, respectively (Table 2; Chen *et al.* 2010; Yamashita *et al.* 2010). Everglades component 3 was found to originate from terrestrial sources and was suggested to be bio-available, whereas Everglades component 6 was microbially derived and sensitive to photo-decomposition (Chen *et al.* 2010). C2, another humic-like component identified for Shark Bay (maximum ex = 250(400) nm, em = 512 nm) is similar to Everglades component 5, which was identified as being a terrestrial humic-like, humic acid-like component (Table 2). The third humic-like component, C3 (maximum ex = 260(365) nm, em = 452 nm), is spectrally similar to Everglades component 1, which is a ubiquitous humic-like component (Table 2). C4, the protein-like component (maximum ex = 240(280) nm, <350 nm), is spectrally similar to the two protein-like components in the

Everglades model and may be comprised of a combination of both tryptophan-like and tyrosine-like fluorophores (Table 2).

Spatially, the three humic-like PARAFAC components, C1, C2, and C3, were distributed similarly, with concentrations decreasing from the southern Shark Bay coastline out into the ocean influenced northern section of the bay (Fig. 3). C1 and C2 have higher relative abundances near the outlet of the Wooramel River. C2 and C3 have a region of higher concentration in the southern area of the Peron Peninsula which features a high density of seagrass meadows (Figs. 3 and 1; Walker *et al.* 1988). However, C1 relative abundance does not show the same increase in this area. The protein-like PARAFAC component, C4, increased in relative abundance from the Shark Bay shoreline out into the northern, ocean-influenced region of the bay, having the opposite trend as the three humic-like components (Fig. 3). Near Faure Sill there is an increase in the C4 relative abundance, but the absolute fluorescence intensity of the C4 signal is not higher in the offshore marine area. The relative abundance increase for C4 in this area is caused by a decrease in the humic-like fluorescence intensity of the other three humic-like PARAFAC components.

Stable carbon isotope analysis of the SPE DOM in the Wooramel River shows a $\delta^{13}C$ of -25.08% . The $\delta^{13}C$ values for the samples collected in Shark Bay saline surface waters were significantly more enriched compared to the Wooramel River DOM with a mean of -19.3% (range of -20.9% to -18.0% ; $n = 4$). Because of the limited number of samples we did not assess the spatial changes of $\delta^{13}C$ values.

Salinity values and optical properties including S_R , SUVA₂₅₄ and PARAFAC components were statistically evaluated using principal component analysis (PCA; Fig. 4), where principal

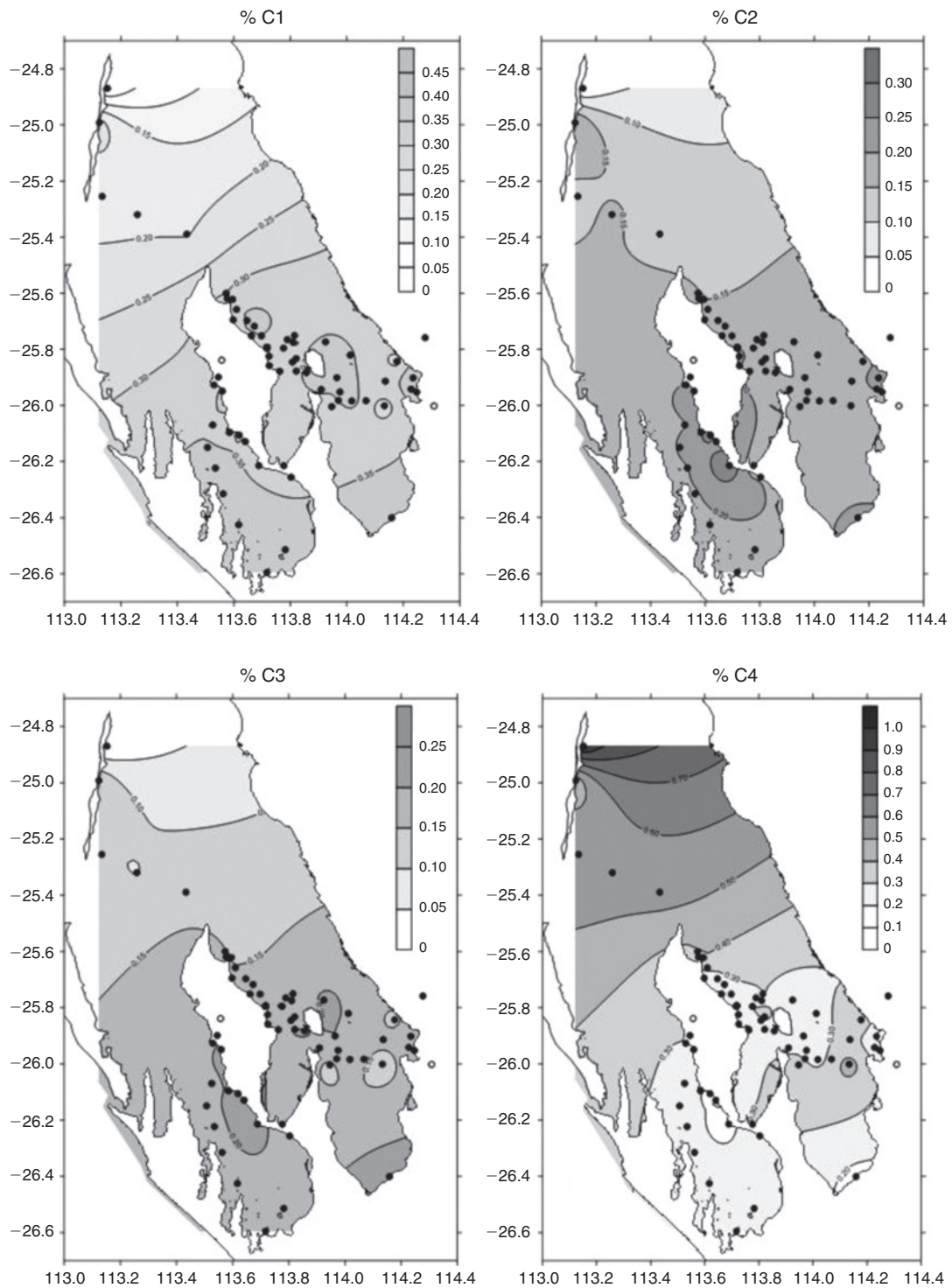


Fig. 3. Relative abundance of the four parallel factor analysis (PARAFAC) components.

component 1 (PC1) and Principal Component 2 (PC2) account for 53% and 28% of the variability, respectively. The cluster analysis resulted in six groups of samples spanning a continuum from marine, to mixed saline, to freshwater, and to groundwater (Fig. 4). The loadings of the PARAFAC components along PC1 are such that the relative abundance of C4 correlates most

negatively and the relative abundance of C3 is the most positively correlated, with C2 and C1 slightly less positive than C3 (Fig. 4). PC1 is correlated negatively to both relative abundance ($P < 0.05$, $r = -0.432$) and intensity ($P < 0.05$, $r = -0.957$) of the protein-like PARAFAC component, C4. Therefore, PC1 is driven by DOM source material with higher marine

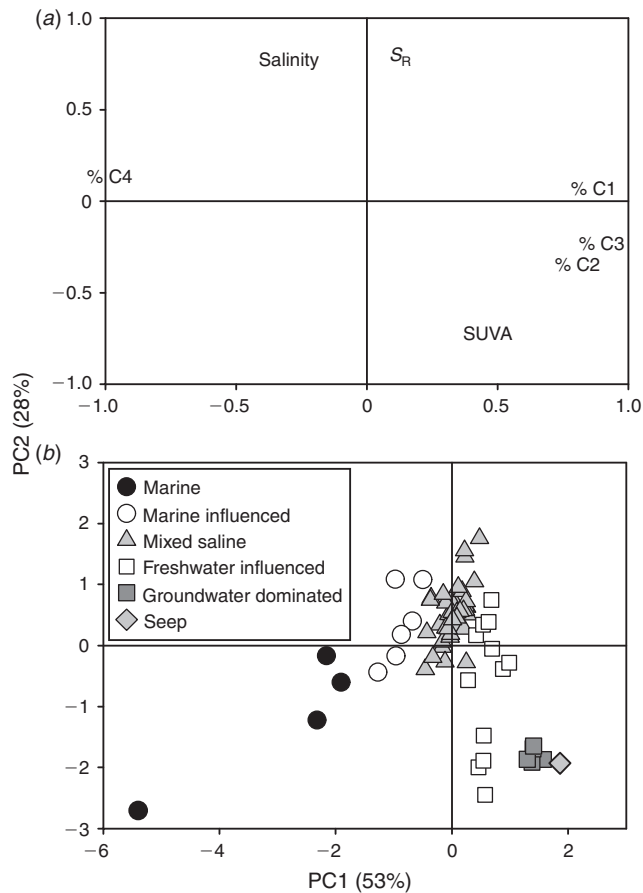


Fig. 4. Graphical representations of principal component analysis of the excitation/emission fluorescence spectroscopy and parallel factor analysis (EEM-PARAFAC) relative abundances. (a) Loadings plot for the four PARAFAC components and water quality parameter and (b) score plot for water samples grouped using a cluster analysis and classified on the basis of geographic location and water quality parameters.

loadings having higher relative abundances of protein-like C4 and more negative PC1 values. The loadings along PC2 are such that $SUVA_{254}$ is the most negatively correlated parameter and S_R and salinity are the most positively correlated parameters (Fig. 4). PC2 seems to be driven by DOM processing with lower molecular weight, more saline samples having higher PC2 values. The scores plot shows that the mixed saline samples are the most positive along PC2 and the marine and groundwater samples are more negative.

Discussion

This study presents the first characterisation of DOM in Shark Bay, Australia using optical properties and ^{13}C stable isotopes. The lack of a specific microbial humic-like component was an unexpected result of this study because there is very little terrestrial run-off into Shark Bay since the surrounding rivers are ephemeral and this region is a desert. Such components are usually characterised as having a shift to lower maximum emission wavelengths, and have been reported in many other water bodies (Cory and McKnight 2005; Stedmon and Markager

2005; Murphy *et al.* 2008; Yamashita *et al.* 2010). It is possible that DOM transformations in Shark Bay, such as microbial or photochemical processing, may degrade the microbial products or transform them into more humic-like fluorophores (Kieber *et al.* 1997; Miller *et al.* 2009). The low $SUVA_{254}$ values in high DOC and high salinity areas may also be a result of photo-bleaching of chromophoric DOM (i.e. reduced aromaticity) concomitant with evaporative DOM enrichment, and/or the result of low $SUVA_{254}$ DOM inputs by seagrass (i.e. carbohydrate enrichment). It is also possible that there is a more terrestrial DOM signature in Shark Bay because of the high turbidity and photo-dissolution of POM to DOM, which has been shown to produce more terrestrial humic-like fluorescence than microbial or protein-like fluorescence in the Everglades and Florida Bay ecosystems (Pisani *et al.* 2011; Shank *et al.* 2011). Lastly, little is known about the potential influence of groundwater-surface water exchange as another source of DOM to the bay.

Another potentially important source of terrestrial-like fluorescence in Shark Bay may come from a non-terrestrial source: detrital seagrass derived DOM, which has been shown to have an optical signature similar to terrestrial DOM (Stabenau *et al.* 2004). The prevalence of seagrass communities in Shark Bay suggests that both degrading seagrass detritus and seagrass primary productivity may contribute to the DOM pool in the bay. In contrast to the optical properties of detrital seagrass, seagrass primary productivity-derived DOM has been reported as highly bioavailable (Ziegler and Benner 2000) but mostly non-absorbing (i.e. enriched in carbohydrates; Maie *et al.* 2005) and enriched in protein-like fluorescence (Maie *et al.* 2012). Therefore, seagrass detritus derived DOM (vs primary productivity derived DOM) may be more prevalent in Shark Bay contributing to the observed, terrestrial humic-like fluorescence signature. The ecological importance of detrital seagrass on trophic dynamics in Shark Bay has also been suggested (Belicka *et al.* 2012).

The fourth component of the PARAFAC model, a protein-like component (C4), may be associated with microbial activity or primary productivity, as Faure Sill, a location with high C4 relative abundance, is a location with relatively high water column chlorophyll concentration that may produce C4 fluorophores through primary productivity, at least on a seasonal basis (Kimmerer *et al.* 1985; Romera-Castillo *et al.* 2010). However, in the offshore areas of Shark Bay with high C4 relative abundances (but no concomitant increase in C4 intensity) the increase in C4 relative abundance is likely because of a lack of humic-like fluorescence signal being transported out into the northern section of Shark Bay rather than an increase in the C4 signal caused by primary productivity. Lower relative abundance of C4 (as compared to C1, C2 and C3) in DOM rich areas may be caused by higher bioavailability of this DOM component (i.e. protein-like). Shark Bay is a phosphorus limited system that depends almost entirely on internally fixed nitrogen (Smith 1984; Smith and Atkinson 1984). Despite the general P-limited nature of the Shark Bay ecosystem as a whole, detailed analysis of the relative abundance of N and P to seagrasses across the bay indicate broad areas of nitrogen limitation near the mouth of the bay, which contrast with the P-limited nature of the more isolated, longer water residence time, higher salinity

interior portions of the bay (Burkholder *et al.* in press). Therefore, it is likely that DON (i.e. C4) is an important nitrogen source in Shark Bay, and as such may not accumulate in high abundance in this environment but is likely present in low abundance throughout Shark Bay.

In addition to the mixed terrestrial and microbial signal seen in the fluorescence spectra of the Shark Bay samples, the stable isotope analysis supports a mixed DOM source. The relatively depleted $\delta^{13}\text{C}$ value for the Wooramel river is similar to the Taylor River in the freshwater Everglades (-26.75%) and both are in line with terrestrial values for ultrafiltered DOM (UDOM) reported by Harvey and Mannino (2001). The SPE DOM from Taylor River, a tributary to Florida Bay, was very similar to the values reported for ultrafiltered DOM (UDOM; >1000 Dalton) from Taylor River showing that the two methods of DOM isolation produce comparable samples for stable isotope analysis (Maie *et al.* 2006b). DOM from seagrass ecosystems tends to be relatively enriched in ^{13}C compared to DOM collected from the surface ocean or from freshwater sources (Maie *et al.* 2006b) suggesting that seagrass DOM is an important contributor to the DOC pool in Shark Bay. However, macroalgae within Shark Bay may also contribute to the DOM pool and have been reported to have intermediate $\delta^{13}\text{C}$ values ranging from -12.0% to -24.1% (Burkholder *et al.* 2011). Our saline water DOM $\delta^{13}\text{C}$ values fall within this range; however, the fluorescence analysis and ecosystem characteristics ($\sim 70\%$ vs 30% seagrass vs macroalgae biomass) make macroalgae as a dominant DOC sources an unlikely scenario for Shark Bay. It is most likely that the DOM comprises a mixture of macroalgae, seagrass, and terrestrial inputs. However, with the limited number of samples collected for $\delta^{13}\text{C}$ of DOC in study, we were unable to reliably model isotope mixing and quantitatively assess DOM source strengths. Therefore, future studies of DOM character and source in Shark Bay would benefit greatly from a greater spatial sampling and end-member characterisation of DOM for ^{13}C stable isotope analysis.

The PCA results (Fig. 4) show that there is a gradient along PC1 (i.e. DOM source) for the different clusters of samples with low PC1 scores for the marine samples, indicating a greater abundance of microbial inputs, and higher PC1 scores for the freshwater-influenced samples, indicating a greater contribution of humic-like terrestrial DOM. As stated above, this is likely driven by a lack of terrestrial or autochthonous microbial inputs in the marine samples rather than an additional source of protein-like DOM at the marine end-member. The freshwater-influenced and mixed saline surface water samples are centred along PC1, indicating that they contain a mixture of humic-like and protein-like PARAFAC components. The freshwater-influenced samples have lower PC1 and higher PC2 scores, indicating a change in source and/or character, compared to the groundwater samples which have terrestrial humic-like character, including lower S_R , lower PC2 values, and higher SUVA₂₅₄ values. Overall, the PCA indicates that the DOM has a mixed autochthonous (seagrass and macroalgae) and allochthonous (terrestrial, riverine and groundwater) source, and is likely photo-degraded or diluted with marine-derived DOM leading to the characteristics seen in the mixed saline samples.

Interestingly, the characterisation of DOM in Shark Bay has highlighted some substantial differences with Florida Bay,

another subtropical seagrass dominated coastal area located in Everglades National Park, Florida, USA. The $\delta^{13}\text{C}$ values measured in Shark Bay were not as enriched as those measured in Florida Bay ($\delta^{13}\text{C} = -14.4\%$; Maie *et al.* 2006b). DOM and CDOM studies of Florida Bay suggest that a significant portion (Maie *et al.* 2005; Maie *et al.* 2006a), possibly as high as 40–50% (Stabenau *et al.* 2004), of DOM is seagrass-derived. Much of the remaining DOM pool in Florida Bay was attributed to allochthonous inputs through hydrological transport (Jaffé *et al.* 2008; Maie *et al.* 2012). Unlike Florida Bay, the total fluorescence in Shark Bay was not significantly correlated with DOC concentration. Potentially, photo-exposure results in the bleaching of fluorophores without photolytically removing as much DOC from the organic pool (Moran *et al.* 2000). Alternatively, there may also be a regionally significant source of low absorbance and fluorescence DOM in Shark Bay, such as the exudation of carbohydrates during primary production by macroalgae or seagrass (Ziegler and Benner 2000; Maie *et al.* 2005; Maie *et al.* 2006c), compared to CDOM production by decomposing seagrass detritus or terrestrial inputs (Stabenau *et al.* 2004).

There are some similarities, however, in regards to DOM processing. The same positive trend between DOC and salinity was reported in Florida Bay indicating that in both ecosystems evaporation influences DOM concentration (Fourqurean *et al.* 1993). A similar pattern of lower S_R values (higher molecular weight) in Shark Bay groundwater was reported for Florida Bay groundwater ($S_R = 1.1$ – 1.2 and surface water $S_R = 1.3$, except for one groundwater site with high S_R values 1.5 – 1.6 ; Chen *et al.* 2010). In Shark Bay, the higher S_R (lower molecular weight) measured in surface water samples may be because of photo-exposure as S_R has been shown to increase with photo-degradation of DOM (Helms *et al.* 2008), and/or an enrichment of more humified, larger molecular weight DOM in groundwater (see SUVA₂₅₄ values in 1).

Climate change, resulting in sea level rise and increased terrestrial run-off could have divergent impacts on the Shark Bay ecosystem altering the relative inputs of different DOM (and CDOM) sources, and consequently result in significant changes of ecosystem functions and environmental degradation of this system. The development of ecological models for managing natural resources and predicting long-term disturbance effects on coastal seagrass ecosystems require more detailed biogeochemical studies to constrain DOM dynamics in protected environments, such as Shark Bay. Both similarities and differences in DOM sources and composition were identified between Florida Bay and Shark Bay, however, it is clear that sub-tropical seagrass ecosystems feature complex biogeochemical processes that require detailed, site-specific studies. As such, along with more detailed ^{13}C isotope studies of DOC to quantify specific source strengths, the potential contribution of humic-like DOM from groundwater to Shark Bay needs to be assessed.

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