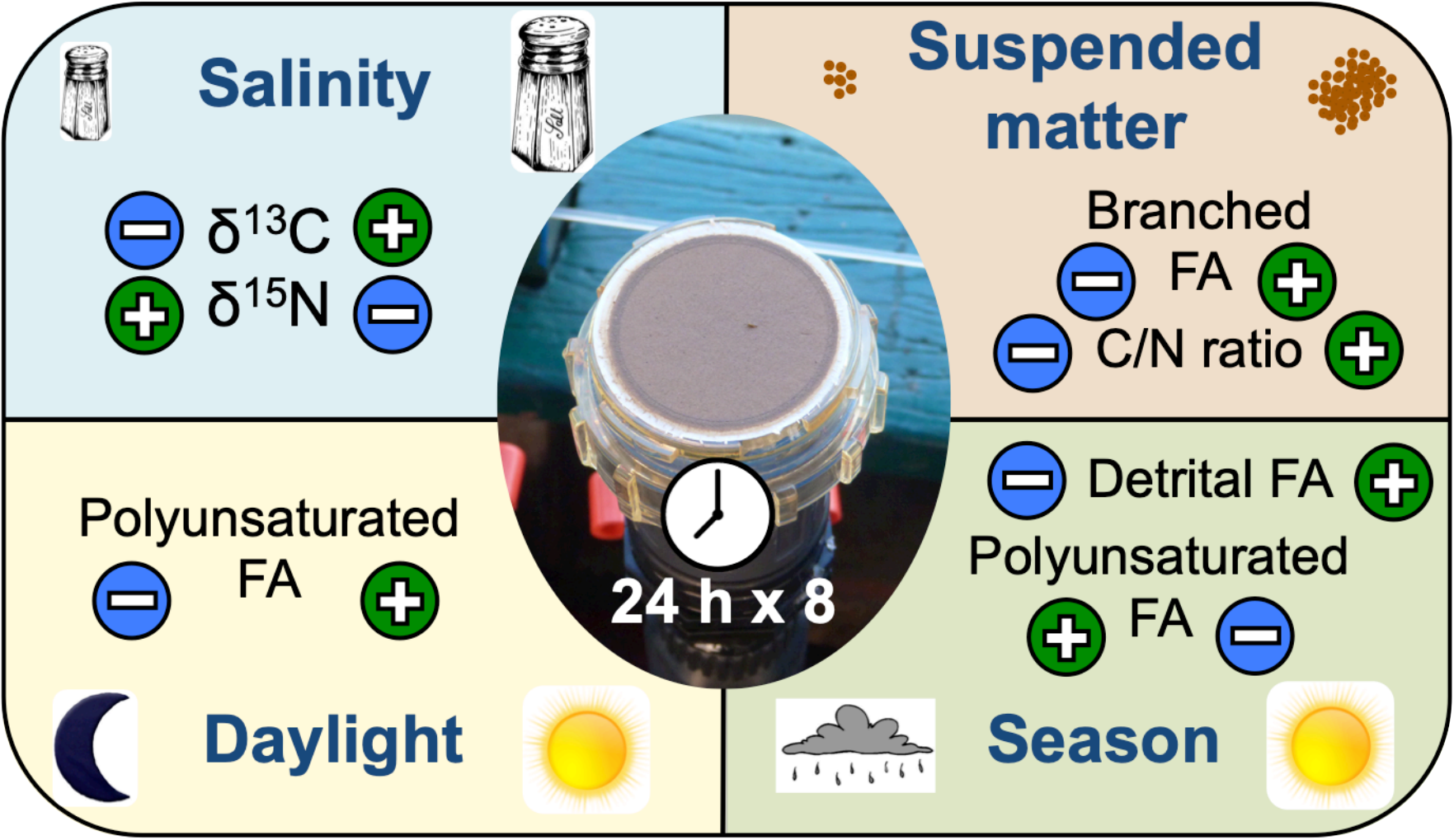
Short-term changes in the quality of suspended particulate matter in a human impacted and mangrove dominated tropical estuary (Can Gio, Vietnam)

Frank David, Cyril Marchand, Najet Thiney, Tran-Thi Nhu-Trang, Tarik Méziane


Salinity
δ13C
δ15N
Suspended matter
Branched FA
C/N ratio
24 h x 8
Polyunsaturated FA
Detrital FA
Polyunsaturated FA
Daylight
Season

Title: Short-term changes in the quality of suspended particulate matter in a human impacted and mangrove dominated tropical estuary (Can Gio, Vietnam).

Running head: Short-term changes in SPM quality in the Can Gio mangrove estuary.

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Abstract

Suspended particulate matter (SPM) is a key component of coastal food webs and a key variable of nutrient budgets. Understanding its variability accross short time scales in estuaries may help ecologists understand seasonal and diurnal migration of estuarine organisms, and answer how their nutritional requirements are fulfilled. It may also inform biogeochemists regarding the factors that influence import and export of nutrients between terrestrial and coastal ecosystems. This study aimed to link the dynamics of fatty acids, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and C/N ratios of SPM, revealing OM quality, to rapidly varying factors (SPM concentration, salinity and presence of daylight) and comparing this variability to the seasonal variation induced by the alternation of dry and wet seasons in the studied region. Our results revealed that these rapidly varying factors had a strong influence on the bacterial and the phytoplanktonic compartments of SPM. They suggest that tidally resuspended particles are the site of intense heterotrophic activity and that estuarine phytoplankton store lipids during the daytime up to substantially modifying SPM quality. Our study also shows higher freshness of SPM during the wet season. We expect this study to raise the interest of both biologists and biogeochemists to introduce daily variability of SPM in food webs and nutrient budgets modelling.

Keywords: fatty acids, stable isotopes, bacteria, phytoplankton, resuspension, lipid storage, South East Asia.

1. Introduction

Suspended particulate matter (SPM) plays an important role in tropical estuaries, both as a component of coastal food webs (Riera et al. 2000, Meziane and Tsuchiya 2002, Alfaro et al. 2006) and as a key variable of nutrient budgets (Kristensen et al. 2008, Cai 2011, Hofmann et al. 2011). Lipids and especially fatty acids (FA) are used as environmental tracers to discriminate sources of organic matter (OM) in aquatic ecosystems (Bodineau et al. 1998, Mortillaro et al. 2011, Antonio and Richoux 2015). These organic compounds are synthesised in specific proportions by living organisms, possibly revealing the contribution of several taxa to bulk OM (Dalsgaard et al. 2003, Bergé and Barnathan 2005). Nevertheless, very few FA are exclusive to any kind of OM. Such biomarkers should preferentially be coupled with other tracers, such as isotopic and elemental compositions of SPM, that are also able to discriminate OM sources and identify biogeochemical processes in estuaries (Middelburg and Herman 2007, Mortillaro et al. 2011, 2016, Bergamino et al. 2014).

The SPM composition in estuaries, and therefore its nutritional quality, is impacted by seasonal changes, with generally a higher contribution of terrestrial OM during the wet season (Mortillaro et al. 2011, Boëchat et al. 2014). However, the opposite trend has also been observed, with reduced autochthonous primary production during the dry season (Xu and Jaffé 2007). The SPM composition also varies as a function of salinity (Bodineau et al. 1998, Shilla et al. 2011, Antonio and Richoux 2015), generally showing the dilution of terrestrial OM with marine OM (Middelburg and Herman 2007, He et al. 2014). Natural populations of estuarine phytoplankton exhibit high diurnal physiological variability in response to light or nutrient limitation, notably with regard to lipids concentration (Madariaga 2002, Halsey and Jones 2015). Finally, in mangrove ecosystems tidal flooding strongly affects SPM concentrations at short temporal scales (Schwarzer et al. 2016). We previously measured strong variations in both the concentration and the composition of SPM within a creek of the

Can Gio mangrove during a tidal cycle, thus revealing the importance of high frequency sampling to correctly examine SPM in this ecosystem (David et al. 2018a).

In the present study, we measured the quality of SPM during 24 h tidal cycles within the main channel of the Can Gio mangrove during two seasons at four sites distributed from the downstream end of Ho Chi Minh City (Southern Vietnam) to the South China Sea coast. We studied a creek almost fully drained at each tide in David et al. (2018a), whereas in the present study we enlarged our investigation scale to a ~600 m wide and 10 to 20 m deep highly dynamic estuary. Our objective was to link the dynamics of fatty acids, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and C/N ratios of SPM, revealing OM quality, to rapidly varying factors (SPM concentration, salinity and presence of daylight) and comparing this variability to the seasonal variation induced by the alternation of dry and wet seasons in this region. At a broader scale, our study intends to partition the effect of these factors, that can be seen as proxies of vertical, diurnal, spatial and seasonal variability on different compartments of SPM, which may in turn help biologists and biogeochmists to focus on given sources of SPM quality variability according to their research questions.

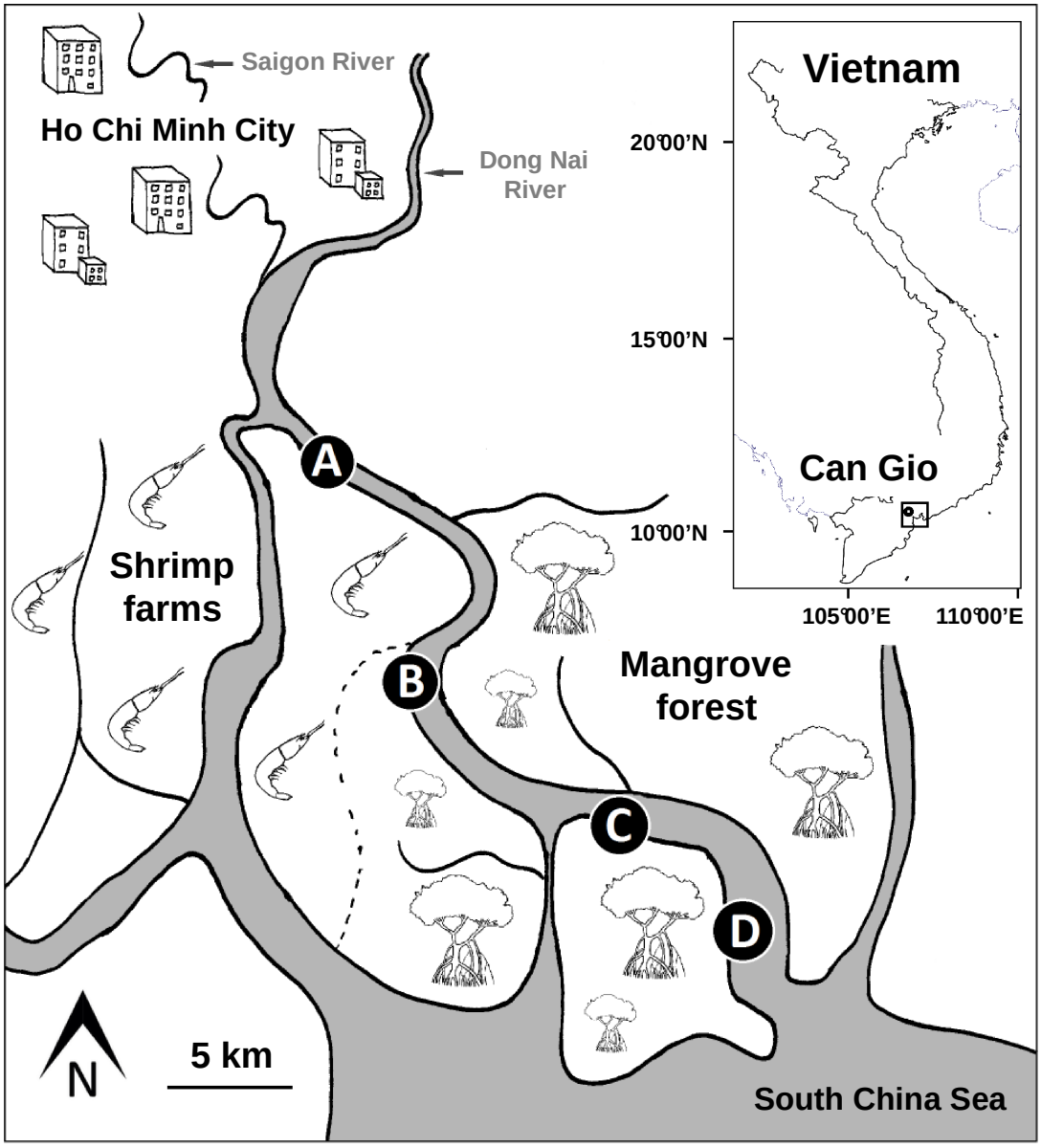
2. Materials and methods

2.1 Study area

The Can Gio mangrove is located at the downstream end of Ho Chi Minh City (~13 million inhabitants) and flooded by the Saigon-Dong Nai Rivers, discharging annually 37.4×10^6 m^3 of freshwater to the South China Sea and whose basin covers a total catchment area of 40.6×10^3 km^2 (12% of the total terrestrial area of Vietnam; Ringler et al. 2002). The climate in Can Gio is monsoonal with a wet season from June to October and a dry season from November to May. Tidal amplitude is variable over time and ranges between 2 to 4 m

89 depending on season and distance from the sea (Nam et al. 2014). In 2000, the UNESCO
90 designated the 719.6 km^2 of the Can Gio district as the first mangrove biosphere reserve in
91 Vietnam and a clear land use regulation was established. The west border of the mangrove is
92 fringed by shrimp farms and salt evaporation ponds, covering roughly 20% of the total
93 biosphere reserve surface area, while the rest of the district is preserved from deforestation
94 and mostly covered with mature trees of the species *Rhizophora apiculata*.

95 We selected the study sites to examine SPM within the main estuarine channel of the
96 mangrove and to cover the entire salinity gradient, from almost freshwater to the coastal
97 ocean (Fig. 1): A) at the downstream end of Ho Chi Minh City (10°39'55"N 106°47'30"E); B)
98 between shrimp farms and the mangrove forested area (10°34'19"N 106°50'11"E); C) in the
99 centre of the mangrove protected core (10°31'04"N 106°53'13"E); and D) between the
100 mangrove forested area and the South China Sea coast (10°29'32"N 106°56'55"E). All sites
101 have steep eroded banks, a width of about 600 m and a depth ranging from 10 to 20 m. These
102 characteristics make the estuary not favourable for benthic productivity.



103

104 Fig. 1: Map of the Can Gio mangrove estuary (Southern Vietnam). A, B, C and D

105 indicate the sampling stations along the estuary

2.2 Sampling strategy

We monitored surface SPM over 24 h time series during the dry (January-February) and monsoon (September-October) periods in 2015. Salinity was measured using a Yellow Spring Instrument® meter (YSI 6920) immersed 30 cm below water surface and calibrated before each survey. Values were not recorded at site C during the dry season due to probe malfunction and were estimated using a linear relationship established on the three other sites between alkalinity measured on the filtered water samples and salinity (salinity = 16.1 × alkalinity (mmol C L^{-1}) – 7.1; R^2 = 0.99; n = 39; David et al. 2018b). The maximum difference between calculated and probe measured salinity was 1.8, which was relatively low given the range of the monitored salinity gradient (0 to 26).

Five samples of surface water were taken at two-minutes intervals every two hours (13 sampling events per 24 h time series) using a 10 L bucket. Samples were immediately vacuum-filtered through pre-combusted (5 h at 450°C) and pre-weighted glass fibre filters (Whatman® GF/F 0.7 μm) until complete clogging of the filters. It required 250 mL to 1.2 L of estuarine water and allowed the collection of 10 to 80 mg of SPM, depending on turbidity. All filters were immediately stored at -25°C until analyses.

2.3 Sample processing

Filters were first freeze-dried and weighted for SPM determination. Then, four filters of SPM per sampling event were used as replicates for the analysis of fatty acids and the remaining filter was used for dual ($\delta^{13}C$ and $\delta^{15}N$) stable isotopes analysis and C/N ratio determination. We measured stable isotopes and C/N ratio on the four sites during both seasons, while FA were measured on the four sites during the dry season but only on site B and D during the monsoon season.

We extracted lipids following a slightly modified protocol of Bligh and Dyer (1959), as described in Meziane et al. (2007). Tricosanoic acid (23:0) was used as an internal standard and 5 µg of methyl tricosanoate provided by Sigma-Aldrich® was added to every sample prior to extraction. Lipids were extracted with 4 mL of a water:methanol:chloroform mixture (1:2:1, v:v:v) enhanced by two 20 min steps of sonication. Chloroform and water was added to the mixture to reach equal proportions of the three solvents and allow the formation of a aqueuous-organic bilayer system. The lipid fraction, contained in the chloroform, was retrieved after phases were separated by centrifugation (3000 rpm, 1400 rcf, 5 min), and evaporated under nitrogen (N_2) flux. Dried lipid extracts were saponified using a methanol:sodium hydroxide (2N) mixture (2:1, v:v) during 1 h 30 min at 90°C. Fatty acid esters were then methylated into fatty acid methyl esters (FAME) using boron trifluoride-methanol (BF_3-CH_3OH) and stored at -25°C. FAME were quantified by gas chromatography analysis (Varian 3800-GC), using a flame ionisation detector. The oven temperature was set at 60°C and held for 1 min, raised at 40°C min^{-1} to 150°C and held for 3 min, and then increased to 240°C at 3°C min^{-1} and held for 25 min. We identified fatty acids using coupled gas chromatography mass spectrometry (Varian 450-GC; Varian 220-MS) and comparison of GC retention times with commercial standards (Supelco® 37 component FAME mix). An example of GC/MS chromatogram has been provided in Appendix 1. We reported the values as % of total FA or absolute concentrations (µg L^{-1} or µg mg SPM^{-1}).

We analysed stable isotope ratios at the University of California Davis Stable Isotope Facility (Department of Plant Sciences, UC Davis, Davis, California) using a Vario EL Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.). Samples were prepared in tin capsules by scraping the SPM clogging the filters and after 4 h of HCl 37% fumigation to remove all carbonates. Then, UC Davis performed the analysis. Carbon

and nitrogen stable isotope ratios were reported in parts per thousand (‰), using standard delta notation ($\delta^{13}C$ and $\delta^{15}N$), and are relative to V-PDB (Vienna PeeDee Belemnite) and atmospheric air, respectively. We calculated the C/N ratio using the mass abundance of carbon and nitrogen in a given sample.

2.4 Data analyses

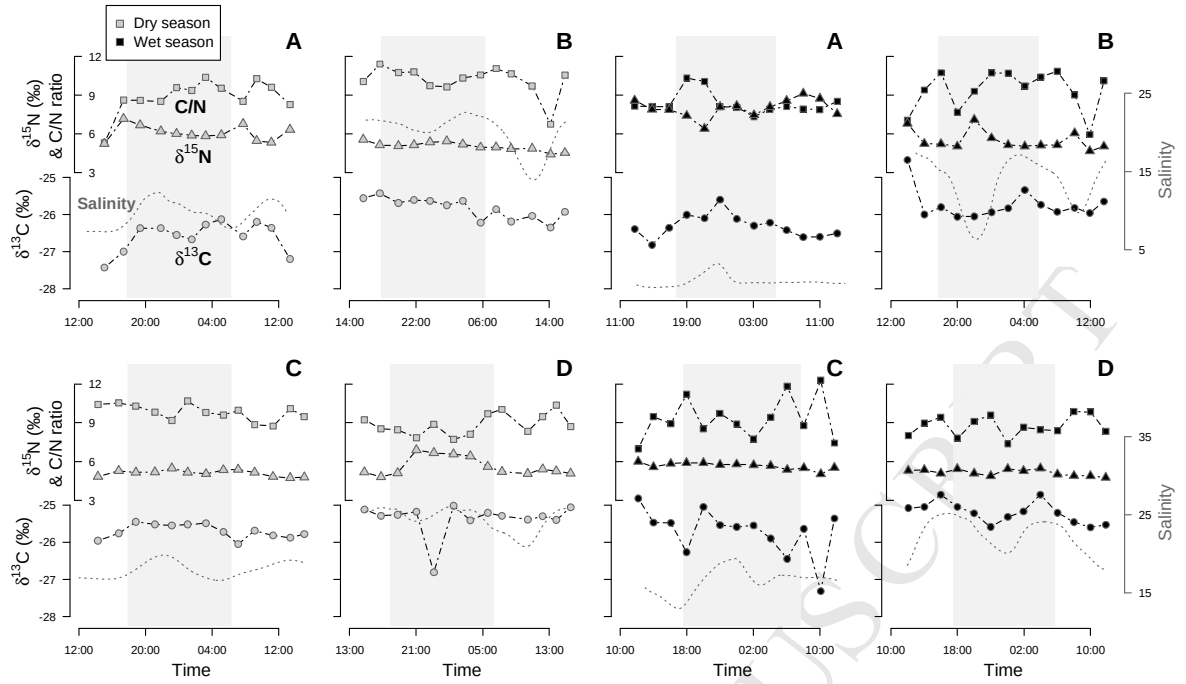
Three FA biomarkers were selected: 1) proportion of iso- and anteiso-branched chain FA (%BrFA), as indicators of bacteria (Mortillaro et al. 2011, Boëchat et al. 2014); 2) proportion of 15:0 + 16:0 + sum of 16:1 + 18:0 + sum of 18:1 (%detritalFA), commonly derived from decaying organic material (Wakeham 1995, Canuel 2001, Boëchat et al. 2014); and 3) proportion of polyunsaturated FA (%PUFA), indicative of fresh autochtonous organic matter (Wakeham 1995, Canuel 2001). The latter was assumed to be mostly phytoplankton in this ecosystem where no macroalgae was observed and where depth and turbidity prevents benthic productivity. In addition, $\delta^{13}C$ and C/N ratio were employed to partition terrestrial vs. marine OM, while $\delta^{15}N$ was used to understand its diagenesis (Middelburg and Herman 2007) and as an integrated N-load measurement (McClelland and Valelia 1998).

We challenged the effect of SPM concentration, salinity (taken as a proxy of fresh vs. marine waters mixing ratio), daylight presence and season on FA biomarkers, stable isotopes and C/N ratios using sequential analysis of covariance (Type I ANCOVA). Suspended particulate dry mass and salinity were considered as quantitative variables, while daylight presence and season were set as qualitative variables. Sequential ANCOVA were used to test whether SPM tracers were correlated to these variables. We chose the order in which the terms were introduced in the model according to the expected effect of each factor, allowing to test the effect of each subsequent variable relative to the preceding model. In the model using FA, we introduced SPM first because data were not equally distributed among seasons

(Table 1), while we started with salinity in the model using stable isotope ratios for the same reason. We tested all other factors in pairs and they showed no correlation with one another. Suspended particulate matter data were natural log transformed before analyses to alleviate heteroscedasticity and improve normality. We reported models sum of squares (SS), Fisher test value (F value) and probability of obtaining the same value considering the null hypothesis true (p). Results of the four FA analyses per sampling event were averaged before running the ANCOVA. Statistical analyses and graphical representations were performed using R (R Core Team 2017).

3. Results

Salinity ranged from 7 to 26 during the dry season and from 0 to 25 during the wet season (Fig. 2). Suspended particulate matter concentrations ranged from 14 to 293 mg L^{-1} and exhibited important tide-induced variability, with highest SPM values measured just after the maximum current velocity was reached (data discussed in David et al. 2018b). $\delta^{13}C$ ranged from -27.6‰ to -24.5‰ with lowest values measured at site A during the dry season (Fig. 2). $\delta^{15}N$ ranged from 2.7‰ to 9.1‰ with higher values measured at site A during the wet season (Fig. 2). Finally, C/N ratios ranged from 5.3 to 12.3 (Fig. 2).



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198 Fig. 2: Dynamics of C/N ratio (squares), $\delta^{15}N$ (triangles), $\delta^{13}C$ (bubbles) and salinity (dotted
199 grey line) during the 24 h tidal cycles in the Can Gio mangrove estuary (Southern Vietnam).
200 Letters in the upper righthand corner indicate the samping site. Shaded areas correspond to
201 night time

202

203 We identified up to 39 FA in the SPM samples of the Can Gio mangrove estuary
204 (Table 1). Predominant FA were saturated fatty acids (SFA) 16:0, 18:0 and 14:0 and
205 monounsaturated fatty acids (MUFA) 18:1ω9, 16:1ω7, 18:1ω7 and 22:1ω9. The ANCOVA
206 revealed that season was the most significant variable affecting %detritalFA and %PUFA
207 (Table 2). Values of %detritalFA were higher during the dry season, whiles values of %PUFA
208 were higher during the wet season (Table 1 and Fig. 3). The %BrFA and $\delta^{13}C$ were also
209 affected by season as second most explanatory variable (Tables 2 and 3), with lower values
210 measured during the dry season (Fig. 2 and 3). Salinity was the most significant variable
211 affecting $\delta^{13}C$ and $\delta^{15}N$, with $\delta^{13}C$ increasing with salinity and $\delta^{15}N$ decreasing with salinity
212 (Table 3 and Fig. 4). It also influenced %detritalFA and C/N ratio as second most explanatory

213 variable and to a lesser extent %PUFA (Table 2 and 3), with %detritalFA and %PUFA
214 increasing with salinity (Fig. 5) and C/N ratio decreasing with salinity (data not showed). The
215 amount of SPM had the most explanatory power for the %BrFA and C/N ratio (Tables 2 and
216 3), with values increasing with SPM concentration (Fig. 6). The daylight presence was the
217 second most explanatory variable affecting %PUFA, with higher values measured during the
218 day (Fig. 7) and an explained variability proportion (SS/Total SS) close to that of season
219 (Table 2).

220 Table 1: Mean (±SD) fatty acid composition of SPM during the 24 h tidal cycles in the Can

221 Gio mangrove estuary

Fatty acids (%)	Dry Season				Wet Season	
	Site A (n = 13)	Site B (n = 13)	Site C (n = 13)	Site D (n = 13)	Site B (n = 13)	Site D (n = 13)
Saturated						
12:0	2.0 ± 1.8	2.0 ± 1.5	2.0 ± 1.7	1.7 ± 1.4	2.6 ± 1.5	2.5 ± 1.4
13:0	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.7 ± 0.3	0.6 ± 0.3
14:0	6.0 ± 1.6	5.6 ± 1.5	6.4 ± 1.8	7.1 ± 2.0	8.7 ± 1.5	9.0 ± 1.0
15:0	2.0 ± 0.7	2.2 ± 0.7	2.2 ± 0.9	2.7 ± 0.9	2.8 ± 0.4	2.4 ± 0.4
16:0	32.6 ± 5.3	28.9 ± 4.3	33.3 ± 4.7	34.8 ± 4.2	28.4 ± 2.2	30.0 ± 2.1
17:0	1.0 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	1.2 ± 0.3	1.4 ± 0.2	1.3 ± 0.3
18:0	16.3 ± 4.8	16.5 ± 4.3	16.8 ± 5.5	13.2 ± 4.0	7.8 ± 1.3	7.8 ± 1.7
19:0	0.3 ± 0.3	0.5 ± 0.3	0.4 ± 0.3	0.3 ± 0.2	0.6 ± 0.2	0.5 ± 0.2
20:0	0.7 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.5 ± 0.1	0.7 ± 0.4
21:0	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
22:0	0.7 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.3 ± 0.1
24:0	0.9 ± 0.5	1.1 ± 0.6	0.7 ± 0.5	0.8 ± 0.6	0.2 ± 0.2	0.1 ± 0.1
26:0	0.3 ± 0.2	0.5 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	n.d.	n.d.
∑SFA	**62.9 ± 9.3**	**60.3 ± 8.3**	**64.7 ± 8.4**	**63.4 ± 7.5**	**54.3 ± 2.9**	**55.3 ± 3.7**
Monounsaturated						
14:1ω5	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.3	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
16:1ω9	2.1 ± 1.9	2.2 ± 2.0	2.1 ± 2.1	3.0 ± 1.8	2.2 ± 0.9	1.5 ± 0.5
16:1ω7	4.3 ± 1.7	5.2 ± 1.5	5.2 ± 1.9	5.0 ± 1.7	8.5 ± 1.0	10.3 ± 1.5
17:1ω9	0.4 ± 0.4	0.7 ± 0.4	0.4 ± 0.4	0.6 ± 0.4	0.3 ± 0.2	0.2 ± 0.1
17:1ω7	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	0.7 ± 0.1	0.9 ± 0.2
18:1ω9	10.3 ± 3.5	12.5 ± 4.2	11.7 ± 3.9	11.5 ± 3.9	12.7 ± 2.3	10.7 ± 1.6
18:1ω7	2.5 ± 0.8	3.9 ± 1.1	3.3 ± 1.0	2.6 ± 0.7	5.7 ± 1.0	6.4 ± 1.0
20:1ω11	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
20:1ω9	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:1ω9	7.9 ± 4.8	3.5 ± 3.1	1.1 ± 0.9	1.0 ± 1.4	0.9 ± 0.6	0.2 ± 0.3
∑MUFA	**28.4 ± 8.4**	**29.2 ± 7.7**	**25.0 ± 6.9**	**24.9 ± 6.2**	**31.6 ± 3.0**	**30.7 ± 2.8**
Polyunsaturated						
16:2ω6	n.d.	n.d.	n.d.	n.d.	0.3 ± 0.1	0.2 ± 0.1
16:2ω4	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.8 ± 0.4
16:3ω4	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.3	0.8 ± 0.4	1.1 ± 0.5
16:4ω3	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
18:2ω6	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.4 ± 0.6	1.3 ± 0.4	1.3 ± 0.3
18:3ω3	0.8 ± 0.7	0.4 ± 0.5	0.5 ± 0.4	0.8 ± 0.4	0.5 ± 0.5	0.7 ± 0.3
18:4ω3	0.6 ± 0.6	0.4 ± 0.5	0.6 ± 0.5	1.1 ± 0.6	0.4 ± 0.4	0.6 ± 0.3
20:4ω6	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.3	0.3 ± 0.1	0.6 ± 0.2	0.7 ± 0.3
20:5ω3	1.0 ± 0.4	1.2 ± 0.5	1.5 ± 0.6	1.9 ± 0.8	1.9 ± 0.6	2.5 ± 0.7
22:6ω3	0.6 ± 0.3	0.7 ± 0.4	0.7 ± 0.3	1.1 ± 1.0	0.5 ± 0.3	0.7 ± 0.3
∑PUFA	**5.1 ± 2.2**	**5.2 ± 2.2**	**5.9 ± 2.1**	**7.9 ± 2.8**	**7.3 ± 2.2**	**8.9 ± 2.4**
Branched						
14:0iso	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.1	0.6 ± 0.1
15:0iso	1.0 ± 0.3	1.5 ± 0.4	1.2 ± 0.3	1.0 ± 0.3	2.0 ± 0.5	1.5 ± 0.3
15:0anteiso	0.8 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	1.2 ± 0.3	0.9 ± 0.2
16:0iso	0.5 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
17:0iso	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.9 ± 0.1
17:0anteiso	0.5 ± 0.2	0.7 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	0.9 ± 0.3	0.7 ± 0.2
∑BrFA	**3.6 ± 0.9**	**5.3 ± 1.0**	**4.4 ± 0.9**	**3.8 ± 0.8**	**6.7 ± 1.2**	**5.2 ± 0.9**
∑FA (µg mgSPM^{-1})	1.0 ± 0.5	0.3 ± 0.2	0.5 ± 0.2	0.8 ± 0.4	0.6 ± 0.3	0.7 ± 0.2
SPM (mg L^{-1})	27.2 ± 11.6	141.5 ± 83.2	65.8 ± 28.3	55.1 ± 30.0	150.9 ± 86.9	62.6 ± 27.2
% detritalFA	69.8 ± 5.0	71.1 ± 3.6	74.4 ± 3.6	72.7 ± 2.9	68.1 ± 2.8	69.1 ± 2.7
Salinity	9.7 ± 1.5	20.2 ± 2.3	22.6 ± 1.3	24.6 ± 1.5	13.9 ± 3.4	22.1 ± 2.4
n Day/Night	6/7	6/7	7/6	6/7	5/8	6/7

Σ detritalFA 15:0 + 16:0 + 16:1 + 18:0 + 18:1
n.d. = not detected

222 Table 2: Summary of ANCOVA between fatty acids (%) and environmental variables

Category		Decomposition and wastes						Autochtonous production		
Indicator		%BrFA			%detritalFA			%PUFA		
	df	SS	Fvalue	p	SS	Fvalue	p	SS	Fvalue	p
ln(SPM)	1	82.5	385.2	<0.001	8.7	1.4	0.25	0.0	0.0	0.90
Salinity	1	7.1	33.1	<0.001	90.0	14.1	<0.001	39.0	15.4	<0.001
Season	1	16.6	77.7	<0.001	147.1	23.0	<0.001	83.1	32.7	<0.001
Day/Night	1	4.8	22.3	<0.001	3.6	0.6	0.45	76.8	30.2	<0.001
Ln(SPM) × Salinity	1	0.9	4.0	<0.05	11.3	1.8	0.19	0.1	0.0	0.85
Ln(SPM) × Season	1	0.5	2.4	0.13	12.0	1.9	0.18	2.5	1.0	0.33
Ln(SPM) × D/N	1	0.2	1.1	0.31	2.1	0.3	0.57	5.7	2.3	0.14
Salinity × Season	1	0.4	1.7	0.19	36.2	5.6	<0.05	3.9	1.5	0.22
Salinity × D/N	1	0.1	0.4	0.56	11.5	1.8	0.18	1.8	0.7	0.41
Season × D/N	1	0.1	0.3	0.59	11.3	1.8	0.19	0.8	0.3	0.58
Residuals	67	14.3			429.1			170.2		
Total	77	127.4			762.9			383.8		

Σ BrFA 14:0-i + 15:0-i + 15:0-ai + 16:0-i + 17:0-i + 17:0-ai

Σ detritalFA 15:0 + 16:0 + 16:1 + 18:0 + 18:1

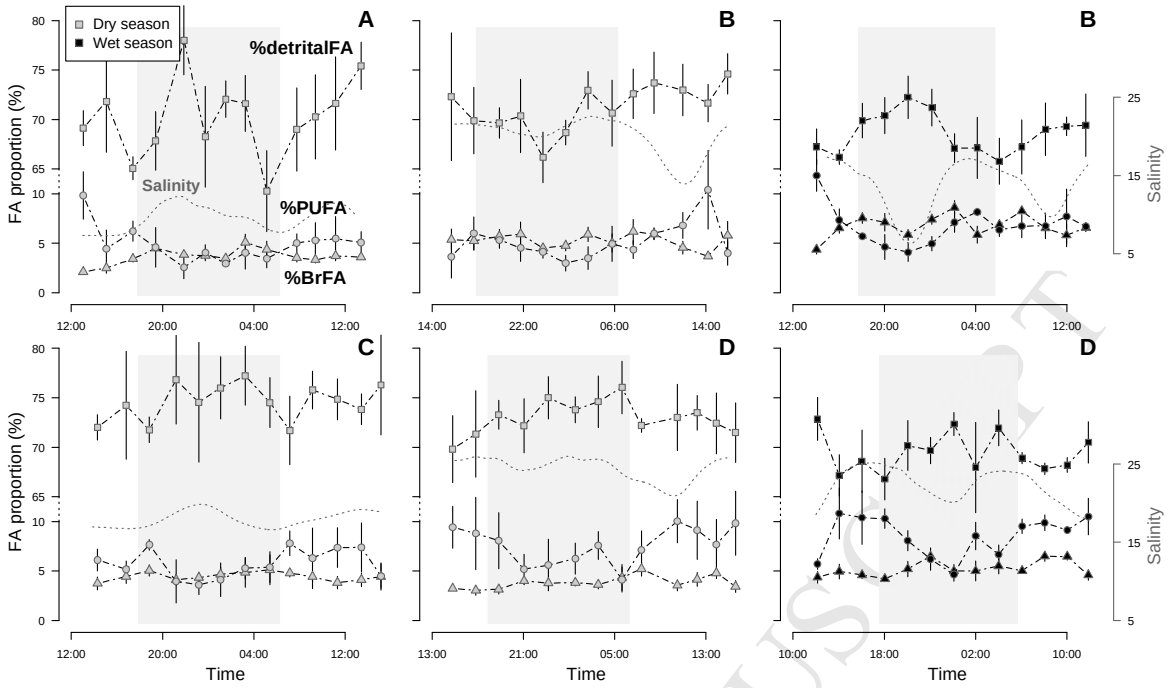
Σ PUFA 16:2ω4 + 16:3ω4 + 18:2ω6 + 18:3ω3 + 18:4ω3 + 20:5ω3 + 20:4ω6 + 22:5ω3 + 22:6ω3

223

224 Table 3: Summary of ANCOVA between stable isotopes and C/N ratio and environmental

225 variables

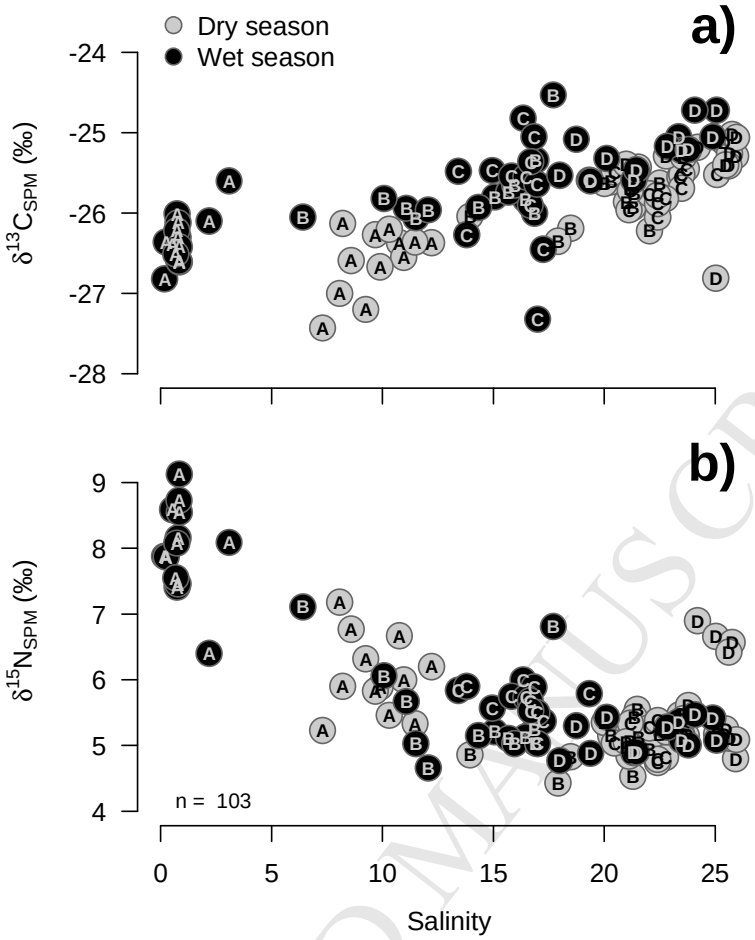
Indicator		δ^{13}C-SPM			δ^{15}N-SPM			C/N ratio		
	df	SS	F value	p	SS	F value	p	SS	F value	p
Salinity	1	13.4	95.1	<0.001	63.0	300.7	<0.001	10.4	9.9	<0.01
ln(SPM)	1	0.3	1.8	0.18	5.9	28.3	<0.001	39.6	37.7	<0.001
Season	1	4.9	35.1	<0.001	0.0	0.0	0.8	2.9	2.7	0.10
Day/Night	1	0.1	0.5	0.50	1.4	6.6	<0.05	2.3	2.2	0.14
Salinity × Ln(SPM)	1	0.0	0.0	0.84	1.0	5.0	<0.05	0.1	0.1	0.71
Salinity × Season	1	0.7	4.7	<0.05	16.2	77.2	<0.001	1.4	1.4	0.25
Salinity × D/N	1	0.4	3.0	0.09	1.1	5.2	<0.05	1.3	1.2	0.27
Ln(SPM) × Season	1	0.2	1.5	0.23	0.2	1.1	0.3	2.2	2.1	0.15
Ln(SPM) × D/N	1	0.0	0.0	0.85	2.3	11.1	<0.01	0.2	0.2	0.63
Season × D/N	1	0.3	2.3	0.14	0.5	2.5	0.1	0.4	0.4	0.55
Residuals	92	13.0			19.3			96.6		
Total	102	33.3			110.9			157.4		



226

227 Fig. 3: Dynamics of %detritalFA (squares; proportion of 15:0 + 16:0 + sum of 16:1 + 18:0 +
228 sum of 18:1), %PUFA (bubbles; proportion of polyunsaturated FA), %BrFA (triangles;
229 proportion of iso- and anteiso-branched chain FA) and salinity (dotted grey line) during the 24
230 h tidal cycles in the Can Gio mangrove estuary (Southern Vietnam). Error bars correspond to
231 standard deviation of the four filters (from different water samples) analysed at each sampling
232 event. Letters in the upper righthand corner indicate the samping site. Shaded areas
233 correspond to night time

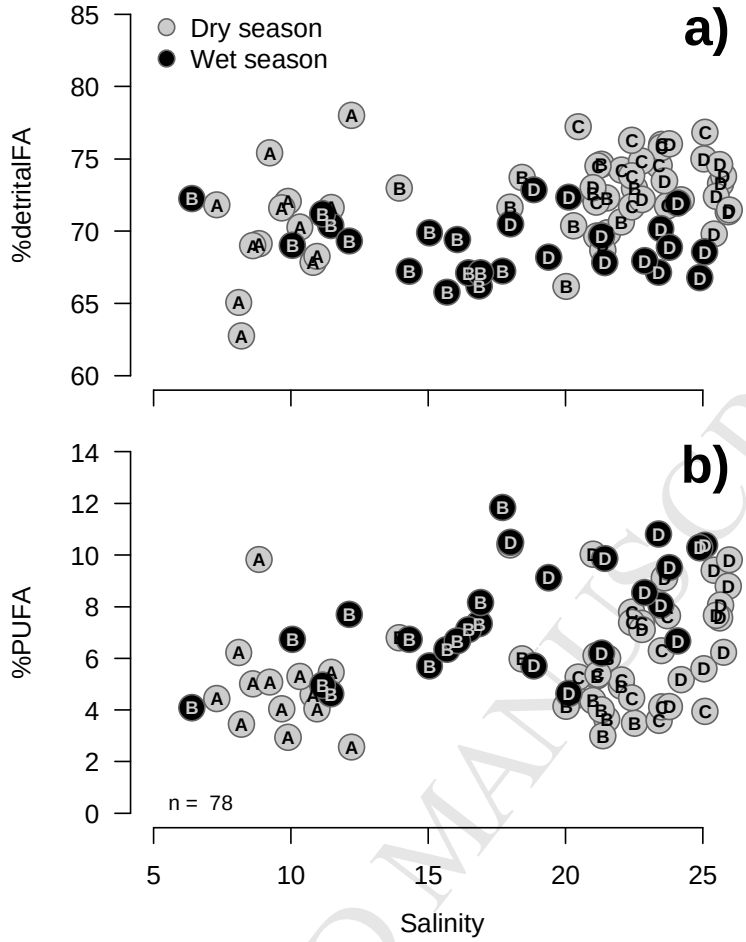
234



235

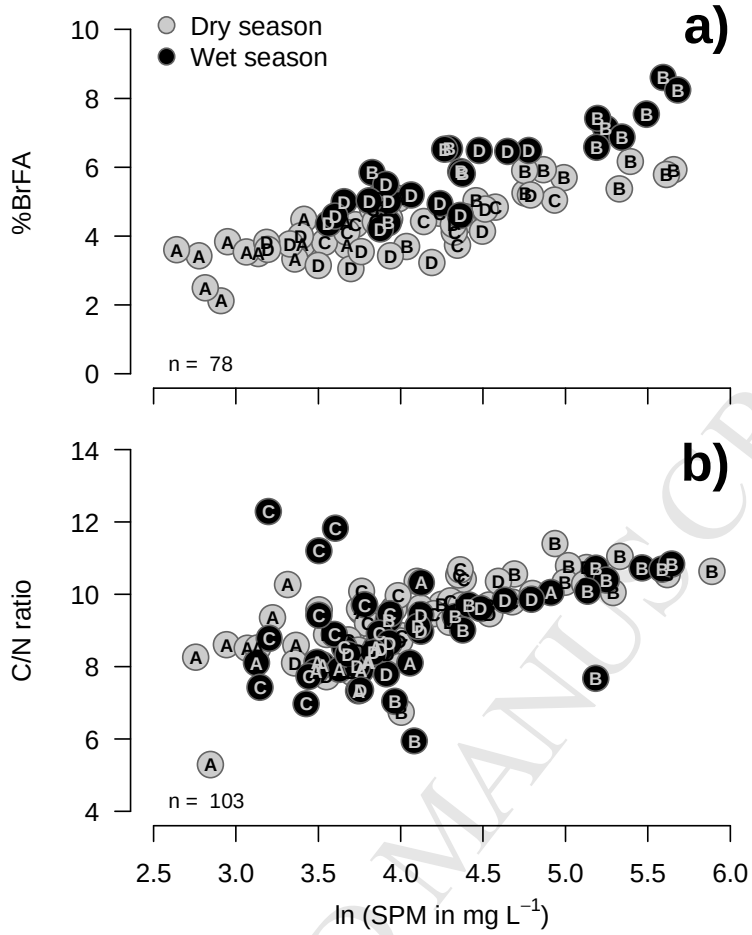
236 Fig. 4: a) $\delta^{13}C$ and b) $\delta^{15}N$ of SPM expressed as a function of salinity in the Can Gio

237 mangrove estuary. Letters in bubbles correspond to the sites where samples were collected



238

239 Fig. 4: a) %detritalFA (proportion of 15:0 + 16:0 + sum of 16:1 + 18:0 + sum of 18:1) and b)
240 %PUFA (proportion of polyunsaturated FA) in SPM expressed as a function of salinity in the
241 Can Gio mangrove estuary. Bubbles correspond to the mean value of the four filters (from
242 different water samples) analysed at each sampling event. Letters in bubbles correspond to the
243 sites where samples were collected



244

245 Fig. 6: a) %BrFA (proportion of iso- and anteiso-branched chain FA) and b) C/N ratio in SPM
246 expressed as a function of SPM concentrations in the Can Gio mangrove estuary. Bubbles
247 correspond to the mean value of the four filters (from different water samples) analysed at
248 each sampling event (for %BrFA only). Letters in bubbles correspond to the sites where
249 samples were collected

250

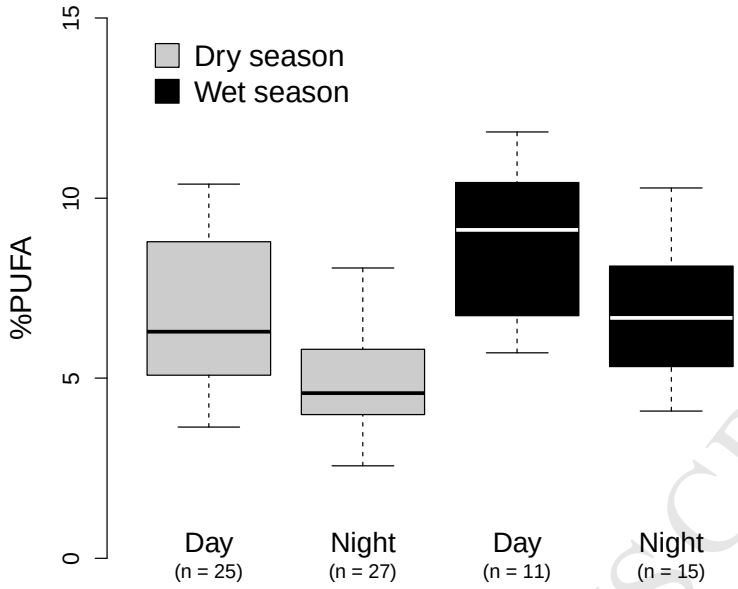


Fig. 7: Boxplot of %PUFA (proportion of polyunsaturated FA) in SPM separated according to season and presence of daylight in the Can Gio mangrove estuary

4. Discussion

4.1 Overall composition of SPM

Suspended particulate matter in the Can Gio mangrove estuary had a similar proportion of detritalFA to other anthropogenically impacted sub-tropical and temperate mangrove estuaries of Japan and New Zealand and tropical systems of Brazil (Table 4), where OM was mostly of detrital origin (Alfaro et al. 2006, Sakdullah and Tsuchiya 2008, Mortillaro et al. 2011, Boëchat et al. 2014). The detritalFA biomarker as stated in this study indicates an advanced decomposition state of OM, which is a common feature in estuarine mangrove areas (Kristensen et al. 2008). The range of BrFA proportions in SPM is relatively large whatever the study area and does not cluster any kind of ecosystem (Table 4). The PUFA proportions in SPM were low (2.6 to 11.8%) but still in the range observed in other large tropical rivers such

as the Brasilian Rio das Mortes, where Boëchat et al. (2014) concluded that algae and other autochthonous autotrophic organisms contributed poorly to the overall SPM pool. Considering the high water turbulence and turbidity of the estuary, with current velocity up to 1.7 m/s and estimated light penetration of less than 1 m (David et al. 2018b), such results were expected. The FA composition of SPM in this large estuary was also relatively similar to that of our previous study in a mangrove creek (David et al. 2018a), except PUFA proportion that was much higher once during the tidal cycle in David et al. (2018a) and attributed to a pulse of phytoplankton that grew within the mangrove channels. The Can Gio mangrove estuary thus conveys highly decomposed SPM to the South China sea coast, exhibiting varying levels of bacterial indicators and a small proportion of phytoplanktonic biomarkers.

Table 4: Overview of 21 literature data on SPM fatty acid composition (%) in aquatic ecosystems

Location	Ecosystem	% detritalFA	% BrFA	% PUFA	n	Reference
Dong Nai River estuary, Vietnam	Mangrove estuary	62.9 - 78.0	2.1 - 8.6	2.6 - 11.8	6	This study
Can Gio mangrove, Vietnam	Mangrove creek	64.1 - 78.4	3.6 - 7.1	5.9 - 21.8	14	David et al. 2018a
Manko River estuary, Japan	Mangrove estuary	64.4 - 74.0		6.9 - 15.8	4	Sakdullah and Tsuchiya 2009
Manko River estuary, Japan	Mangrove estuary	47.9 - 87.7	0.5 - 4.9	14.1 - 47.7	12	Shilla et al. 2011
Matapouri Estuary, New Zealand	Mangrove estuary	56.4 - 84.3		5.4 - 10.4	3	Alfaro et al. 2006
Amazon River, Brasil	Tropical river	60.3 - 67.4	3.7 - 5.4	11.5 - 19.1	6	Mortillaro et al. 2011
Rio das Mortes, Brasil	Tropical river	56.8 - 65.0	4.0 - 6.7	4.3 - 6.6	4	Boëchat et al. 2014
Camaleão Lake, Brasil	Tropical lake	61.1	10.4	8.6	1	Mortillaro et al. 2016
Gulf of Mexico, Mexico	Tropical shelf	22.2 88.0	0.0 - 10.0	1.6 - 27.6	18	Carréon-Palau et al. 2017
Florida Bay, Florida (USA)	Subtropical shelf		0.6 - 3.9	0.2 - 35.0	18	Xu and Jaffé 2007
Chesapeake Bay, Virginia (USA)	Temperate estuary	34.3 - 80.1	0.0 - 13.1	4.1 - 57.1	42	Canuel 2001
Kowie River estuary, South Africa	Temperate estuary	52.3 - 70.7	3.0 - 9.6	6.0 - 20.3	12	Antonio and Richoux 2016
Krka River, Italy	Temperate estuary	60.2 - 70.6	1.6 - 8.2	5.6 - 31.8	13	Scribe et al. 1991
Morlaix River estuary, France	Temperate estuary	55.6 - 73.6	3.6 - 6.6	7.1 - 25.2	13	Quéméneur and Marty 1994
San Franciso Bay, California (USA)	Temperate estuary	21.5 - 59.9	0.7 - 7.6	9.6 - 55.4	30	Canuel 2001
York River estuary, Virginia (USA)	Temperate estuary	48.7 - 62.1		18.6 - 32.4	6	McCallister et al. 2006
Biscay Bay, France	Temperate shelf	45.3 - 75.6	1.3 - 5.2	5.2 - 37.4	8	Chouvelon et al. 2015
Chausey Archipelago, France	Temperate shelf	64.6 - 76.9	2.8 - 3.8	7.5 - 19.3	6	Moynihan et al. 2016
Elkhorn Slough, California (USA)	Temperate shelf	28.5 - 40.7	3.0 - 6.1	15.7 - 23.6	3	Fischer et al. 2014
Yangtze River estuary, China	Temperate shelf	36.9 - 66.0		9.3 - 53.9	2	Wang et al. 2015
Pilley's Tickle Bay, Canada	Subarctic shelf		2.4 - 5.2	32.0 - 54.0	4	Budge et al. 2001
Trinity Bay, Canada	Subarctic shelf	28.4 - 35.7	0.5 - 0.9	29.4 - 54.8	4	Budge and Parrish 1998
Beaufort Sea, Alaska (USA)	Arctic shelf	34.9 - 81.9	0.9 - 3.6	8.5 - 42.3	12	Connelly et al. 2015

Σ BrFA 14:0-i + 15:0-i + 15:0-ai + 16:0-i + 17:0-i + 17:0-ai
Σ detritalFA 15:0 + 16:0 + 16:1 + 18:0 + 18:1
Σ PUFA 16:2ω4 + 16:3ω4 + 18:2ω6 + 18:3ω3 + 18:4ω3 + 20:5ω3 + 20:4ω6 + 22:5ω3 + 22:6ω3

279 *4.3 Changes in the SPM composition due to the water mixing ratio*

280 We used salinity as a conservative tracer to identify the mixing ratio of fresh vs.
281 marine waters in the estuary. The increase of $\delta^{13}C$ values along the increasing salinity
282 gradient (Fig. 4) indicates a shift from OM of terrestrial origin to OM of marine origin
283 (Middelburg and Herman 2007). This observation is strengthened by the %PUFA increase
284 correlated to the increasing salinity gradient (Fig. 5b), suggesting that the contribution of
285 freshly produced vs. decomposing OM is higher in marine waters compared to the upper
286 watershed. Actually, PUFA are rapidly degraded in decaying material and the PUFA
287 proportion can be used as an indicator of OM freshness (Budge et al. 2001). Dominating
288 PUFA affected by the salinity gradient were 20:5ω3, 16:2ω4 and 16:3ω4 (Table 1) that are
289 diatom indicators in marine ecosystems (Dalsgaard et al. 2003).

290 The $\delta^{15}N$ values of suspended particles in estuaries result from the balance between
291 external inputs of ammonium vs. nitrate and heterotrophic processing of OM (Middelburg and
292 Herman 2007). In addition, the isotopic value of $\delta^{15}N$ in estuarine particles and organisms can
293 provide an integrated N-loading measurement (McClelland and Valelia 1998). In the present
294 study, the $\delta^{15}N$ decreased along the increasing salinity gradient (Fig. 4b). This decrease most
295 probably reflected a high nutrient loading due to anthropogenic activity near Ho Chi Minh
296 City, and its dilution with nitrogen-poor marine waters when proximity to the sea increased
297 (Nguyen et al. 2019).

298 The above paragraphs illustrated the mixing of freshwater SPM with marine SPM
299 along the increasing salinity gradient. However, the variability that could be explained by the
300 mixing ratio of fresh vs. marine waters, approximated by salinity, actually remained low
301 compared to the total variability exhibited by the dataset, especially for FA biomarkers and
302 C/N ratio (SS salinity / Total; Tables 2 and 3). In addition, although the time-based plots
303 illustrated the rapid changes of measured parameters during tidal cycles (Fig. 2 and 3), they

304 did not show a clear trend related to the tidal stage. We thus suggest that other rapidly
305 changing factors, such as tide-induced resuspension or daylight, may contribute to short-term
306 (few hours) changes in the SPM composition and we partitioned the effect of each of these
307 factors using sequential ANCOVA (Tables 2 and 3).

308

309 *4.4 Short-term changes in the SPM composition*

310 In the present study, we assumed that during the 24 h time series upstream inputs of
311 SPM were roughly stable and that short-term increases in SPM concentrations were mostly
312 due to particle resuspension, as generally observed in turbid estuaries (Wang et al. 2013).
313 Increasing SPM concentrations were correlated to increasing %BrFA in SPM (Fig. 6a),
314 indicating that bacterial contribution to the overall pool of OM was higher in resuspended
315 particles (with short settling-time) compared to longer settling-time particles. In a tidally
316 flushed mangrove creek, high proportions of BrFA in SPM (up to 7.1%; David et al. 2018a)
317 revealed sediment erosion. Although inputs from the mangrove forest may enrich the OM
318 pool in the estuary, the tracers we employed in this study could not partition the OM
319 originating from the mangrove ecosystem from that of the higher watershed. This
320 differenciation would have been more appropriately done using triterpenoids such as taraxerol
321 (He et al. 2014). Nevertheless, we concluded from a previous study that OM originating from
322 mangrove detritus was poorly flushed during ebb in a mangrove creek (David et al. 2018a).
323 We thus considered SPM as a whole and focused on its short-term quality changes within the
324 estuary. We suggest that resuspended particles are the site of an intense heterotrophic activity,
325 due to their elevated %BrFA, and that their resuspension and aggregation with others, due to
326 flocculation (Eisma 1986, Verney et al. 2009), enhance the processing of OM in the estuary.

327 The C/N ratio increase with increasing SPM concentrations suggests that the
328 contribution of higher plant organic fragments increased with particle resuspension (Fig. 6b).

Plant-derived OM has a C/N ratio much higher than bacterial cells (>20 vs. 4-5; Middelburg and Herman 2007) and one may expect increasing proportions of bacterial biomarkers (e. g. %BrFA in resuspended particles) to be associated with lower C/N ratio. However, FA are minor constitutens of SPM (<1%; Table 1) and although bacterial abundance most probably increased in resuspended particles, their influence on the C/N ratio remained negligible. Resuspended particles are thus dominated by detrital OM but they might bare an intense mineralisation activity due to their high bacterial load.

The day/night (D/N) factor had the second most explanatory power for the %PUFA variable, just after season (Table 2), with higher values measured during daytime illustrating autotrophic production in surface waters during the day (Fig. 7). Considering the high water turbulence and turbidity of the estuary (see David et al. 2018b for more details), such results were surprising. In turbid estuaries, light is often the limiting factor for autotrophic production. Phytoplankton spends most of the time in darkness and photosynthesis only occurs in short intermittent periods when phytoplankton is transported in the euphotic zone (Lancelot and Muylaert 2011). In experimental conditions under diel light cycle, lipid content of the species *Dunaliella bioculata* was twice as high in the presence of light compared to dark (Halsey and Jones 2015). We thus suggest that despite most probable low primary production, because of high turbidity and as revealed by the small contribution of PUFA in the Can Gio mangrove estuary, phytoplankton diel lipid storage notably modified the quality of SPM in surface water during the day. Such day/night variations explained almost as much variability of %PUFA in our dataset than season (Table 2).

4.5 Seasonal variability in the SPM composition

The changing of seasons was the most explanatory variable for %PUFA and %detritalFA in SPM. The higher %PUFA measured during the wet season indicates that OM

354 exhibited higher freshness during this season (Tables 1 and 2). This higher freshness was also
355 highlighted by the lower detritalFA contribution to the overall FA, the higher BrFA
356 contribution and the higher $\delta^{13}C$ values during the wet season (Tables 1, 2 and 3 and Fig. 2
357 and 4). This latter indicator also shows that at equivalent salinity the contribution of OM of
358 terrestrial origin was lower during the wet season compared to OM of estuarine or marine
359 origin which are enriched in ^{13}C (Middelburg and Herman 2007). Such results were
360 unexpected since wet season in tropical ecosystems is generally associated with higher soil
361 leaching and higher inputs of terrigenous OM to estuaries (Mortillaro et al. 2011, Boëchat et
362 al. 2014), which are assumed to be in a more advanced state of decomposition than
363 autochthonously produced OM. In our study area, the watershed receives 90% of its annual
364 precipitation during the wet season and the river discharge may be up to 30 times higher (in
365 August) than lower values (in March) measured during the dry season (Nippon Koie 1996).
366 The intrusion of OM of marine origin in the estuary is thus necessarily lower during the wet
367 season. Higher inputs from the watershed may nevertheless increase bacterial activity and
368 bacterial loads in the estuary during the monsoon season, as observed in the Okinawan Manko
369 estuary (Shilla et al. 2011). We suggest that the higher bacterial load increases the
370 mineralisation rate of OM, and thus the availability of nutrients, resulting in higher
371 autotrophic production in the Can Gio mangrove estuary during the wet season.

372

373 **5. Conclusions**

374 Our study reveals that SPM varies across short time scale in the Can Gio mangrove
375 estuary, essentially due to tide-induced particle resuspension and changes in the physiological
376 state of phytoplankton. To date, most studies dealing with SPM in estuaries emphasized
377 spatial and/or seasonal variability (e. g. Canuel 2001, Alfaro et al. 2006, Shilla et al. 2011).
378 We believe that efforts should be made in the future to include the daily variability of SPM in

379 both food web models and nutrients budgets. Actually, we showed in this study that PUFA
380 proportion, an indicator of freshness itself revealing the nutritional quality of SPM, was
381 almost as much affected by the presence of daylight than by season, while spatial variability
382 was the second most influencial factor. These results are in accordance with a previous study
383 in the temperate Urdaibai estuary in Spain (Madariaga 2002). Such findings may help
384 ecologists understanding seasonal and diurnal migration of estuarine organisms, along with
385 answering how their nutritional requirements are fulfilled (e. g. Riera et al. 2000). Similarly,
386 we showed that tide-induced resuspension was the dominant variable affecting C/N ratio of
387 SPM, which may have implications on nutrient budgets stoichiometry. In addition,
388 resuspension may influence particle retention time in the estuary, which will affect
389 decomposition and atmospheric releases of greenhouse gases (e. g. CO_2, N_2O) along with
390 making their quantification more difficult.

391 Fatty acid profiling represents a high workload (e. g. compared to multi-parameters
392 probe measurements or spectrophotometric determination of chlorophyll a) and one could ask
393 whether the same conclusions could have been reached using stable isotopes supplemented
394 with chlorophyll a data. Our study shows that FA are more sensitive to daily changes in SPM
395 composition than stable isotopes, and since we suggest that increasing PUFA relative
396 abundance in surface water during the day originates from lipid storage rather than cell
397 division, chlorophyll a data would probably not highlight such phenomenon. We thus believe
398 that FA represent an effective tool to evaluate factors affecting SPM quality in estuaries at
399 short-term scales.

400

401

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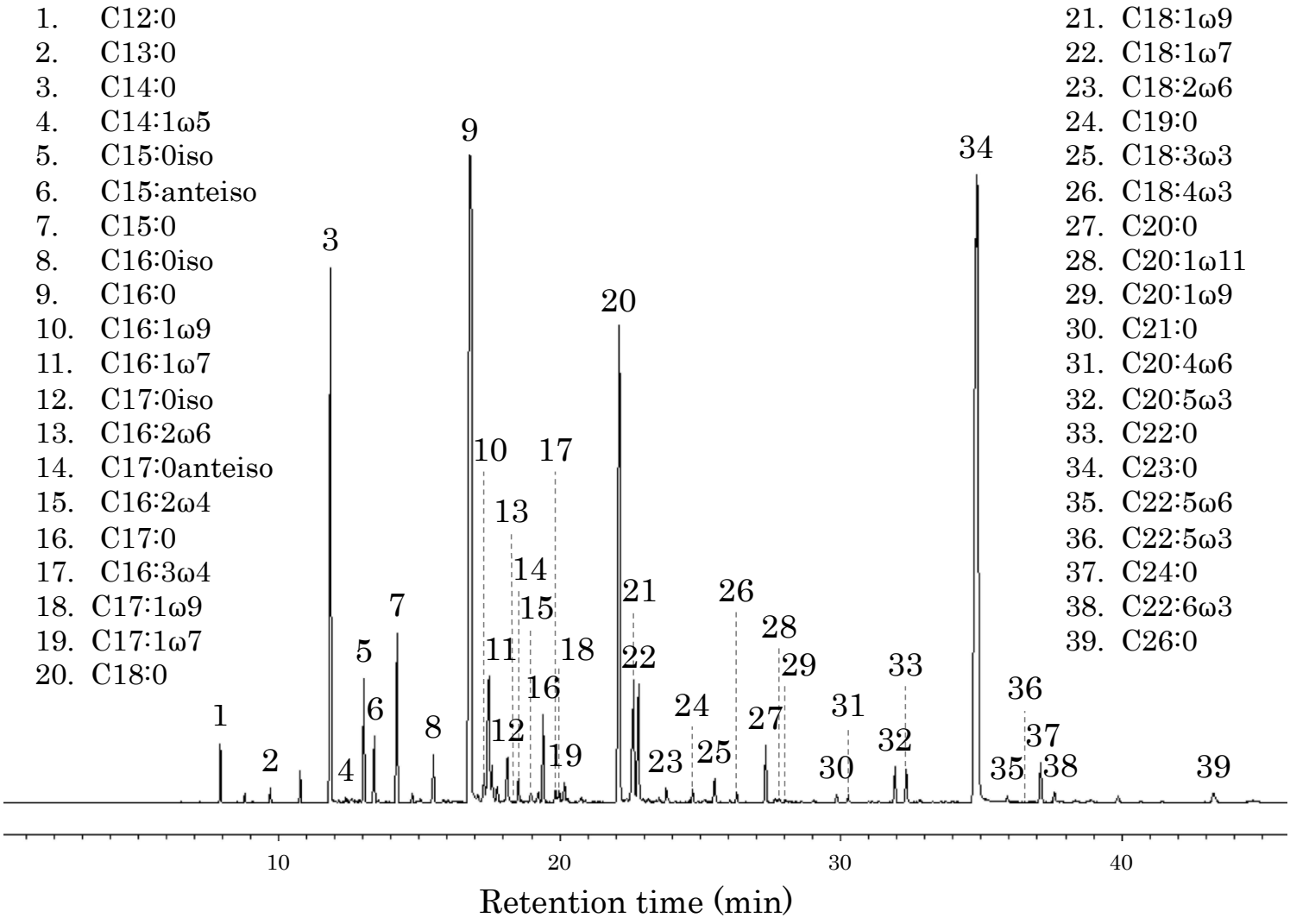
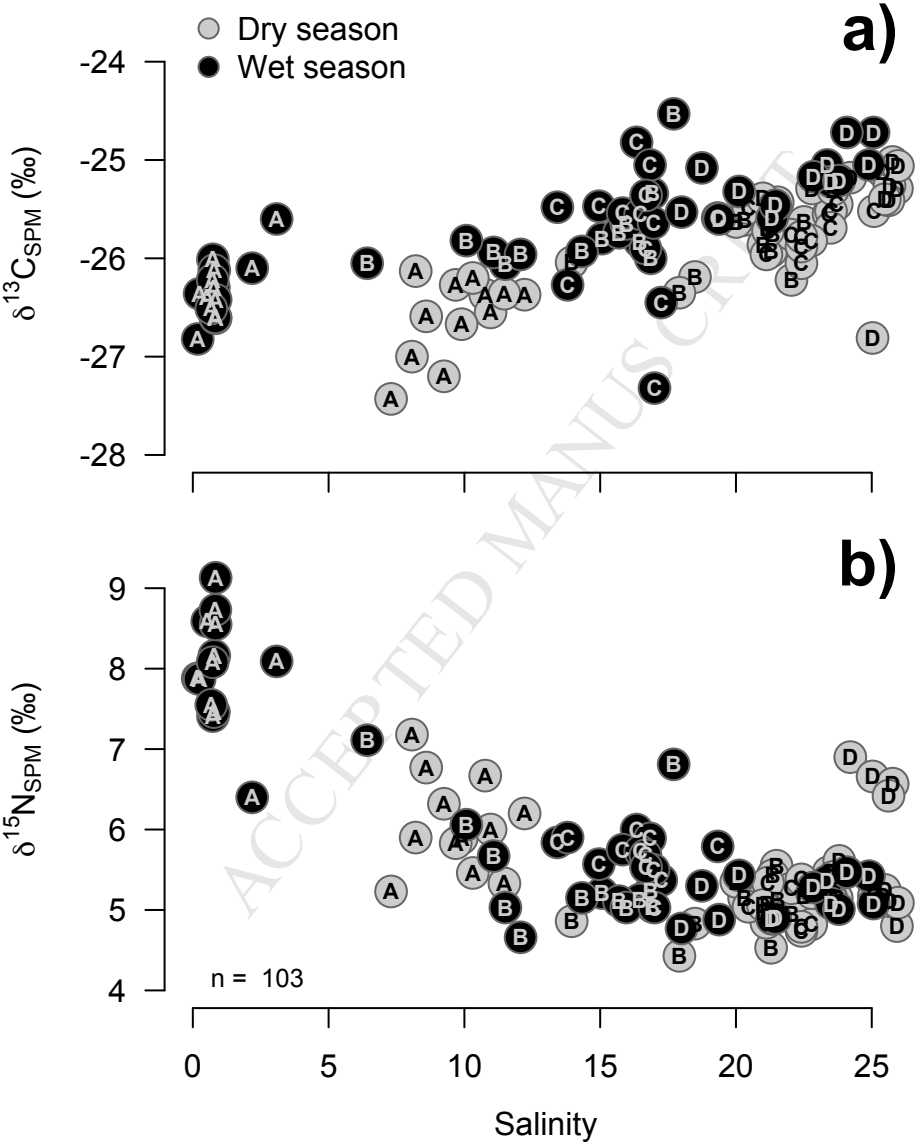
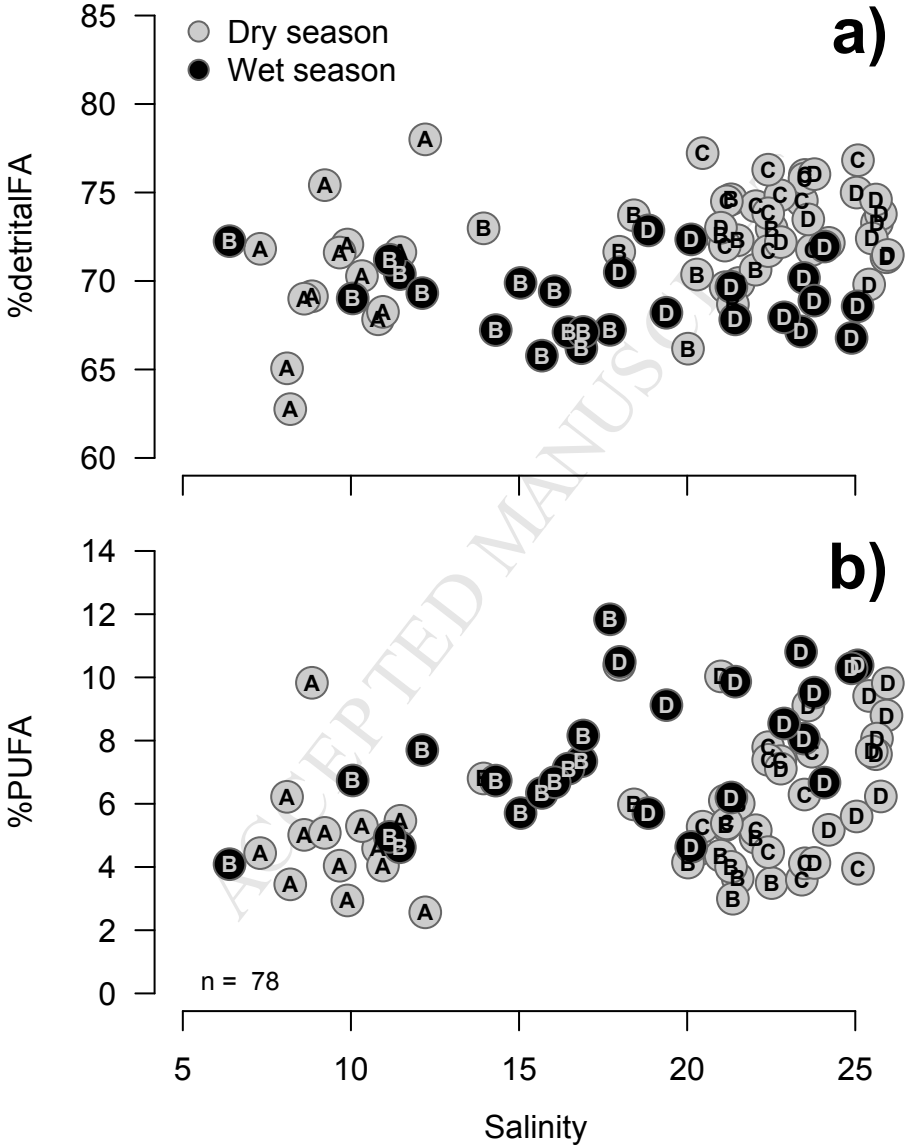


Appendix 1: Example of GC/MS ion chromatogram (m/z = 74) of fatty acids detected in suspended particulate matter of the Can Gio mangrove estuary.





Highlights

- We highlight suspended particulate matter daily variability in a tropical estuary
- Tide-resuspended particles are rich in bacterial fatty acid biomarkers
- Phytoplankton stores lipid during daytime up to substantially modifying SPM quality
- Higher freshness of SPM was measured during the wet season