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

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Salinity



- $\delta^{13}\text{C}$
- $\delta^{15}\text{N}$



Suspended matter



Branched

- FA
- C/N ratio



24 h x 8

Polyunsaturated

- FA

- Detrital FA

Polyunsaturated

- FA



Daylight



Season



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

3
4 Running head: Short-term changes in SPM quality in the Can Gio mangrove estuary.

5
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20 **Abstract**

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

37
38 Keywords: fatty acids, stable isotopes, bacteria, phytoplankton, resuspension, lipid
39 storage, South East Asia.

40 1. Introduction

41 Suspended particulate matter (SPM) plays an important role in tropical estuaries, both
42 as a component of coastal food webs (Riera et al. 2000, Meziane and Tsuchiya 2002, Alfaro et
43 al. 2006) and as a key variable of nutrient budgets (Kristensen et al. 2008, Cai 2011, Hofmann
44 et al. 2011). Lipids and especially fatty acids (FA) are used as environmental tracers to
45 discriminate sources of organic matter (OM) in aquatic ecosystems (Bodineau et al. 1998,
46 Mortillaro et al. 2011, Antonio and Richoux 2015). These organic compounds are synthesised
47 in specific proportions by living organisms, possibly revealing the contribution of several taxa
48 to bulk OM (Dalsgaard et al. 2003, Bergé and Barnathan 2005). Nevertheless, very few FA
49 are exclusive to any kind of OM. Such biomarkers should preferentially be coupled with other
50 tracers, such as isotopic and elemental compositions of SPM, that are also able to discriminate
51 OM sources and identify biogeochemical processes in estuaries (Middelburg and Herman
52 2007, Mortillaro et al. 2011, 2016, Bergamino et al. 2014).

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

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

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

80

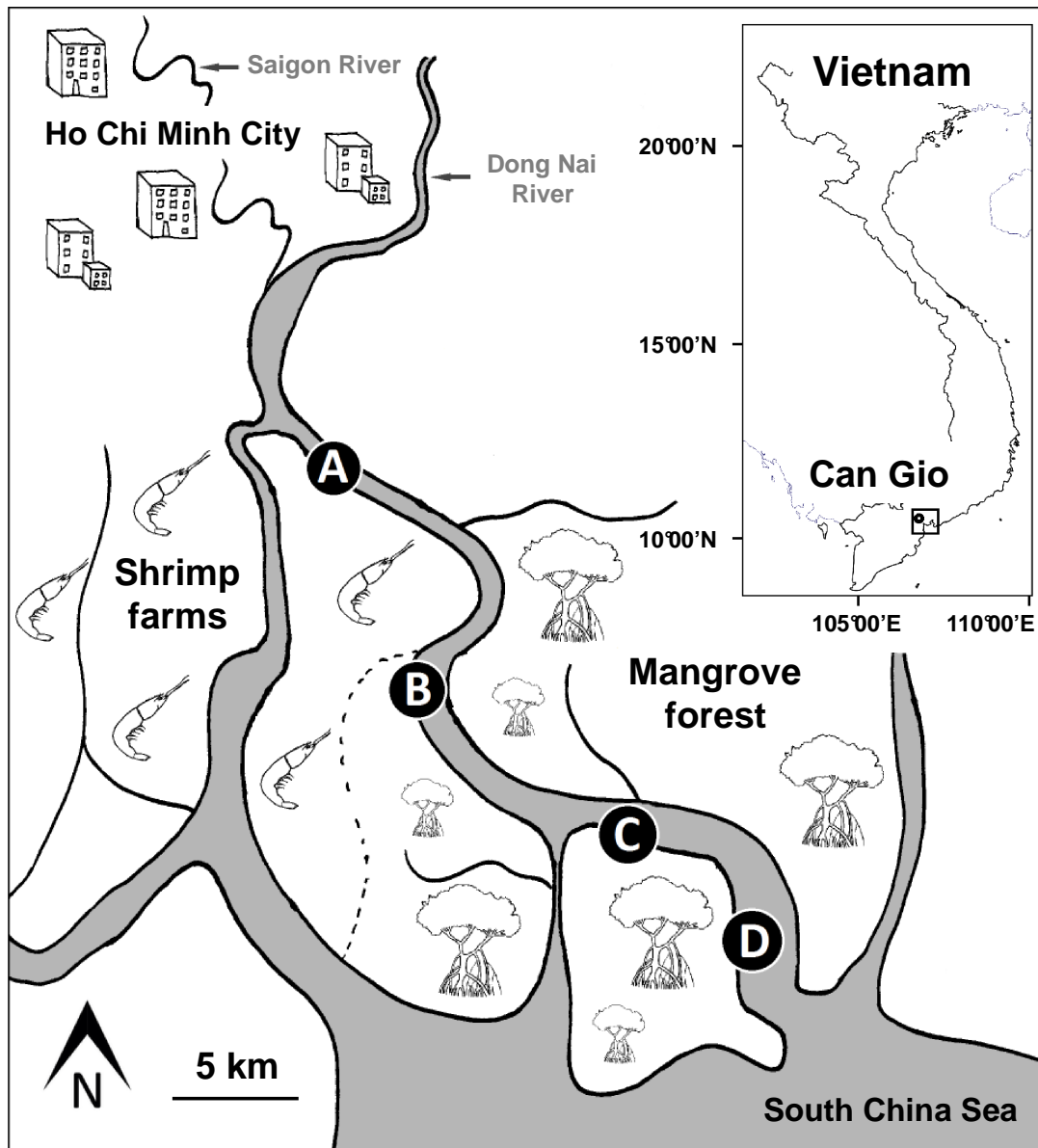
81 **2. Materials and methods**

82 *2.1 Study area*

83 The Can Gio mangrove is located at the downstream end of Ho Chi Minh City (~13
84 million inhabitants) and flooded by the Saigon-Dong Nai Rivers, discharging annually $37.4 \times$
85 10^6 m^3 of freshwater to the South China Sea and whose basin covers a total catchment area of
86 $40.6 \times 10^3 \text{ km}^2$ (12% of the total terrestrial area of Vietnam; Ringler et al. 2002). The climate
87 in Can Gio is monsoonal with a wet season from June to October and a dry season from
88 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 km² 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

105

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

106 2.2 Sampling strategy

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

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

123 2.3 Sample processing

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

130 We extracted lipids following a slightly modified protocol of Bligh and Dyer (1959),
131 as described in Meziane et al. (2007). Tricosanoic acid (23:0) was used as an internal standard
132 and 5 μg of methyl tricosanoate provided by Sigma-Aldrich[®] was added to every sample prior
133 to extraction. Lipids were extracted with 4 mL of a water:methanol:chloroform mixture
134 (1:2:1, v:v:v) enhanced by two 20 min steps of sonication. Chloroform and water was added
135 to the mixture to reach equal proportions of the three solvents and allow the formation of a
136 aqueous-organic bilayer system. The lipid fraction, contained in the chloroform, was
137 retrieved after phases were separated by centrifugation (3000 rpm, 1400 rcf, 5 min), and
138 evaporated under nitrogen (N_2) flux. Dried lipid extracts were saponified using a
139 methanol:sodium hydroxide (2N) mixture (2:1, v:v) during 1 h 30 min at 90°C. Fatty acid
140 esters were then methylated into fatty acid methyl esters (FAME) using boron trifluoride-
141 methanol ($\text{BF}_3\text{-CH}_3\text{OH}$) and stored at -25°C. FAME were quantified by gas chromatography
142 analysis (Varian 3800-GC), using a flame ionisation detector. The oven temperature was set at
143 60°C and held for 1 min, raised at 40°C min^{-1} to 150°C and held for 3 min, and then increased
144 to 240°C at 3°C min^{-1} and held for 25 min. We identified fatty acids using coupled gas
145 chromatography mass spectrometry (Varian 450-GC; Varian 220-MS) and comparison of GC
146 retention times with commercial standards (Supelco[®] 37 component FAME mix). An example
147 of GC/MS chromatogram has been provided in Appendix 1. We reported the values as % of
148 total FA or absolute concentrations ($\mu\text{g L}^{-1}$ or $\mu\text{g mg SPM}^{-1}$).

149 We analysed stable isotope ratios at the University of California Davis Stable Isotope
150 Facility (Department of Plant Sciences, UC Davis, Davis, California) using a Vario EL Cube
151 elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a
152 PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.). Samples
153 were prepared in tin capsules by scraping the SPM clogging the filters and after 4 h of HCl
154 37% fumigation to remove all carbonates. Then, UC Davis performed the analysis. Carbon

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

159

160 *2.4 Data analyses*

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

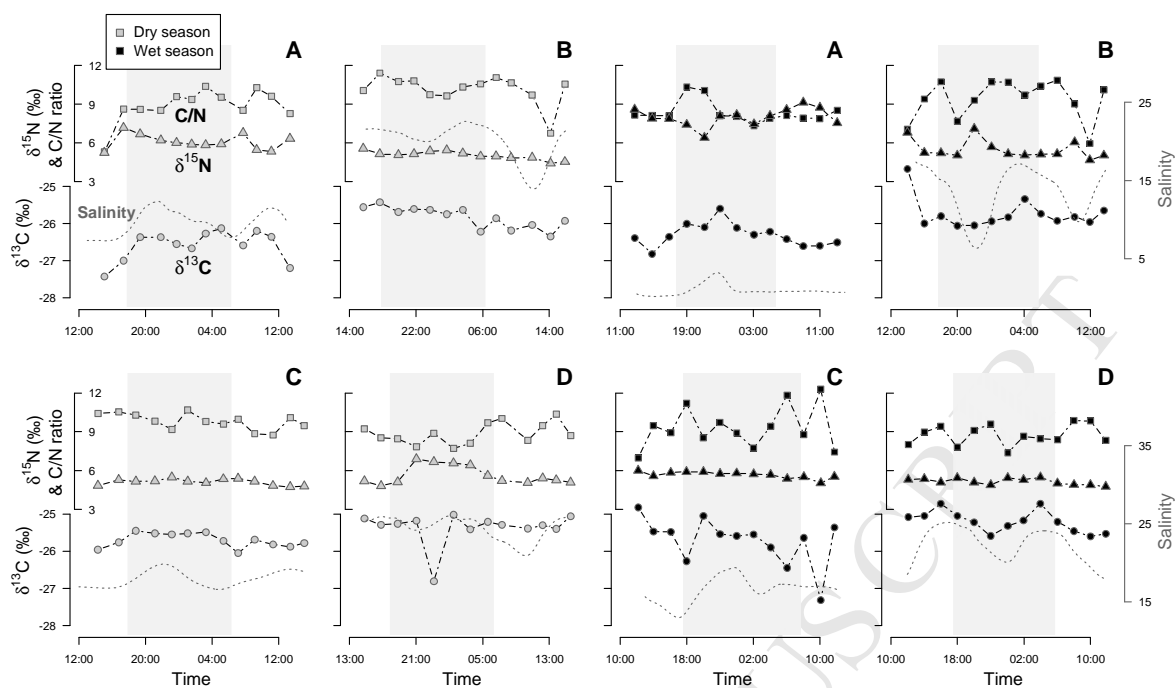
171 We challenged the effect of SPM concentration, salinity (taken as a proxy of fresh vs.
172 marine waters mixing ratio), daylight presence and season on FA biomarkers, stable isotopes
173 and C/N ratios using sequential analysis of covariance (Type I ANCOVA). Suspended
174 particulate dry mass and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

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

188

189 3. Results

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



197

198 Fig. 2: Dynamics of C/N ratio (squares), $\delta^{15}\text{N}$ (triangles), $\delta^{13}\text{C}$ (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 sampling 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) 18:1 ω 9, 16:1 ω 7, 18:1 ω 7 and 22:1 ω 9. 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, while values of %PUFA
 208 were higher during the wet season (Table 1 and Fig. 3). The %BrFA and $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, with $\delta^{13}\text{C}$ increasing with salinity and $\delta^{15}\text{N}$ 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 (\pm 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)
<i>Saturated</i>						
12:0	2.0 \pm 1.8	2.0 \pm 1.5	2.0 \pm 1.7	1.7 \pm 1.4	2.6 \pm 1.5	2.5 \pm 1.4
13:0	0.2 \pm 0.2	0.3 \pm 0.2	0.3 \pm 0.2	0.2 \pm 0.1	0.7 \pm 0.3	0.6 \pm 0.3
14:0	6.0 \pm 1.6	5.6 \pm 1.5	6.4 \pm 1.8	7.1 \pm 2.0	8.7 \pm 1.5	9.0 \pm 1.0
15:0	2.0 \pm 0.7	2.2 \pm 0.7	2.2 \pm 0.9	2.7 \pm 0.9	2.8 \pm 0.4	2.4 \pm 0.4
16:0	32.6 \pm 5.3	28.9 \pm 4.3	33.3 \pm 4.7	34.8 \pm 4.2	28.4 \pm 2.2	30.0 \pm 2.1
17:0	1.0 \pm 0.2	1.3 \pm 0.3	1.2 \pm 0.2	1.2 \pm 0.3	1.4 \pm 0.2	1.3 \pm 0.3
18:0	16.3 \pm 4.8	16.5 \pm 4.3	16.8 \pm 5.5	13.2 \pm 4.0	7.8 \pm 1.3	7.8 \pm 1.7
19:0	0.3 \pm 0.3	0.5 \pm 0.3	0.4 \pm 0.3	0.3 \pm 0.2	0.6 \pm 0.2	0.5 \pm 0.2
20:0	0.7 \pm 0.2	0.9 \pm 0.2	0.7 \pm 0.2	0.7 \pm 0.2	0.5 \pm 0.1	0.7 \pm 0.4
21:0	0.2 \pm 0.1	0.3 \pm 0.2	0.2 \pm 0.1	0.2 \pm 0.1	0.1 \pm 0.1	0.0 \pm 0.0
22:0	0.7 \pm 0.2	0.8 \pm 0.3	0.6 \pm 0.2	0.6 \pm 0.2	0.5 \pm 0.1	0.3 \pm 0.1
24:0	0.9 \pm 0.5	1.1 \pm 0.6	0.7 \pm 0.5	0.8 \pm 0.6	0.2 \pm 0.2	0.1 \pm 0.1
26:0	0.3 \pm 0.2	0.5 \pm 0.3	0.4 \pm 0.2	0.4 \pm 0.2	n.d.	n.d.
ΣSFA	62.9 \pm 9.3	60.3 \pm 8.3	64.7 \pm 8.4	63.4 \pm 7.5	54.3 \pm 2.9	55.3 \pm 3.7
<i>Monounsaturated</i>						
14:1 ω 5	0.2 \pm 0.2	0.2 \pm 0.2	0.2 \pm 0.3	0.3 \pm 0.2	0.4 \pm 0.1	0.3 \pm 0.1
16:1 ω 9	2.1 \pm 1.9	2.2 \pm 2.0	2.1 \pm 2.1	3.0 \pm 1.8	2.2 \pm 0.9	1.5 \pm 0.5
16:1 ω 7	4.3 \pm 1.7	5.2 \pm 1.5	5.2 \pm 1.9	5.0 \pm 1.7	8.5 \pm 1.0	10.3 \pm 1.5
17:1 ω 9	0.4 \pm 0.4	0.7 \pm 0.4	0.4 \pm 0.4	0.6 \pm 0.4	0.3 \pm 0.2	0.2 \pm 0.1
17:1 ω 7	0.4 \pm 0.2	0.6 \pm 0.1	0.6 \pm 0.2	0.4 \pm 0.2	0.7 \pm 0.1	0.9 \pm 0.2
18:1 ω 9	10.3 \pm 3.5	12.5 \pm 4.2	11.7 \pm 3.9	11.5 \pm 3.9	12.7 \pm 2.3	10.7 \pm 1.6
18:1 ω 7	2.5 \pm 0.8	3.9 \pm 1.1	3.3 \pm 1.0	2.6 \pm 0.7	5.7 \pm 1.0	6.4 \pm 1.0
20:1 ω 11	0.3 \pm 0.1	0.3 \pm 0.1	0.4 \pm 0.2	0.3 \pm 0.1	0.2 \pm 0.1	0.1 \pm 0.1
20:1 ω 9	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1
22:1 ω 9	7.9 \pm 4.8	3.5 \pm 3.1	1.1 \pm 0.9	1.0 \pm 1.4	0.9 \pm 0.6	0.2 \pm 0.3
ΣMUFA	28.4 \pm 8.4	29.2 \pm 7.7	25.0 \pm 6.9	24.9 \pm 6.2	31.6 \pm 3.0	30.7 \pm 2.8
<i>Polyunsaturated</i>						
16:2 ω 6	n.d.	n.d.	n.d.	n.d.	0.3 \pm 0.1	0.2 \pm 0.1
16:2 ω 4	0.2 \pm 0.1	0.3 \pm 0.2	0.4 \pm 0.2	0.5 \pm 0.2	0.6 \pm 0.2	0.8 \pm 0.4
16:3 ω 4	0.2 \pm 0.1	0.4 \pm 0.2	0.5 \pm 0.2	0.6 \pm 0.3	0.8 \pm 0.4	1.1 \pm 0.5
16:4 ω 3	0.2 \pm 0.1	0.2 \pm 0.2	0.2 \pm 0.2	0.3 \pm 0.1	0.4 \pm 0.1	0.3 \pm 0.1
18:2 ω 6	1.3 \pm 0.5	1.3 \pm 0.5	1.3 \pm 0.5	1.4 \pm 0.6	1.3 \pm 0.4	1.3 \pm 0.3
18:3 ω 3	0.8 \pm 0.7	0.4 \pm 0.5	0.5 \pm 0.4	0.8 \pm 0.4	0.5 \pm 0.5	0.7 \pm 0.3
18:4 ω 3	0.6 \pm 0.6	0.4 \pm 0.5	0.6 \pm 0.5	1.1 \pm 0.6	0.4 \pm 0.4	0.6 \pm 0.3
20:4 ω 6	0.2 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.3	0.3 \pm 0.1	0.6 \pm 0.2	0.7 \pm 0.3
20:5 ω 3	1.0 \pm 0.4	1.2 \pm 0.5	1.5 \pm 0.6	1.9 \pm 0.8	1.9 \pm 0.6	2.5 \pm 0.7
22:6 ω 3	0.6 \pm 0.3	0.7 \pm 0.4	0.7 \pm 0.3	1.1 \pm 1.0	0.5 \pm 0.3	0.7 \pm 0.3
ΣPUFA	5.1 \pm 2.2	5.2 \pm 2.2	5.9 \pm 2.1	7.9 \pm 2.8	7.3 \pm 2.2	8.9 \pm 2.4
<i>Branched</i>						
14:0iso	0.3 \pm 0.1	0.4 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1	0.9 \pm 0.1	0.6 \pm 0.1
15:0iso	1.0 \pm 0.3	1.5 \pm 0.4	1.2 \pm 0.3	1.0 \pm 0.3	2.0 \pm 0.5	1.5 \pm 0.3
15:0anteiso	0.8 \pm 0.2	1.1 \pm 0.2	0.9 \pm 0.2	0.8 \pm 0.2	1.2 \pm 0.3	0.9 \pm 0.2
16:0iso	0.5 \pm 0.1	0.8 \pm 0.2	0.6 \pm 0.2	0.5 \pm 0.1	0.7 \pm 0.2	0.5 \pm 0.1
17:0iso	0.6 \pm 0.1	0.8 \pm 0.2	0.8 \pm 0.2	0.7 \pm 0.2	0.9 \pm 0.2	0.9 \pm 0.1
17:0anteiso	0.5 \pm 0.2	0.7 \pm 0.2	0.6 \pm 0.3	0.4 \pm 0.1	0.9 \pm 0.3	0.7 \pm 0.2
ΣBrFA	3.6 \pm 0.9	5.3 \pm 1.0	4.4 \pm 0.9	3.8 \pm 0.8	6.7 \pm 1.2	5.2 \pm 0.9
Σ FA (μ g mgSPM ⁻¹)	1.0 \pm 0.5	0.3 \pm 0.2	0.5 \pm 0.2	0.8 \pm 0.4	0.6 \pm 0.3	0.7 \pm 0.2
SPM (mg L ⁻¹)	27.2 \pm 11.6	141.5 \pm 83.2	65.8 \pm 28.3	55.1 \pm 30.0	150.9 \pm 86.9	62.6 \pm 27.2
% detritalFA	69.8 \pm 5.0	71.1 \pm 3.6	74.4 \pm 3.6	72.7 \pm 2.9	68.1 \pm 2.8	69.1 \pm 2.7
<hr/>						
Salinity	9.7 \pm 1.5	20.2 \pm 2.3	22.6 \pm 1.3	24.6 \pm 1.5	13.9 \pm 3.4	22.1 \pm 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 Indicator	df	Decomposition and wastes						Autochthonous production		
		%BrFA			%detritalFA			%PUFA		
		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

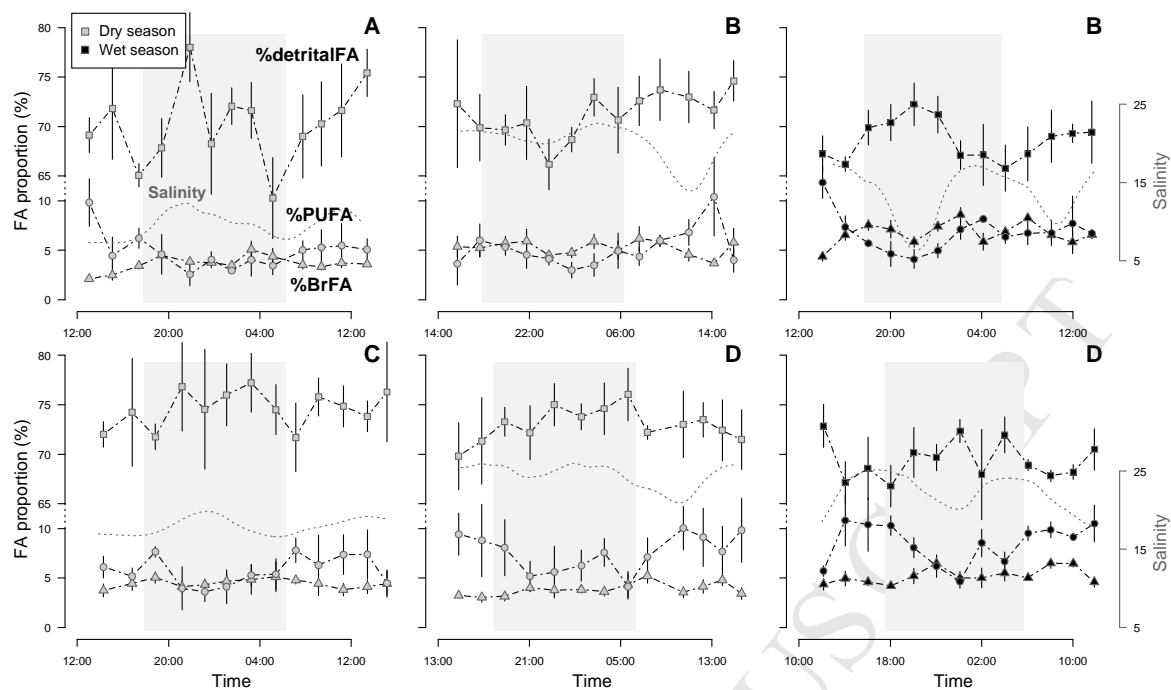
Σ PUFA 16:2ω4 + 16:3ω4 + 18:2ω6 + 18:3ω3 + 18:4ω3 + 20:5ω3 + 20:4ω6 + 22:5ω3 + 22:6ω3

223

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

225 variables

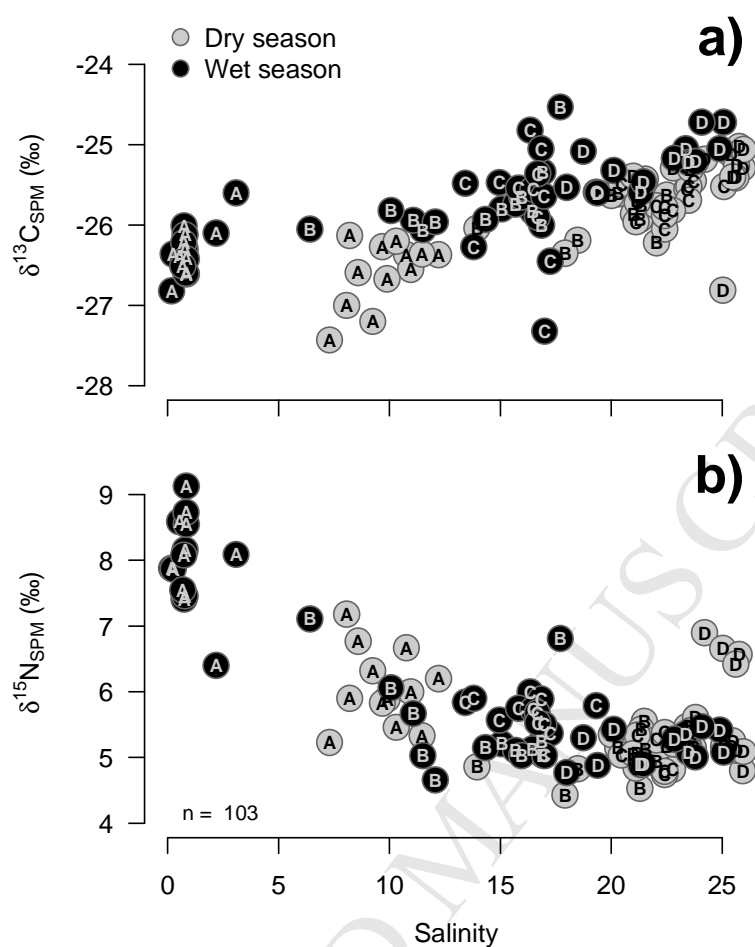
Indicator	df	$\delta^{13}\text{C-SPM}$			$\delta^{15}\text{N-SPM}$			C/N ratio		
		SS	F value	p	SS	F value	p	SS	F value	p
Salinity	1	13.4	95.1	<0.001	63.0	300.7	<0.001	10.4	9.9	<0.01
ln(SPM)	1	0.3	1.8	0.18	5.9	28.3	<0.001	39.6	37.7	<0.001
Season	1	4.9	35.1	<0.001	0.0	0.0	0.8	2.9	2.7	0.10
Day/Night	1	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

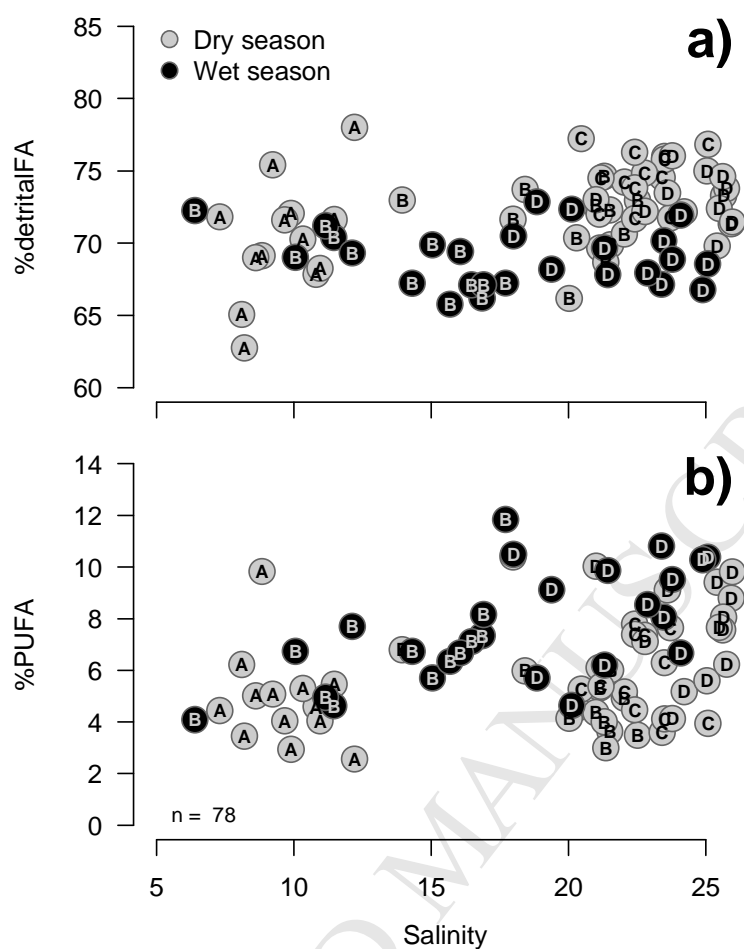
227 Fig. 3: Dynamics of %detritalFA (squares; proportion of 15:0 + 16:0 + sum of 16:1 + 18:0 +
 228 sum of 18:1), %PUFA (bubbles; proportion of polyunsaturated FA), %BrFA (triangles;
 229 proportion of iso- and anteiso-branched chain FA) and salinity (dotted grey line) during the 24
 230 h tidal cycles 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 sampling site. Shaded areas
 233 correspond to night time

234



235

236 Fig. 4: a) $\delta^{13}\text{C}$ and b) $\delta^{15}\text{N}$ 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

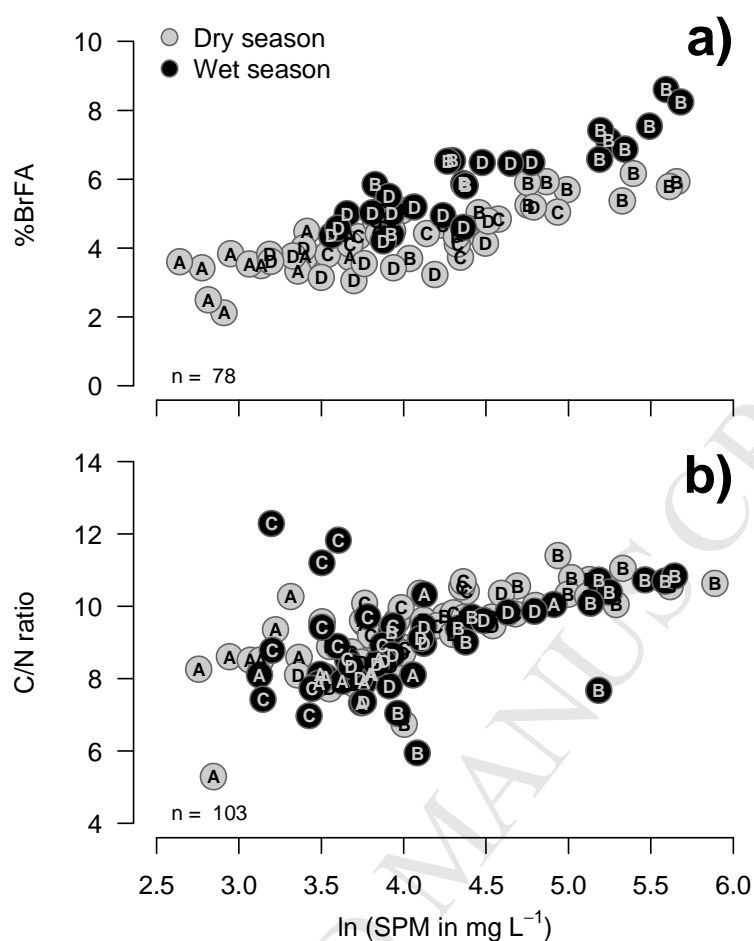
239 Fig. 4: a) %detritalFA (proportion of 15:0 + 16:0 + sum of 16:1 + 18:0 + sum of 18:1) and b)

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

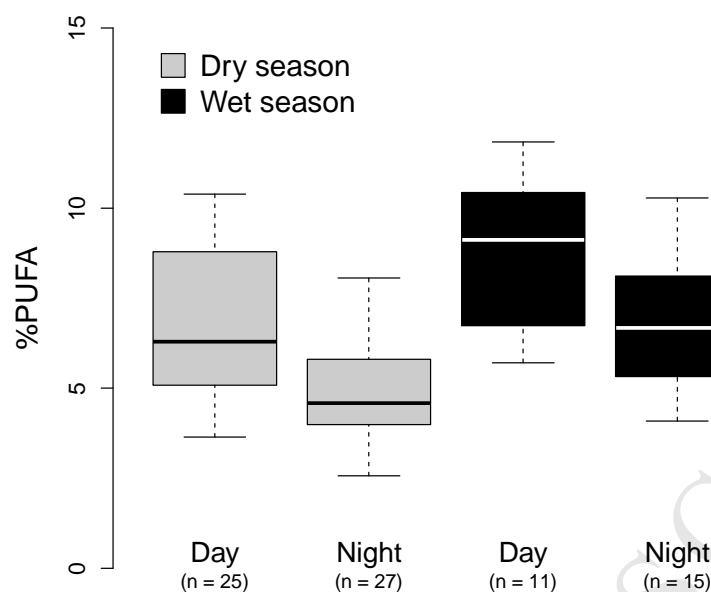
243 sites where samples were collected



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



251
 252 Fig. 7: Boxplot of %PUFA (proportion of polyunsaturated FA) in SPM separated according to
 253 season and presence of daylight in the Can Gio mangrove estuary

255 4. Discussion

256 4.1 Overall composition of SPM

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

266 as the Brazilian Rio das Mortes, where Boëchat et al. (2014) concluded that algae and other
 267 autochthonous autotrophic organisms contributed poorly to the overall SPM pool.
 268 Considering the high water turbulence and turbidity of the estuary, with current velocity up to
 269 1.7 m/s and estimated light penetration of less than 1 m (David et al. 2018b), such results
 270 were expected. The FA composition of SPM in this large estuary was also relatively similar to
 271 that of our previous study in a mangrove creek (David et al. 2018a), except PUFA proportion
 272 that was much higher once during the tidal cycle in David et al. (2018a) and attributed to a
 273 pulse of phytoplankton that grew within the mangrove channels. The Can Gio mangrove
 274 estuary thus conveys highly decomposed SPM to the South China sea coast, exhibiting
 275 varying levels of bacterial indicators and a small proportion of phytoplanktonic biomarkers.
 276

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

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

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

∑ 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 $\delta^{13}\text{C}$ 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 20:5 ω 3, 16:2 ω 4 and 16:3 ω 4 (Table 1) that are
289 diatom indicators in marine ecosystems (Dalsgaard et al. 2003).

290 The $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ in estuarine particles and organisms can
293 provide an integrated N-loading measurement (McClelland and Valelia 1998). In the present
294 study, the $\delta^{15}\text{N}$ 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 differentiation 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).

329 Plant-derived OM has a C/N ratio much higher than bacterial cells (>20 vs. 4-5; Middelburg
330 and Herman 2007) and one may expect increasing proportions of bacterial biomarkers (e. g.
331 %BrFA in resuspended particles) to be associated with lower C/N ratio. However, FA are
332 minor constituents of SPM (<1%; Table 1) and although bacterial abundance most probably
333 increased in resuspended particles, their influence on the C/N ratio remained negligible.
334 Resuspended particles are thus dominated by detrital OM but they might bear an intense
335 mineralisation activity due to their high bacterial load.

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

350

351 *4.5 Seasonal variability in the SPM composition*

352 The changing of seasons was the most explanatory variable for %PUFA and
353 %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 $\delta^{13}\text{C}$ 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 influential 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. CO₂, N₂O) 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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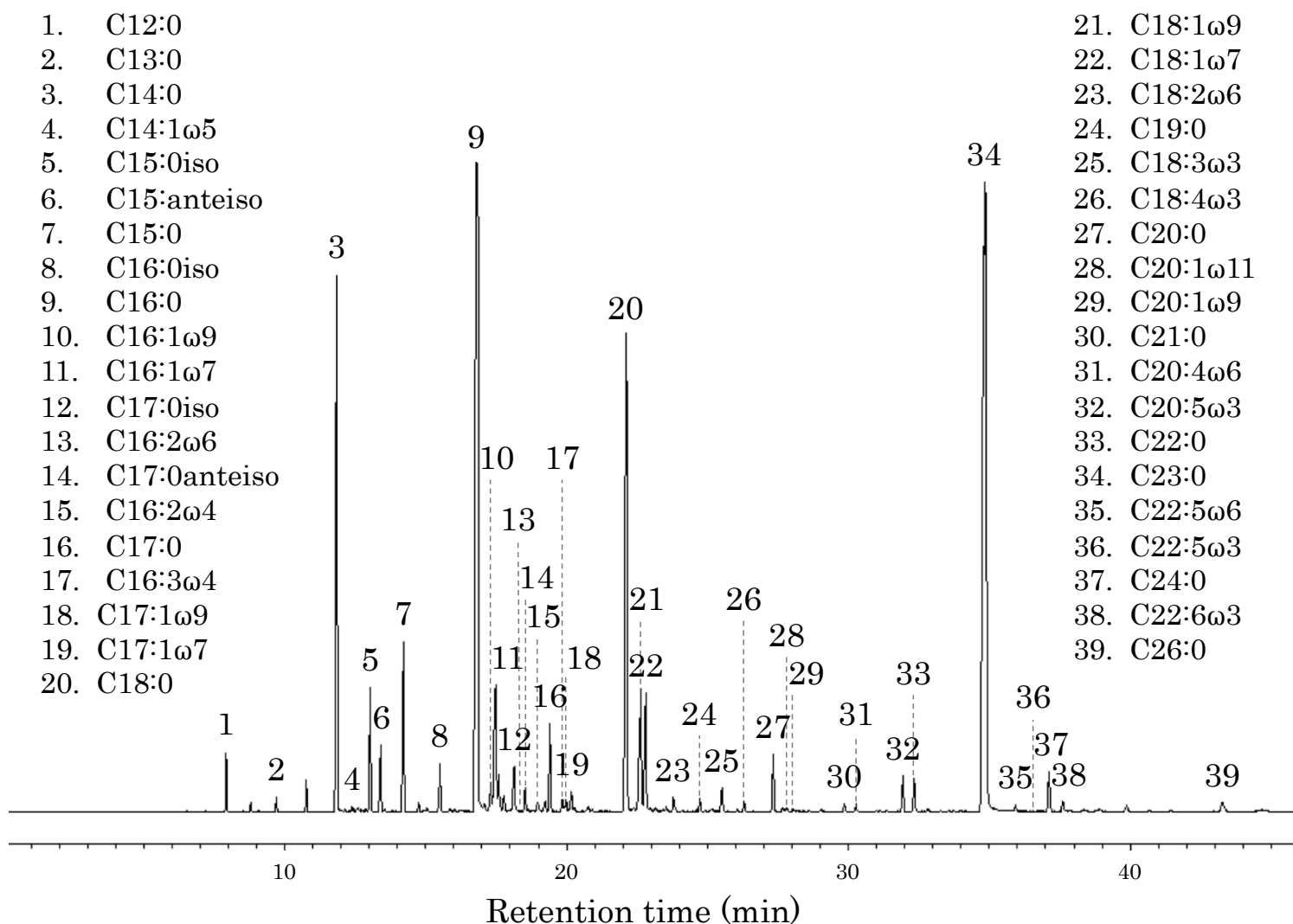
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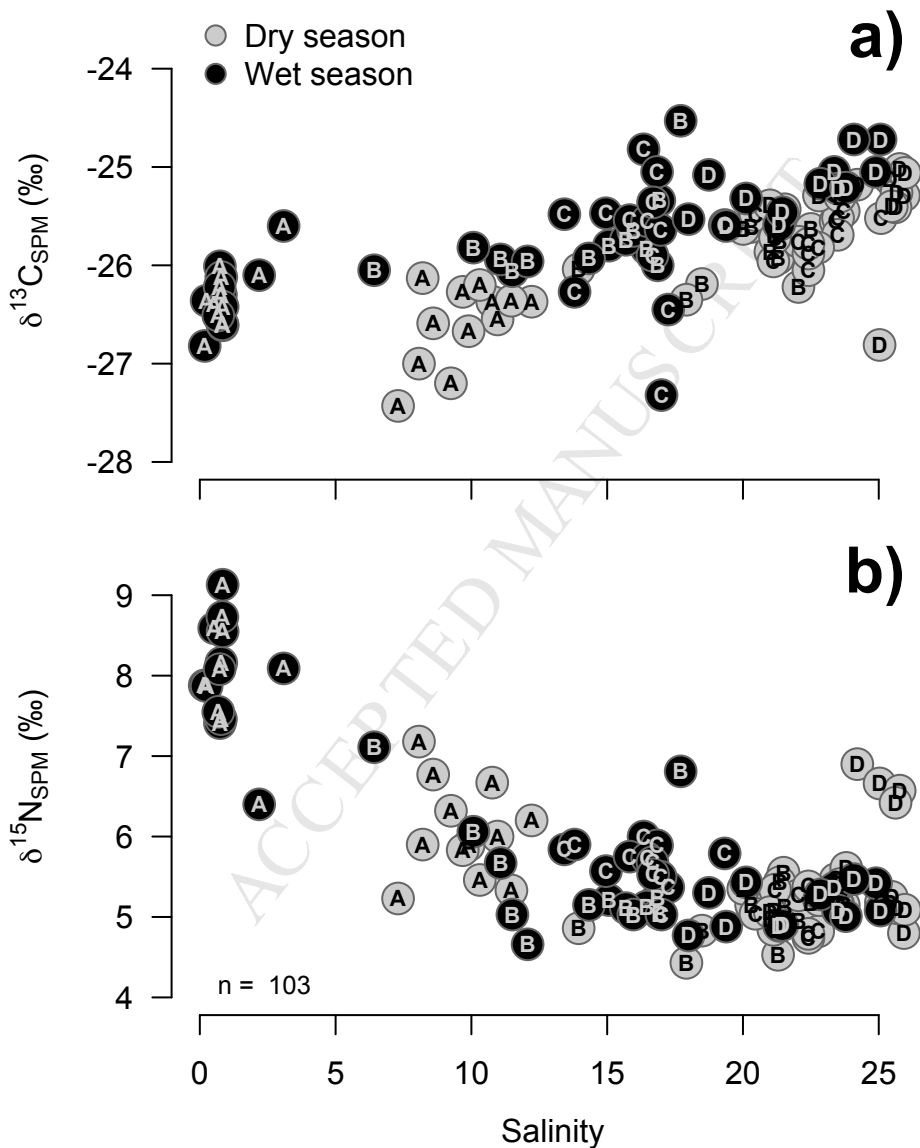
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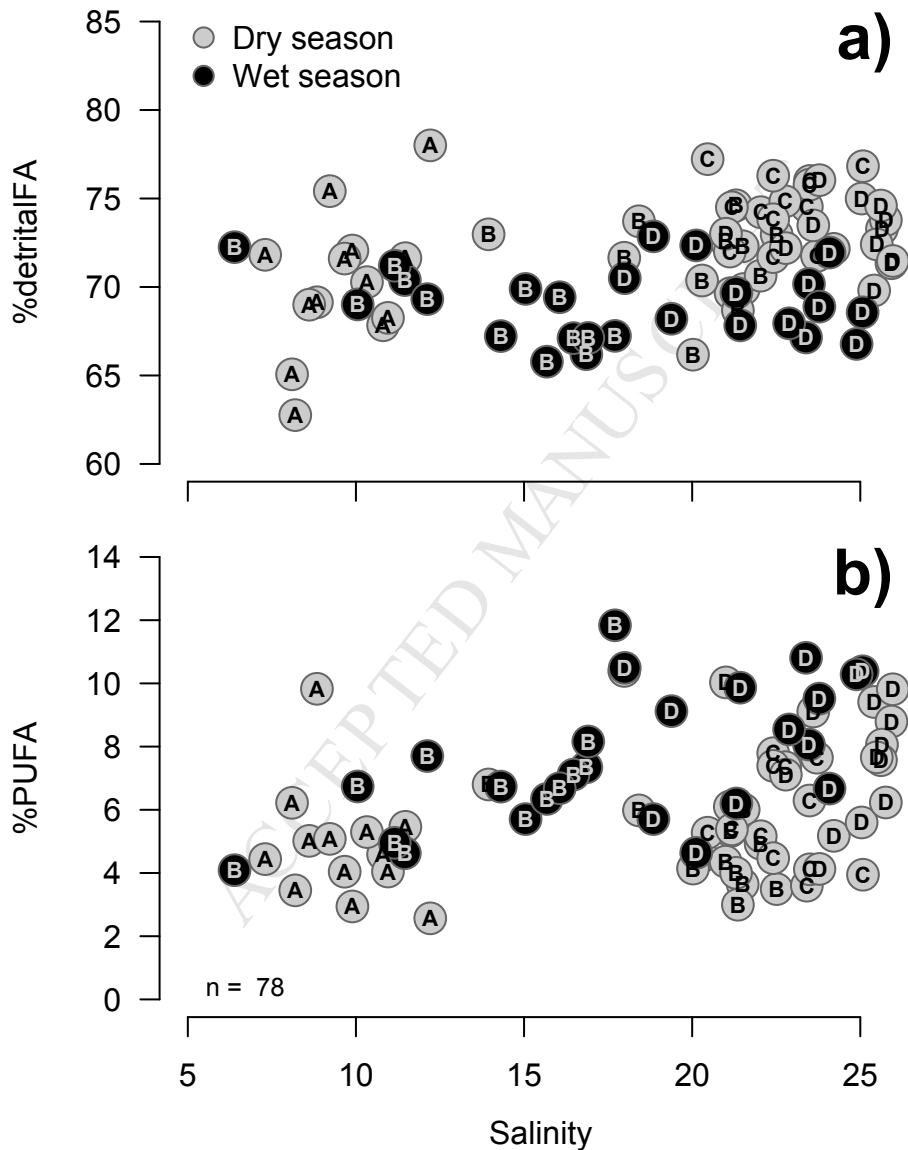
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Appendix 1: Example of GC/MS ion chromatogram ($m/z = 74$) of fatty acids detected in suspended particulate matter of the Can Gio mangrove estuary.





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