Prokaryotic abundance, cell size and extracellular enzymatic activity in a human impacted and mangrove dominated tropical estuary (Can Gio, vietnam)

Frank David, Tarik Meziane, Cyril Marchand, Guillaume Rolland, Aurélie Pham, Nguyen Thanh-Nho, Dominique Lamy

HAL Id: hal-03137158

https://hal.sorbonne-universite.fr/hal-03137158

Submitted on 10 Feb 2021

1   Title: Prokaryotic abundance, cell size and extracellular enzymatic activity

2   in a human impacted and mangrove dominated tropical estuary (Can Gio,

3   Vietnam).

4

5   Running head: Prokaryotic activity in a tropical estuary.

6

7   Authors: Frank DAVID[1a], Tarik MEZIANE[a], Cyril MARCHAND[b,c], Guillaume

8   ROLLAND[a], Aurélie PHAM[a], Nguyen THANH-NHO[c,d] and Dominique LAMY[a]

9

10   [1] Corresponding author: frank.david@live.fr; orcid.org/0000-0002-6145-

11   4618

12

13   [a] Laboratoire Biologie des Organismes et Ecosystèmes Aquatiques

14   (BOREA), Muséum National d'Histoire Naturelle, CNRS 7208, IRD 207, SU, UCN,

15   UA, 61 rue Buffon, 75005 Paris, France

16   [b] Université de la Nouvelle-Calédonie, ISEA, Nouméa, New Caledonia,

17   France

18   [c] IMPMC, Institut de Recherche pour le Développement (IRD), Sorbonne

19   Université, CNRS, MNHN, Nouméa, New Caledonia, France

20   [d] Faculty of Environmental and Food Engineering, Nguyen Tat Thanh

21   University, Ho Chi Minh City, Viet Nam

22

23

26   **1. Abstract**

27   Extracellular enzymatic activities constitute the first and limiting step of

28   the whole process of organic matter (OM) cycling in aquatic ecosystems. This

29   study aims to identify the factors controlling prokaryotes ability to hydrolyse

30   OM in an Indo-Pacific tropical mangrove ecosystem (Can Gio, Vietnam).

31   Prokaryotic abundance and leucine-aminopeptidase exo-proteolytic activity

32   (EPA) were measured at vertical (from the sea-surface microlayer to bottom

33   waters), spatial (along a transect within the estuary) and seasonal (wet and dry

34   season) scales. Prokaryotic abundance ranged from 1.2 to 5.7 x 10^9 cells L^{-1} and

35   EPA ranged from 24 to 505 nmol $L^{-1} h^{-1}$ that was relatively similar to other

36   highly productive ecosystems. The estuary was poorly stratified, most probably

37   because of high water turbulence. Yet, exo-proteolytic activity was significantly

38   higher in bottom waters, where higher loads of suspended particulate matter

39   were measured. Seasonal and spatial differences in EPA suggest that the nature

40   of OM transported by the Can Gio mangrove estuary affect EPA. The latter

41   seems to be increased by two "uncommon" situations: the input of fresh and

42   labile OM (*e.g.* shrimp farm effluents) or the lack of labile OM and the need to

43   hydrolyse refractory compounds (*e.g.* during the dry season).

2. Introduction

Prokaryotes and their ability to mineralise organic matter (OM) are essential for controlling the fluxes of carbon and nutrients in coastal waters. Yet, when, where, and how OM is decomposed into nutrients and CO_2 is not fully understood in tropical mangrove ecosystems (Cai 2011). Extracellular enzymatic activities constitute the limiting step of the whole process of OM cycling (Arnosti 2011) and thus, the turnover rates of various compounds have been used to evaluate the efficiency of the microbial community to mineralise OM in coastal waters (Patel et al. 2000, Cunha and Almeida 2006, Bhaskar and Bhosle 2008, Lamy et al. 2009). The leucine-aminopeptidase activity (exo-proteolytic activity, EPA) is one of the most commonly used indicators of OM hydrolysis. It represents the general ability of prokaryotes to hydrolyse proteins (Hoppe 1993) and thus it informs on the efficiency of the ecosystem to recycle nitrogen, which is generally the first limiting nutrient in marine production (Fernandes 2011).

In the present study we explored vertical, spatial and seasonal variations in the prokaryotic compartment of the Can Gio mangrove water column. Prokaryotic abundances, cell sizes and EPA were measured at three layers of the water column: (1) surface micro-layer (SML), (2) sub-surface layer and (3) bottom layer. Our measurements were distributed from the downstream end of Ho Chi Minh City to the South China Sea. We hypothesised that (i) the SML prokaryotic compartment would be enriched and more active than underlying waters, but rather similar or even reduced compared to the bottom water layer (enriched in suspended particles); and that (ii) the prokaryotic compartment and more particularly the EPA would vary at short spatial (a few kilometers) and temporal (within one month) scales in the estuary, due to short-term and spatial variations in suspended particulate matter (SPM) composition and

71 origin (David et al. 2019). Revealing short-term spatial and temporal variations

72 in the ability of prokaryotes to mineralise OM and therefore making nutrients

73 available for primary producers will contribute to understanding OM cycling in

74 tropical estuaries.

75

76 **3. Materials and methods**

77 3.1. Study area

78 The Can Gio mangrove is located at the downstream end of Ho Chi Minh

79 City (Southern Vietnam; ~13 million inhabitants) and flooded by the Saigon-

80 Dong Nai River. The river discharges annually 37.4×10^6 m^3 of freshwater into

81 the South China Sea and its basin covers a total catchment area of 40.6×10^3

82 km^2 (12% of the total terrestrial area of Vietnam; Ringler et al. 2002). The

83 climate in Can Gio is monsoonal with a wet season from June to October and a

84 dry season from November to May. Tidal amplitude is variable over time and

85 ranges between 2 to 4 m depending on seasons and the distance from the sea

86 (Nam et al. 2014). In 2000, the UNESCO designated the 719.6 km^2 of the Can

87 Gio district as the first mangrove biosphere reserve in Vietnam and a land-use

88 regulation was established. The north-west border of the mangrove is fringed

89 by shrimp farms and salt producing lands, covering roughly 20% of the total

90 reserve surface area, while the rest of the district is preserved from

91 deforestation and mostly covered with mature trees of the species *Rhizophora*

92 *apiculata*.

93 We selected the study sites on the main estuarine channel to be at the

94 interface between land uses (Fig. 1): A) at the downstream end of Ho Chi Minh

95 City (10°39'55"N 106°47'30"E); B) between shrimp farms and the forested

96 mangrove area (10°34'19"N 106°50'11"E); C) in the centre of the mangrove

97 core area (10°31'04"N 106°53'13"E); D) and between the forested mangrove

98 area and the South China Sea coast (10°29'32"N 106°56'55"E). At all these sites,

99 the estuary has steep eroded banks, a depth of about 10-15 m and a width of

100 about 600 m. Site E exhibited very different physical characteristics. It was

101 located in a 1-3 m deep and 30 m large tidal creek within the mangrove core

102 area, which does not receive freshwater upstream inputs (10°30'24"N

103 106°52'57"E; Fig. 1).

104

105 ### 3.2.   Sampling strategy

106 The study sites were sampled once per season, each one at one week

107 intervals and in alphabetical order. Samplings were performed from January

108 14th to February 3rd 2015 during the dry season and from September 22nd to

109 October 20th during the wet season. Exact sampling dates, corresponding tidal

110 coefficient and tidal variations can be found in David et al. 2018. Salinity, pH

111 and water temperature were measured at 5-min intervals during 24 h tidal

112 cycles using a Yellow Spring Instrument® meter (YSI 6920) and dissolved oxygen

113 (DO) was monitored similarly with a Hobo® data logger (HOBO U26-001), both

114 immersed 30 cm below the water surface and calibrated before each survey.

115 We collected sub-surface water from the bow of a sampling boat using a

116 10 L plastic bucket immersed 10 cm below the water level. We sampled bottom

117 water using a 2 L weighted bottle with a stopper pulled roughly 1 m above the

118 bottom of the estuary. Samples from the surface micro-layer were taken using

119 a glass plate sampler. The glass plate was immersed vertically and withdrawn at

120 an approximate rate of 20 cm s^{-1}, following the recommendations of Harvey

121 and Burzell (1972). It was then wiped between two polytetrafluoroethylene

122 squeegees fixed face to face to remove the adhering SML, which was falling by

123 gravity into a polyethylene bottle. This method was expected to recover the

124   first 60-100 μm of the water column, which constitutes a water-air interface

125   where biological processes may differ from the rest of the water column. We

126   repeated the procedure of dipping, withdrawing and wiping the plate to obtain

127   a sufficient volume of sample for the analyses.

128   Sub-samples of water were vacuum-filtered through pre-combusted (5 h

129   at 450°C) and pre-weighted glass fibre filters (Whatman® GF/F 0.7 μm).

130   Suspended particulate matter concentration was measured gravimetrically

131   after freeze-drying the filters. Three sub-samples for bacterial counting and cell

132   size measurements were immediately preserved in 4.5 mL sterile Abdos Cryo

133   Vials®, buffered with 0.2 μm pre-filtered formalin (4 % final concentration) and

134   further stored at -25°C. Four and five replicates were analysed for EPA during

135   the dry season and the wet season, respectively (Table 1). Replicates consisted

136   of different water samples that were collected at 5-min intervals.

137

138   3.3.   Sample processing

139   Preserved samples were defrosted at ambient temperature and filtered

140   on 0.2 μm polycarbonate filters. Bacteria were stained using the 4'-6-diamino-

141   2-phenylindole (DAPI) fluorescent stain, following the method described by

142   Porter and Feig (1980). Ten to twelve randomly selected fields (to count at

143   least 500 cells) were photographed using a Leica epifluorescence microscope

144   coupled with a digital camera measuring light emission at 450 nm after

145   excitation at 350 nm. Prokaryotes numeration and cell size measurements

146   were performed with the open-source image-processing program ImageJ v1.5.

147   We binarised monochromatic pictures to automatically count nearly circular

148   aggregates of fluorescent pixels, which we assumed to be prokaryote cells. We

149   measured cell size using a Feret box enclosing the cell and considering that the

150 cell diameter roughly equals the average distance between maximal and

151 minimal Feret diameters (Loferer-Kröbbacher et al. 1998, Merkus et al. 2009).

152   The EPA was measured in the unfiltered fraction using the fluorigenic

153 substrate analogue L-leucine-methylcoumarinylamide (Leu-MCA; Hoppe 1993).

154 The substrate was added to 1.8 mL of water sub-samples and incubated on

155 board during 1 h at *in situ* temperature and low natural light intensity. At the

156 end of the incubation period, the enzymatic cleavage activity was stopped

157 using Sodium Dodecyl Sulfate (1 % final concentration). Controls in duplicates

158 were run similarly, except that the stopper solution was added before the

159 substrate. The cleavage of Leu-MCA resulted from the exoproteolytic activity

160 and was linearly related to the MCA fluorescence. Saturation curves were

161 carried out to determine the saturating substrates concentration and 800 µM

162 final concentrations were used for all samples. Since the substrate was

163 saturating in our study, results corresponded to potential activity rates. The

164 fluorescence was measured using a Varian Cary Eclipse spectrofluorometer

165 (excitation/emission of 380/440 nm) and transformed to hydrolysis activity

166 using a standard curve established with different concentrations of the

167 fluorochrome MCA. All laboratory analysis were performed within 9 months

168 after samples recollection.

169

170   3.4.   Data analyses

171   We performed univariate multiple comparisons using non-parametric

172 Kruskal-Wallis test due to variance heterogeneity and we identified groups of

173 samples differing from one another using Wilcoxon pairwise comparisons

174 (α = 5%, modified by Holm correction for multiple analyses). Statistical analyses

175 and graphical representations were performed using R 3.3.2 (R Core Team

176 2017).

177   **4. Results and discussion**

178   Salinity along the 40 km of the Can Gio mangrove estuary ranged from 0

179   to 26; water temperature ranged from 26 to 31 °C; dissolved oxygen saturation

180   ranged from 17 to 83 % and pH ranged from 6.5 to 7.8 (David et al. 2018,

181   Taillardat et al. 2019). All parameters were linearly correlated to salinity, which

182   increased from site A to site D and was roughly similar at sites C, D and E.

183   Dissolved oxygen saturation and pH increased with salinity and water

184   temperature decreased during the dry season and remained roughly stable

185   during the wet season. Both pH and dissolved oxygen reflected the mixing of

186   fresh and sea waters without significant perturbations induced by adjacent

187   inputs (e.g. from mangrove creeks). The acidic pH values measured in the

188   upper part of the estuary are likely due to the leaching of the surrounding

189   acidic sulfate soils along with the decay of organic inputs (Thanh-Nho et al.

190   2018). The latter being also responsible for dissolved oxygen levels down to 1.2

191   mgO_2 L^{-1}. These organic inputs may originate from domestic and urban

192   discharges of Ho Chi Minh City urban center and also from the industrial areas

193   located along the Saigon and Dong Nai Rivers (Thanh-Nho et al. 2018)

194

195   4.1.   Overall comparisons

196   Prokaryotic abundance ranged from 1.2 to 5.7 x 10^9 cells L^{-1} (Fig. 2). It

197   was slightly above the values previously reported in other tropical (Bhaskar and

198   Bhosle 2008) and sub-tropical mangroves (Bano et al. 1997, Williams and

199   Jochem 2006). In tidal creeks of the Indus River delta, Bano et al. (1997)

200   measured a prokaryotic abundance ranging from 1 to 4 x 10^9 cells L^{-1}. Bhaskar

201   and Bhosle (2008) counted 0.6 to 3.5 x 10^9 cells L^{-1} in a mangrove dominated

202 estuary from the west coast of India. Williams and Jochem (2006) reported

203 0.27 to 2.91 x 10^9 cells L^{-1} in Florida Bay waterways.

204 The only recent record of exo-proteolytic activity in mangrove waters

205 was provided by Williams and Jochem (2006) in Florida Bay, and to the best of

206 our knowledge, no studies have ever been measuring EPA in Indo-Pacific

207 mangrove waters. The activities we measured, ranging from 24 to 505 nmol L^{-1}

208 h^{-1} in sub-surface waters (Fig. 2), were below those reported by Williams and

209 Jochem (2006) in Florida Bay, which ranged from 70 to 1650 nmol L^{-1} h^{-1}. They

210 were relatively similar to those measured by Rath et al. (1993) in an eutrophic

211 mangrove-influenced station in a barrier reef off Belize (247 to 306 nmol L^{-1} h^{-}

212 ^{1}). Converted to cell-specific activity, the EPA we measured in sub-surface

213 waters ranged from 4.9 to 179.6 amol cell^{-1}, which is one order of magnitude

214 below the values measured by Williams and Jochem (2006) in Florida Bay (52.2

215 to 1571.7 amol cell^{-1}) or by Rath et al. (1993) off Belize (316.0 to 765.7 amol

216 cell^{-1}). Yet, the values of the present study were roughly similar to those

217 reported by Lamy et al. (2009) after a *Phaeocystis globosa* bloom in the eastern

218 English Channel (50 to 239.1 amol cell^{-1}). These results suggest that

219 prokaryotes of the Can Gio mangrove estuary efficiently mineralise proteins

220 and ease the availability of nitrogen for primary producers at a rate similar to

221 that of other highly productive ecosystems.

222

223 4.2. Prokaryotic compartment stratification

224 No significant differences in prokaryotic abundance or cell size were

225 measured between the three water layers during the dry season (Fig. 3),

226 suggesting that the prokaryotic compartment is poorly stratified in the water

227 column. Yet, lower EPA in the surface micro-layer compared to underlying

228 waters and maximum values in the bottom (Fig. 3) suggest that prokaryotes

229 function differently between water layers. The Can Gio mangrove estuary is

230 highly hydrodynamic, with current velocity reaching 1.7 m s^{-1} during ebb and

231 1.3 m s^{-1} in the opposite direction during flood (David et al. 2018). A strong

232 vertical mixing is thus to be expected and probably inhibits the establishment

233 of a prokaryote-enriched SML, as previously observed in a highly hydrodynamic

234 estuary of Portugal (Santos et al. 2011). In a site of the Ria de Aveiro estuary

235 (Portugal), with similar hydrodynamic characteristics as ours, Santos et al.

236 (2011) measured a similar abundance of prokaryotes between water layers but

237 lower prokaryotic productivity in the SML compared to underlying waters,

238 while the opposite was recorded when a structured SML could be established

239 due to lower hydrodynamism. We suggest that although prokaryotes may be

240 homogenously distributed in the upper layers of the water column in highly

241 hydrodynamic estuaries, they are less active at the water-air interface. Our

242 hypothesis that the SML would be more active than underlying waters is thus

243 invalidated, most probably because of the high hydronynamism of the Can Gio

244 mangrove estuary.

245 Higher EPA and suspended particulate matter concentrations in the

246 bottom compared to other layers (Fig. 3) suggest a particle-associated

247 behaviour of prokaryotes in bottom waters. Actually, prokaryotic cells are

248 influenced by the presence of particles in the water column, creating patches,

249 or "hotspots" of biomass where OM is intensely recycled (Long and Azam 2001,

250 Simon et al. 2002). In a tidal estuary exhibiting high loads of suspended matter,

251 particle-attached prokaryotes can reach up to 80% of total abundance (De

252 Souza et al. 2003). It has previously been observed that particle-attached

253 prokaryotes were more active than free-living cells (Grossart et al. 2007a,

254 2007b, Schapira et al. 2012). Although in our study no difference could be

255 evidenced in prokaryote abundance between water layers, the proportion of

256 particle-attached cells may have been higher in the bottom compared to other
257 layers, due to higher loads of SPM (Fig. 3). Actually, a previous study (David et
258 al. 2019) showed a high relative contribution of branched fatty acids, indicative
259 of bacteria, in short settling-time particles, which are more hardly resuspended
260 and tend to remain closer to the estuary bottom. We thus suggest that short
261 settling-time particles are the sites of an intense prokaryotic
262 activity. Prokaryotes may however alternate from a free-living and a particle-
263 attached lifestyle (Grossart et al., 2010, Riemann and Winding, 2001) and both
264 populations may belong to the same community as no difference in cell size
265 was revealed between water layers (Fig. 3). Our hypothesis that the bottom
266 layer would be more active than upper waters is therefore confirmed, most
267 probably because of higher loads of SPM compared to other water layers.

268

269 4.3. Link with organic matter quality

270 During the dry season, EPA was roughly 10 times higher than during the
271 wet season, while prokaryotic abundance did not differ and cells were only
272 slightly smaller (Fig. 2), which clearly indicates that the prokaryotic biomass did
273 not notably vary between seasons. We thus expect the specific activity of
274 prokaryotes to vary spatially and/or temporally rather than their abundances.
275 Exo-proteolytic activity is usually closely related to the prokaryotic abundance
276 (Cunha et al. 2000, Patel et al. 2000) and thus, seasonal stability in bacterial
277 stocks is surprising. Yet, intense top-down pressure (grazing or virus mortality)
278 could explain a decoupling between prokaryotic standing stock and activity
279 (Fuhrman and Noble 1995, De Souza et al. 2003, Pradeep Ram et al. 2018).
280 Aspects of top-down processes on prokaryotes would require further
281 investigations in mangrove ecosystems.

282   In the Can Gio mangrove estuary, organic matter exhibited higher lability

283 during the wet season, associated with a higher proportion of polyunsaturated

284 fatty acids indicative of fresh phytoplankton-derived OM, while during the dry

285 season, OM sources were mostly terrestrial (David et al. 2019). The observed

286 seasonal EPA variability could thus be related to the seasonal variation in the

287 composition of organic pools. Moreover, previous studies demonstrated

288 significant protease stimulation in the presence of terrestrial dissolved OM

289 (Traving et al. 2017) and humic-rich dissolved OM (Stepanauskas et al. 1999).

290 Baltar et al. (2017) demonstrated experimentally that prokaryotes fuelled with

291 mangrove-derived dissolved OM increased their EPA up to 400 nmol L^{-1} h^{-1}

292 after 4 days of incubation. In our study, the renewal of water in the mangrove

293 was most probably higher during the wet season compared to the dry season,

294 due to rain inputs and a higher discharge of the estuary. Thus, OM released by

295 mangrove leaves could be more rapidly diluted than during the dry season.

296 Higher leaching of mangrove leaves was actually measured in the Can Gio

297 mangrove during the wet season (Vinh et al. 2020). As a consequence, the EPA

298 did not increase to the same levels as recorded during the dry season,

299 characterized by heavier loads of OM of terrestrial origin. Similarly, high EPA at

300 site E during the wet season in comparison to the other sites (Fig. 2) was most

301 likely due to the high concentrations of mangrove-derived OM nearby the

302 mangrove forest. The ability of prokaryotes to mineralise OM thus varies at the

303 seasonal scale most probably as a response to OM quality (*i.e.* origin, lability).

304   Finally, the high EPA at site B in sub-surface water during both seasons at

305 high tide (Fig. 2) compared to its relative stability in other sites (except site E)

306 whatever the tidal stage considered suggest that OM differed at this site. Site B

307 is located down the area dedicated to shrimp farming (Fig. 1) and the intensive

308 shrimp production requires punctual water renewal in ponds, releasing

309 wastewaters loaded with OM and nutrients to adjacent ecosystems (Anh et al.

310 2010). Shrimp pond effluents are constituted by fresh and highly labile OM that

311 stimulates bacterial growth (Vivier et al. 2019), which may explain the higher

312 EPA activity measured at site B. Our results suggest that both mangrove-

313 derived OM and shrimp pond effluents increased the release of extracellular

314 enzymes by prokaryotes. The aim of such enzymes is to convert compounds of

315 high molecular weight into monomeric substances, allowing them to be

316 transported through the cytoplasmic membrane. It is thus to be expected that

317 a higher degree of macromolecules polymerisation or organically less labile OM

318 would lead to a higher release of extracellular enzymes by prokaryotes. Baltar

319 et al. (2017) rather suggested that it is the high palatability (that is probably

320 linked to easier assimilation) of mangrove-derived dissolved OM that enhances

321 extracellular enzymes production. According to Findlay et al. (1991),

322 autochthonously produced OM, including exudates, intracellular contents and

323 biomass of primary producers are easily degraded by prokaryotes, while

324 allochthonous sources are relatively more complex and refractory to

325 prokaryote degradation. Our results suggest that EPA is enhanced in both

326 situations: when OM is mostly composed of macromolecules resistant to

327 degradation, and when OM is constituted by unusually high amounts of fresh

328 and labile OM.

329

330   4.4.   Prokaryotes behaviour in mangrove ecosystems

331   Conceptually, there might be a "common" situation for prokaryotes in

332 mangrove ecosystems, where inputs of fresh labile organic matter are

333 sufficient to sustain their nitrogen demand. In the Can Gio mangrove this

334 "common" situation occurs during the wet season. It is probably reinforced by

335 the fact that the strong anthropogenic pressure brings easily available nitrogen

336 to the ecosystem (Nguyen et al. 2019). Consequently, prokaryotes release low

337 amounts of exo-proteolytic enzymes. The situation becomes "uncommon"

338 during the dry season, when fresh and labile OM is getting more scarce.

339 Nitrogen demand is then fullfiled through higher release of exo-proteolytic

340 enzymes. Another "uncommon" situation is the input of OM from shrimps

341 farms, that may modify nutrient balance, and stimulate prokaryotes release of

342 exo-proteolytic enzymes. Yet, the focus on one mangrove ecosystem is

343 insufficient to conclude with certainty on prokaryotes behavior in these

344 understudied type of ecosystems (regarding prokaryotes) that are mangroves.

345 Further studies in other regions would need to confirm these preliminary

346 results in other regions, especially regarding anthropogenic releases that may

347 disturb the system and switch it to an unusual fonctionning. Later on, if this

348 switch is broadly confirmed in other ecosystems, its consequences on trophic

349 webs and nutrient cycling should be assessed.

350

351 **5. Conclusions**

352 To the best of our knowledge, this study is the first to measure exo-

353 proteolytic activity in Indo-Pacific mangrove waters. The surface micro-layer of

354 the estuary, that we expected to serve as a receptacle for anthropogenic

355 pollutants and organic matter, is not well structured because of high water

356 turbulence. Yet, higher loads of suspended particulate matter in bottom waters

357 were correlated to higher EPA in this layer. The nature of OM transported by

358 the Can Gio mangrove estuary may affect the ability of prokaryotes to degrade

359 OM and recycle nitrogen, despite abundance and size of prokaryotic cells did

360 not vary. Releases of extracellular enzymes seem to be increased as a response

361 to "uncommon" situations induced by the input of fresh and labile OM (*e.g.*

362 shrimp farm effluents) or by the lack of labile OM and the need to hydrolyse

363 refractory compounds (*e.g.* during the dry season).

364

365 **6. Acknowledgments**

366

7. References

Anh PT, Kroeze C, Bush SR, Mol APJ (2010) Water pollution by intensive brackish shrimp farming in south-east Vietnam: causes and options for control. Agricultural Water Management 97:872–882.

Arnosti C (2011) Microbial extracellular enzymes and the marine carbon cycle. Annual Review of Marine Science 3:401–425.

Baltar F, Morán XAG, Lønborg C (2017) Warming and organic matter sources impact the proportion of dissolved to total activities in marine extracellular enzymatic rates. Biogeochemistry 133:307–316.

Bano N, Nisa M-U, Khan N, Saleem M, Harrison PJ, Ahmed SI, Azam F (1997) Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. Marine Ecology Progress Series 157:1–12.

Bhaskar PV, Bhosle NB (2008) Bacterial production, glucosidase activity and particle-associated carbohydrates in Dona Paula bay, west coast of India. Estuarine, Coastal and Shelf Science 80:413–424.

Cai W-J. (2011) Estuarine and coastal ocean carbon paradox: CO_2 sinks or sites of terrestrial carbon incineration? Annual Review of Marine Science 3:123–145.

Cunha A, Almeida A (2006) Influence of an estuarine plume and marine sewage outfall on the dynamics of coastal bacterioplankton communities. Aquatic Microbial Ecology 44:253–262.

Cunha A, Almeida A, Alcântara F (2000). Patterns of ectoenzymatic and heterotrophic bacterial activities along a salinity gradient in a shallow tidal estuary. Marine Ecology Progress Series 204:1–12.

395 David F, Meziane T, Tran-Thi N-T, Truong Van V, Thanh-Nho N, Taillardat P,

396   Marchand C (2018) Carbon biogeochemistry and CO_2 emissions in a

397   human impacted and mangrove dominated tropical estuary (Can Gio,

398   Vietnam). Biogeochemistry 138:261–275.

399 David F, Marchand C, Thiney N, Tran-Thi N-T, Meziane T (2019) Short-term

400   changes in the quality of suspended particulate matter in a human

401   impacted and mangrove dominated tropical estuary (Can Gio, Vietnam).

402   Continental Shelf Research 178:59–67.

403 De Souza M-JB, Nair S, Bharathi PL, Chandramohan D (2003) Particle-associated

404   bacterial dynamics in a tropical tidal plain (Zuari estuary, India). Aquatic

405   microbial ecology 33:29–40.

406 Fernandes L (2011). Origin and biochemical cycling of particulate nitrogen in

407   the Mandovi estuary. Estuarine, Coastal and Shelf Science 94:291–298.

408 Findlay S, Pace ML, Lints D, Cole JJ, Caraco NF, Peierls B (1991) Weak coupling

409   of bacterial and algal production in a heterotrophic ecosystem: the

410   Hudson River estuary. Limnology and Oceanography 36:268–278.

411 Fuhrman JA, Noble RT (1995) Viruses and protists cause similar bacterial

412   mortality in coastal seawater. Limnology and Oceanography 40:1236–

413   1242.

414 Grossart H.-P. (2010) Ecological consequences of bacterioplankton lifestyles:

415   changes in concepts are needed: Ecological consequences of

416   bacterioplankton lifestyles. Environmental Microbiology Reports 2, 706–

417   714.

418 Grossart H.-P., Engel A, Arnosti C, De La Rocha C, Murray A, Passow U (2007a)

419   Microbial dynamics in autotrophic and heterotrophic seawater

420   mesocosms. III. Organic matter fluxes. Aquatic Microbial Ecology 49:143–

421   156.

422 Grossart H.-P., Tang KW, Kiørboe T, Ploug H (2007b) Comparison of cell-specific

423   activity between free-living and attached bacteria using isolates and

424   natural assemblages. FEMS Microbiology Letters 266:194–200.

425 Harvey GW, Burzell LA (1972) A simple microlayer method for small samples.

426   Limnology and Oceanography 17:156–157.

427 Hoppe H-G (1993) Use of fluorogenic model substrates for extracellular enzyme

428   activity (EEA) measurement of bacteria. Handbook of methods in aquatic

429   microbial ecology, pp. 423–431.

430 Lamy D, Obernosterer I, Laghdass M, Artigas F, Breton E, Grattepanche J,

431   Lecuyer E, Degros N, Lebaron P, Christaki U (2009) Temporal changes of

432   major bacterial groups and bacterial heterotrophic activity during a

433   *Phaeocystis globosa* bloom in the eastern English Channel. Aquatic

434   Microbial Ecology 58:95–107.

435 Loferer-Kröbbacher M, Klima J, Psenner R (1998) Determination of bacterial cell

436   dry mass by transmission electron microscopy and densitometric image

437   analysis. Applied and Environmental Microbiology 64:688–694.

438 Long RA, Azam F (2001) Microscale patchiness of bacterioplankton assemblage

439   richness in seawater. Aquatic Microbial Ecology 26:103–113.

440 Merkus HG (2009) Particle Size, Size Distributions and Shape, in: Merkus H.G.

441   (Ed.), Particle size measurements: fundamentals, practice, quality.

442   Springer Science & Business Media, pp. 13–42

443 Nam VN, Sinh LV, Miyagi T, Baba S, Chan HT (2014) An overview of Can Gio

444   district and mangrove biosphere reserve, in: Studies in Can Gio

445   Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam. Tohoku Gakuin

446   University, Japan, pp. 1–7.

447 Nguyen TTN, Némery J, Gratiot N, Strady E, Tran VQ, Nguyen AT, Aimé J, Peyne

448   A (2019) Nutrient dynamics and eutrophication assessment in the

449   tropical river system of Saigon – Dongnai (southern Vietnam). Science of

450   the Total Environment 653:370–383.

451 Patel AB, Fukami K, Nishijima T (2000) Regulation of seasonal variability of

452   aminopeptidase activities in surface and bottom waters of Uranouchi

453   Inlet, Japan. Aquatic Microbial Ecology 21:139–149.

454 Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic

455   microflora. Limnology and oceanography 25:943–948.

456 Pradeep Ram AS, Mari X, Brune J, Torréton JP, Chu VT, Raimbault P, Niggemann

457   J, Sime-Ngando T (2018) Bacterial-viral interactions in the sea surface

458   microlayer of a black carbon-dominated tropical coastal ecosystem

459   (Halong Bay, Vietnam). Elementa Science of the Anthropocene 6 (1), 13.

460 R Core Team (2017) R: A language and environment for statistical computing. R

461   Foundation for Statistical Computing, Vienna, Austria. URL

462   https://www.R-project.org/.

463 Rath J, Schiller C, Herndl GJ (1993) Ectoenzymatic activity and bacterial

464   dynamics along a trophic gradient in the Caribbean Sea. Marine Ecology

465   Progress Series 102:89–96.

466 Riemann L, Winding A (2001) Community Dynamics of Free-living and Particle-

467   associated Bacterial Assemblages during a Freshwater Phytoplankton

468   Bloom. Microbial Ecology 42:274–285.

469 Ringler C, Cong NC, Huy NV (2002) Water allocation and use in the Dong Nai
470   River Basin in the context of water institution strengthening. Integrated
471   Water-resources Management in a River-basin Context: Institutional
472   Strategies for Improving the Productivity of Agricultural Water
473   Management 215.

474 Santos L, Santos AL, Coelho FJRC, Gomes NCM, Dias JM, Cunha Â, Almeida A
475   (2011) Relation between bacterial activity in the surface microlayer and
476   estuarine hydrodynamics. FEMS Microbiology Ecology 77:636–646.

477 Schapira M, McQuaid CD, Froneman PW (2012) Free-living and particle-
478   associated prokaryote metabolism in giant kelp forests: Implications for
479   carbon flux in a sub-Antarctic coastal area. Estuarine, Coastal and Shelf
480   Science 106:69–79.

481 Simon M, Grossart H-P, Schweitzer B, Ploug H (2002) Microbial ecology of
482   organic aggregates in aquatic ecosystems. Aquatic microbial ecology
483   28:175–211.

484 Stepanauskas R, Leonardson L, Tranvik LJ (1999) Bioavailability of wetland-
485   derived DON to freshwater and marine bacterioplankton. Limnology and
486   Oceanography 44:1477–1485.

487 Taillardat P, Ziegler AD, Friess DA, Widory D, David F, Ohte N, Nakamura T,
488   Evaristo J, Thanh-Nho N, Truong Van V, Marchand C (2019) Assessing
489   nutrient dynamics in mangrove porewater and adjacent tidal creek using
490   nitrate dual-stable isotopes: A new approach to challenge the Outwelling
491   Hypothesis? Marine Chemistry 214, 103662.

492 Thanh-Nho N, Strady E, Nhu-Trang T, David F, Marchand C (2018) Trace metals
493   partitioning between particulate and dissolved phases along a tropical
494   mangrove estuary (Can Gio, Vietnam). Chemosphere 196, 311–322.

495 Traving SJ, Rowe O, Jakobsen NM, Sørensen H, Dinasquet J, Stedmon CA,
496   Andersson A, Riemann L (2017) The effect of increased loads of dissolved
497   organic matter on estuarine microbial community composition and
498   function. Frontiers in Microbiology 8, 351.

499 Vinh TV, Allenbach M, Linh KTV, Marchand C (2020) Changes in leaf litter
500   quality during its decomposition in a tropical planted mangrove forest
501   (Can Gio, Vietnam). Frontiers in Environmental Science 8, 10.

502 Vivier B, David F, Marchand C, Thanh-Nho N, Meziane T (2019) Fatty acids, C
503   and N dynamics and stable isotope ratios during experimental
504   degradation of shrimp pond effluents in mangrove water. Marine
505   Environmental Research 150, 104751.

506 Williams CJ, Jochem FJ (2006). Ectoenzyme kinetics in Florida Bay: Implications
507   for bacterial carbon source and nutrient status. Hydrobiologia 569, 113–
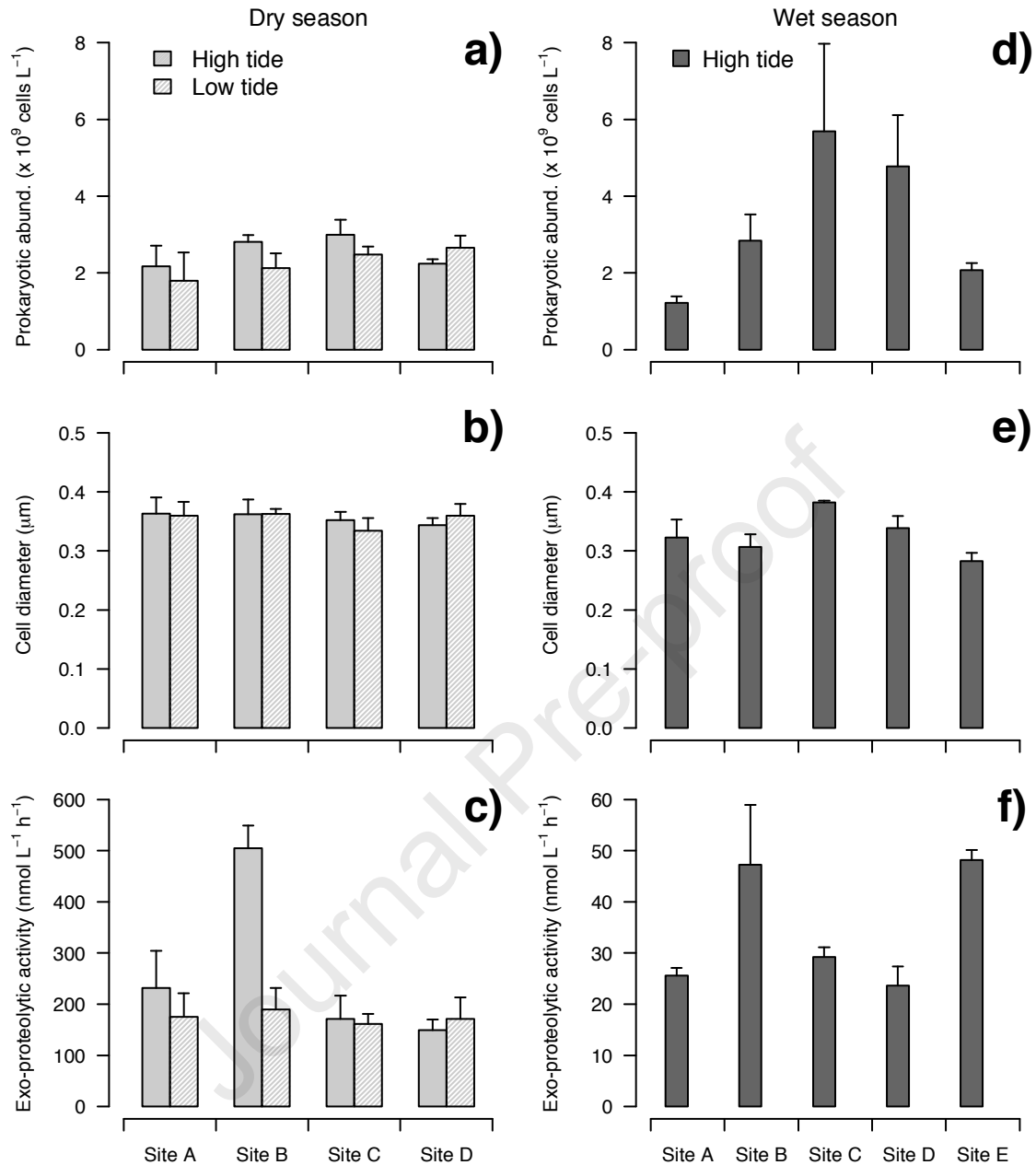508   127.
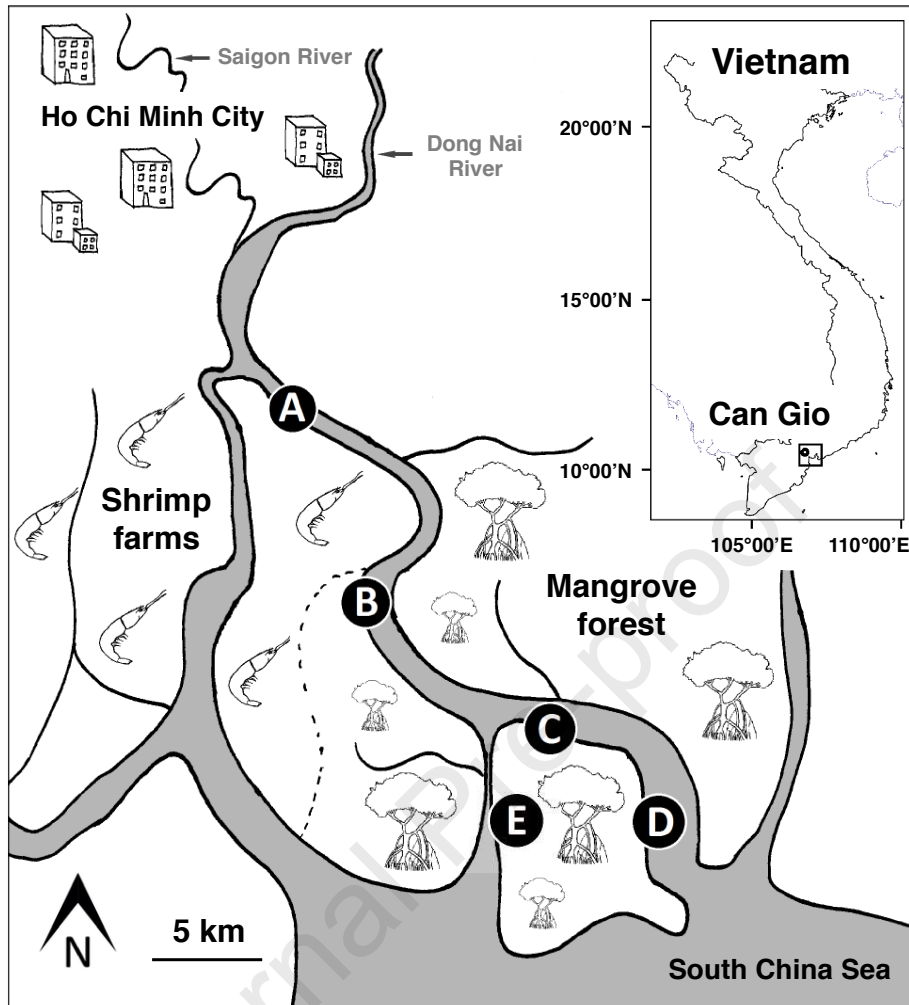
509

510 Fig. 1: Map of the sampling area in the Can Gio mangrove (Southern Vietnam).

511 A, B, C, D and E indicate the sampling stations along the estuary and within the

512 tidal creek

513

Fig. 2: Spatial variability in a) & d) prokaryotic abundance b) & e) cell diameter
and c) & f) leucine-aminopeptidase exo-proteolytic activity in the sub-surface
water layer of the Can Gio mangrove, during dry season (left panel) and wet
season (right panel). For a), b), d) and e) n = 3 for each bar; for c), n = 4 for each
bar; and for f), n = 5 for each bar. Statistical differences between groups were
not tested given the low amount of data.

23
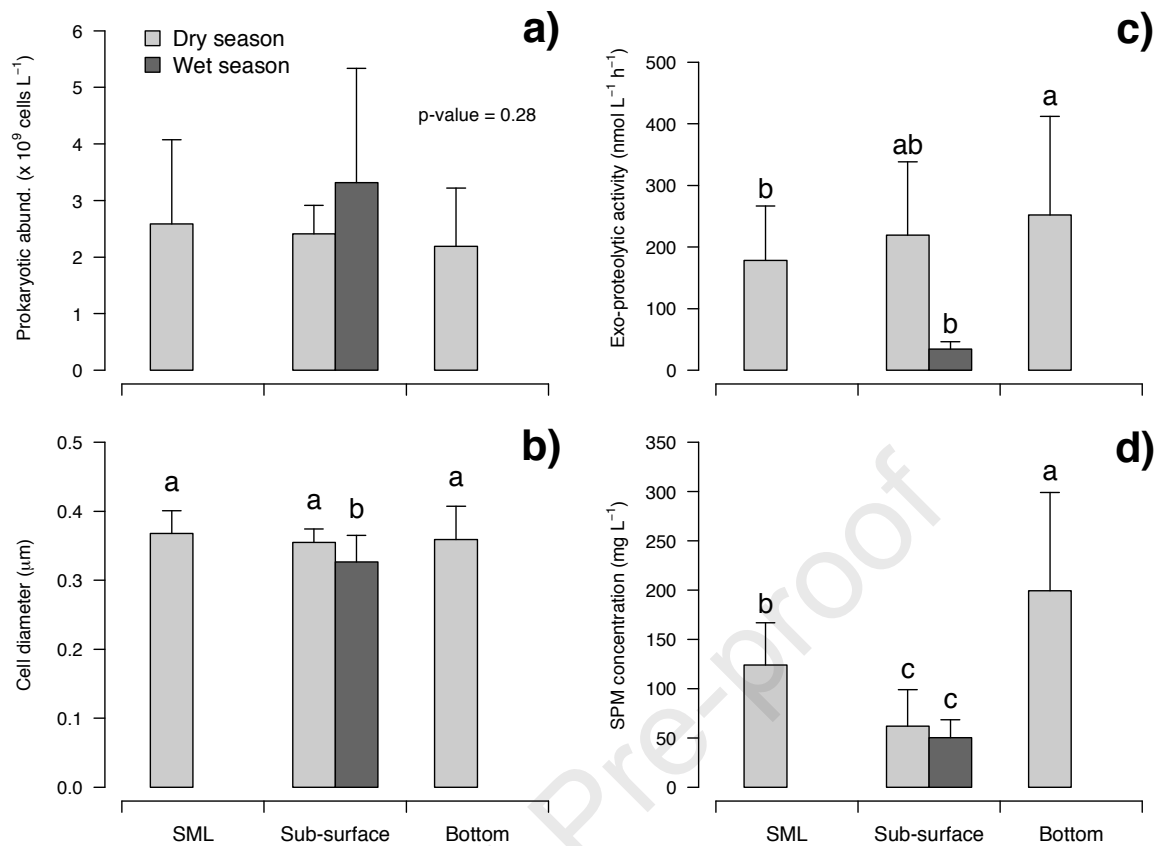
Fig. 3: Vertical variability in a) prokaryotic abundance b) cell diameter c) leucine-aminopeptidase exo-proteolytic activity and d) suspended particulate matter concentration in the three water layers of the Can Gio mangrove. For a) and b), n = 24 for each bar during the dry season and n = 15 during the wet season, and for c) and d), n = 32 for each bar during the dry season and n = 25 during the wet season. Letters indicate significant differences at α = 5% (Kruskal-Wallis rank test plus Wilcoxon pairwise comparisons with Holm-corrected α).

24

529 Table 1: Sampling plan and sample distribution

Season	Study sites	Water layers	Tidal stage	EPA and SPM			Cell counts and size		
				Replicates[a]	Total per layer	TOTAL[b]	Replicates[a]	Total per layer	TOTAL[b]
Dry	A, B, C, D	SML, sub-surface, bottom	High tide + low tide	4	32	96	3	24	72
Wet	A, B, C, D, E	Sub-surface	High tide	5	25	25	3	15	15

[a]Replicates are different water samples collected at 5-min intervals

[b]TOTAL is the total number of samples

530

Highlights

- Exo-proteolytic activity is similar to that of other highly productive ecosystems

- Bottom waters are more active that upper layers, especially the surface micro-layer

- Exo-proteolytic activity is enhanced by the input of labile organic matter

- The need to hydrolyse refractory compounds increases exo-proteolytic activity