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1 **DEPTH DISTRIBUTION OF BENTHIC DINOFLAGELLATES**

2 **IN THE CARIBBEAN SEA**

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13 Running Head: Depth distribution of benthic dinoflagellates

14

15 **Abstract**

16 Monitoring of benthic dinoflagellates is usually conducted between sub-surface and
17 5 m depth, where these organisms are supposed to be in highest abundances. However,
18 only few studies have focused on the small-scale depth distribution of benthic
19 dinoflagellates. In the present study, abundances of dinoflagellates were evaluated on an
20 invasive macrophyte *Halophila stipulacea* in two coastal sites in Guadeloupe along a depth
21 gradient from sub-surface to 3 m at Gosier and until 20 m at Rivière Sens during the
22 tropical wet and dry seasons. Depth did not influence total dinoflagellate abundance but
23 several genera showed particular depth-distribution preferences. The highest abundances
24 of *Ostreopsis* and *Gambierdiscus* species were estimated preferentially in surface waters
25 whereas *Coolia* spp. was found comparatively at depth. *H. stipulacea* biomass was
26 positively correlated with *Ostreopsis* spp. abundance. Our study suggests that sampling of
27 benthic dinoflagellates should be conducted at different water depths taking into account
28 the presence of the macroalgal substrate as well. In the Caribbean area, special attention
29 should be addressed to the presence of *H. stipulacea* which tends to homogenize the
30 marine landscape and constitutes a substrate favourable for dinoflagellates growth.

31

32 **Introduction**

33 Toxic harmful algal bloom occurrence is becoming more frequent and can cause
34 more problems on ecosystems and human health at global scale (Hallegraeff, 1993; Cloern
35 et al., 2005; Glibert et al., 2005; Hallegraeff, 2010). Several species of planktonic and
36 benthic dinoflagellates can produce ecological damages to the environment and human
37 health when they bloom in excess. Decreased levels of oxygen in the water column when
38 the blooms decay and/or production of toxins, can lead to mass mortalities of marine

39 organisms (Shears and Ross, 2009) and/or to intoxication when toxins are transferred and
40 bioaccumulated in the food web (Yasumoto et al., 1987; Holmes and Teo, 2002). Those
41 toxins are accumulated within the food chain (Yasumoto et al., 1977; Adachi and Fukuyo,
42 1979; Lewis and Holmes, 1993; Gleibs and Mebs, 1999; Aligizaki et al., 2011).
43 Phycotoxins potentially generate human intoxications through consumption of marine
44 fishery products contaminated with bio-accumulated toxins (Valdiglesias et al., 2013).
45 There are several poisoning syndromes caused by benthic dinoflagellates including,
46 gastrointestinal (nausea, vomiting, diarrhoea) and/or neurological (tingling, headaches,
47 dizziness, hallucinations, seizures) consequences (Ajani et al., 2017). These poisoning
48 syndromes are mainly due to the presence of *Ostreopsis* spp., *Prorocentrum* spp. and
49 *Gambierdiscus* spp. *Ostreopsis* spp. synthesize potent palytoxin and derivatives (Onuma et
50 al., 1999; Lenoir et al., 2004) probably responsible for clupeotoxin fish poisoning (Onuma
51 et al., 1999; Randall, 2005; Aligizaki et al., 2011) and palytoxicosis (Alcala et al., 1988) in
52 tropical areas. In temperate regions, blooms of *Ostreopsis* spp. are the causal agent of skin
53 and eye irritations (Ciminiello et al., 2006; Tichadou et al., 2010) and respiratory
54 syndromes due to exposure of aerosolized toxins or cells (Ciminiello et al., 2014).
55 *Prorocentrum* spp. produce okadaic acid and dinophysistoxins (Kumagai et al., 1986;
56 Yasumoto et al., 1987; Faust and Gullede, 2002; Nascimento et al., 2016; Luo et al.,
57 2017) causing diarrhetic shellfish poisoning for seafood consumers (Landsberg et al.,
58 2005). Ciguaterins produced by *Gambierdiscus* spp. are responsible of ciguatera fish
59 poisoning (Chinain et al., 2010; Berdalet et al., 2017). This poisoning is the most common
60 non-bacterial food-borne illness (Tester, 1994; Tester et al., 2009) associated with
61 consumption of several fish species (Bagnis, 1981; Tester et al., 2009; Dickey and Plakas,
62 2010). Ciguatera can lead to death in the most severe cases (Friedman et al., 2008). *Coolia*
63 spp. and *Amphidinium* spp., synthesize toxins that can affect marine life but the

64 bioaccumulation of these toxins through marine food chain and human poisoning have not
65 been proven (Holmes et al., 1995; Botana, 2014; Ben-Gharbia et al., 2016). To our
66 knowledge, effects of *Sinophysis* spp. on human health have not been documented yet.

67 Blooms of benthic toxic dinoflagellates generates also economic problems for
68 fishermen and aquaculture (Bagnis, 1981; Shumway, 1990; Bauder et al., 2001; Heredia-
69 Tapia et al., 2002; Berdalet et al., 2015) whose consequences are difficult to quantify
70 (Ahmed, 1991). For instance in Australia and French Polynesia the strategy to protect
71 human health consisted, among other actions, on a decree banning the fishing and selling
72 of several fish species (Bagnis, 1981; Lehane and Lewis, 2000) as their contamination
73 cannot be easily measured (Ahmed, 1991). In Guadeloupe Archipelago selling of several
74 fish species, known to cause sanitary problems, is prohibited. It is presently suspected that
75 new fish species could be potential poisoning vectors representing a supplementary
76 shortfall for Caribbean fisherman. For environmental, sanitary and economic reasons it is
77 important to set up long time-scale monitoring of benthic dinoflagellates considering
78 physicochemical parameters to know precisely the ecological niches of each species and to
79 allow an efficient risk management due to toxic dinoflagellates.

80 *Ostreopsis*, *Prorocentrum*, *Gambierdiscus*, *Coolia*, *Amphidinium*, *Sinophysis*
81 species are present in the Caribbean Sea (Ballantine et al., 1988; Morton and Faust, 1997;
82 Faust, 2009; Chomérat, 2016). Usually, samplings are restricted to shallow depths, from
83 the surface to 5 m depth (Chang et al., 2000; Okolodkov et al., 2007; Parsons and Preskitt,
84 2007; Mangialajo et al., 2008; Shears and Ross, 2009; Rahman Sha et al., 2014) where
85 benthic dinoflagellates are supposed to be in highest abundances because they are
86 considered to be mainly photosynthetic (Taylor, 1985; Faust, 1997; Ten-Hage et al., 2000;
87 Fraga et al., 2008; Fraga and Rodríguez, 2014). However, these organisms are not
88 necessarily restricted to the sub-surface as they can complement their autotrophic

89 behaviour with the uptake of organic matter (Burkholder et al., 2008; Pistocchi et al., 2011;
90 Jauzein et al., 2017).

91 Only few studies focused on depth distribution of benthic toxic dinoflagellates. A
92 first approach is to consider several sites with different depths (Taylor, 1985; Richlen and
93 Lobel, 2011) but observed differences can be due to local environmental conditions rather
94 than depth effects. A way to limit this potential bias is to collect samples at different depths
95 in a single area. The comparison at two different depths of the abundance of
96 *Gambierdiscus* revealed a decrease in the first 10 m (Xu et al., 2014) and stability between
97 depths of 10 and 20 m (Loeffler et al., 2015). A better way to evaluate the depth effect is to
98 set up transects of sampling along a depth gradient. The study by Totti *et al.* (2010) was
99 the only one considering a single substrate with this approach. As macrophytes are not
100 often homogeneously distributed along this gradient, several macrophytes species are
101 usually collected (Delgado et al., 2006; Cohu and Lemée, 2012; Cohu et al., 2013). When
102 dinoflagellate abundances are coming from different macrophytes, dinoflagellates
103 abundance comparisons are difficult because normalizing cell counts to the weight of the
104 macroalgal host introduces a significant source of error due to different surface area to
105 mass ratios of each host algal species (Richlen and Lobel, 2011). Previous studies have
106 suggested a host preference of dinoflagellates depending on the macrophyte morphology
107 (Parsons and Preskitt, 2007; Totti et al., 2010), taxonomic group (Morton and Faust, 1997;
108 Delgado et al., 2005; Monti et al., 2007; Parsons et al., 2017) or species (Ballantine et al.,
109 1985; Delgado et al., 2005). Furthermore, such macroalgae could produce molecules
110 stimulating or inhibiting growth of benthic dinoflagellates (Grzebyk et al., 1994; Morton
111 and Faust, 1997; Accoroni et al., 2015). Such allelopathic interactions between macroalgae
112 and benthic dinoflagellates can induce bias in abundances comparisons. Several methods
113 were recently set up to minimize bias due to host preferences and avoid macrophyte

114 destruction. Artificial substrates need 24h of incubation to have a comparable colonization
115 between them and macrophytes at the same sampling locations (Tester et al., 2014; Jauzein
116 et al., 2016). However, this method is collecting resuspended dinoflagellates without direct
117 contact with the benthic stock of microalgal population (Jauzein et al., 2016) but a positive
118 correlation was found between planktonic and benthic abundances in several studies
119 (Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2011). Benthic dinoflagellates
120 integrator called “BEDI” (Mangialajo et al., 2017) and syringe (Abbate et al., 2012)
121 methods are faster than artificial substrates but they would be difficult to adapt to low
122 abundances of dinoflagellates in tropical regions.

123 The aim of the present study is to describe the natural depth distribution of harmful
124 benthic dinoflagellates at genus level, in the Caribbean Sea. To avoid variations due to
125 spatial ecosystem heterogeneity and variations linked to biotic substrates, a single
126 macrophyte species, *Halophila stipulacea* Fosskal, was regularly sampled along a depth
127 gradient in two sites in Guadeloupe. *H. stipulacea* is an invasive seagrass noticed for the
128 first time in 2002 in Grenada (Ruiz and Ballantine, 2004), now established in the Eastern
129 Caribbean (Willette et al., 2014) and presenting large mono-specific meadows (Willette
130 and Ambrose, 2012). This seagrass is native from the Red Sea and Persian Gulf (den
131 Hartog, 1970). Rapid lateral bed expansion combined with its tolerance for a wide
132 spectrum of environmental conditions allows *H. stipulacea* to exclude dominant seagrasses
133 in the Caribbean (Willette and Ambrose, 2012). To our knowledge enumerations of
134 benthic dinoflagellates have never been done on *Halophila stipulacea*.

135

136 **Material and method**

137 Sampling of *H. stipulacea* was conducted in the morning by scuba diving during the
138 wet season on September 21st and 22nd 2015 and during the dry season on February 1st and
139 2nd respectively at Gosier (16°13'25.1"N, 61°31'50"W) and Rivière Sens (15°58'51.8"N,
140 61°42'59.2"W) in Guadeloupe (French West Indies) (Fig. 1). Sampling was conducted
141 according to the distribution and availability of *H. stipulacea* in each site. In the shallow
142 site (Gosier), samples were collected every 0.5 m from 0 m depth to 3 m depth as *H.*
143 *stipulacea* was absent deeper. In the deep site (Rivière Sens), samples were collected every
144 0.5 m from 4 m to 10 m depth then every 5 m until 20 m depth as *H. stipulacea* was absent
145 from the surface to 4 m depth. Samples were taken in triplicate (n=3) at each sampled
146 depth. In Guadeloupe, tides are semidiurnal with a mean tidal amplitude of 30 cm (tide
147 gauge of Pointe-à-Pitre, REFMAR®).

148 *Abundance of benthic toxic dinoflagellates*

149 For each depth, triplicate samples of *Halophila stipulacea* with their surrounding
150 water were taken carefully in 250 mL plastic flasks avoiding microalgae resuspension from
151 the macroalgae. Acidic Lugol at 1 % (vol/vol) was added in all samples to fix microalgae
152 and 10 seconds agitation allowed to dislodge benthic dinoflagellates present on
153 macrophyte. Samples were passed through a 500 µm mesh to separate the macrophyte
154 from water containing dinoflagellates. To recover a maximum of dinoflagellates, *H.*
155 *stipulacea* was rinsed twice for 10 seconds with 100 mL of filtered seawater and then
156 weighted. Total seawater volume was measured. Samples were stocked in the dark at 4 °C.
157 Benthic dinoflagellates present in 1 mL were counted with a Sedgewick Rafter © counting
158 cell using a standard light microscope less than one week after the sampling. This
159 abundance and macrophyte's fresh weight allowed calculation of the number of benthic

160 toxic dinoflagellates per gram of fresh weight of *H. stipulacea* (cells.gFW⁻¹). An average
161 cellular abundance was estimated per depth, for all species of benthic toxic dinoflagellates
162 ($n = 3$).

163

164 *Density of benthic dinoflagellates and leaf surface of H. stipulacea*

165 All *H. stipulacea* present in 20 x 20 cm square surface were sampled in triplicate at
166 each depth and kept in the dark at -4°C. *H. stipulacea* content of each square was weighed
167 after defrosting, rinsing and drying with absorbent paper and an average biomass of
168 macrophyte per square meter was calculated for each depth. Measurement of *H. stipulacea*
169 weight was used to estimate the density of benthic dinoflagellates per square meter of sea
170 bottom (cells.m⁻²).

$$\text{cells. m}^{-2} = \frac{10\,000 (\textit{H. stipulacea} \text{ biomass})}{400} \times \textit{cells. gFW}^{-1}$$

171

172 Thirty entire limbs of *H. stipulacea* of each triplicate were measured (length and width)
173 with a calliper. Measurement of leaf length and width was used to estimate leaf surface
174 considering rectangular geometric shape of each leaf.

175 *Measurement of ecological factors*

176 A sample of 250 ml of seawater surrounding *H. stipulacea* was used to measure
177 environmental factors at every depths. Temperature was measured immediately with a
178 Checktemps 4 HANNA thermometer and salinity was determined in the laboratory with a
179 Master-S/MilliM ATAGO ® manual refractometer. A SCUBAPRO Aladin Tec 3G dive
180 computer was used to measure depth of sea bottom.

181 *Data analysis*

182 Non-parametric tests were used as variances of *H. stipulacea* biomasses and benthic
183 dinoflagellates abundances were not normally distributed. Kruskal-Wallis tests were
184 utilised to assess *H. stipulacea* biomass and benthic dinoflagellates abundances related to
185 depth. Dunn test is a multiple comparison method permitting to compare the mean of the
186 rank of each treatment after a Kruskal-Wallis test. The normal distribution is used as the
187 asymptotic distribution of the standardized difference of the mean of the ranks. Mann
188 Whitney test was performed to assess (i) differences in temperature and salinity between
189 the wet and the dry seasons, (ii) variations of *H. stipulacea* biomass between both seasons
190 and (iii) to determine influence of seasons on abundances of dinoflagellates. Spearman
191 correlation tests were applied to determine potential relationships between environmental
192 parameters and dinoflagellate abundances. All descriptive analyses are presented as mean
193 \pm standard deviation (SD).

194

195 **Results**

196 *Ecological parameters at Gosier and Rivière Sens*

197 At Gosier, average temperature of water column during the wet and dry seasons
198 varied from $30.9 \pm 0.5^{\circ}\text{C}$ to $27.3 \pm 0.3^{\circ}\text{C}$ and salinity fluctuated from 30 ± 1 to 35 ± 0
199 (Figure 2). At Rivière Sens, seawater temperature varied from $29.4 \pm 0.1^{\circ}\text{C}$ to $26.5 \pm 0.4^{\circ}\text{C}$
200 and salinity from 31 ± 1 to 36 ± 0 during the two sampled seasons. At both sites,
201 temperatures were warmer during the wet than the dry season ($p < 0.01$) and salinity was on
202 average higher during the dry than the wet season ($p < 0.003$).

203 *Biomass and leaf surface of Halophila stipulacea*

204 At Gosier, average biomass of *H. stipulacea* did not change with depth during the
205 wet and dry seasons ($p < 0.051$). *H. stipulacea* biomass averaged $803 \pm 392 \text{ g.m}^{-2}$ and was
206 statistically not different during the two seasons ($p = 0.564$) (Figure 3). At Rivière Sens,
207 average biomass of *H. stipulacea* did not change with depth during the dry season but it
208 was higher at depth during the wet season ($p = 0.008$) (Fig 3). Biomass of *H. stipulacea* was
209 higher at the dry ($1\,075 \pm 445 \text{ g.m}^{-2}$) than the wet season ($528 \pm 329 \text{ g.m}^{-2}$) ($p < 0.0001$).

210 Leaf surface of *H. stipulacea* significantly increased with depth at both sites
211 ($p = 0.0001$). At Gosier, leaf surfaces were statistically higher at 2.5 m depth ($3.4 \pm 1.1 \text{ cm}^2$)
212 than these collected at 0 m and 0.5 m depth ($2.1 \pm 0.7 \text{ cm}^2$). At Rivière Sens, the highest
213 leaf areas were at 15 m and 20 m depths ($2.9 \pm 1.3 \text{ cm}^2$) and smallest between 4 m and 7
214 m, and at 10 m depths ($1.5 \pm 0.5 \text{ cm}^2$) (Figure 3).

215 *Abundances of benthic dinoflagellates and depth distribution*

216 *Ostreopsis* spp. , *Prorocentrum* spp., *Coolia* spp., *Amphidinium* spp. and *Sinophysis*
217 were found during this survey. *Ostreopsis* spp. and *Prorocentrum* spp. were found in
218 higher abundances than the other genera.

219 At Gosier, abundances of benthic dinoflagellates decreased with depth during the wet
220 season ($p = 0.008$). The highest average abundances of benthic dinoflagellates were
221 observed at 0 m depth ($2079 \pm 831 \text{ cells.gFW}^{-1}$) while no cells were found at 3 m depth
222 (Figure 4). *Ostreopsis* was the dominant genus reaching $1\,669 \pm 1\,027 \text{ cells.gFW}^{-1}$ at 0 m
223 depth and $120 \pm 17 \text{ cells.gFW}^{-1}$ at 1 m depth. For similar depths, abundances of
224 *Prorocentrum* were respectively 262 ± 110 and $77 \pm 52 \text{ cells.gFW}^{-1}$. Abundances of
225 *Gambierdiscus* spp. decreased from $113 \pm 104 \text{ cells.gFW}^{-1}$ at 0 m depth to 79 ± 43
226 cells.gFW^{-1} at 2.5 m depth. Maximum abundances were observed at 0 m depth for
227 *Amphidinium* spp. ($18 \pm 16 \text{ cells.gFW}^{-1}$), at 1 m depth for *Coolia* spp. ($22 \pm 21 \text{ cells.gFW}^{-1}$)

228 ¹) and at 1.5 m depth for *Sinophysis* spp. (9 ± 16 cells.gFW⁻¹). Abundances of benthic
229 dinoflagellates changed also with depth during the dry season ($p=0.026$). The highest
230 abundances were found at 1.5 m depth while no cell was found at 2.5 m. *Prorocentrum*
231 spp. dominated other genera of benthic dinoflagellates (Figure 4). The highest average
232 abundances of *Ostreopsis* spp and *Prorocentrum* spp. were respectively at 0.5 m depth (75
233 ± 62 cells.gFW⁻¹) and 1.5 m depth (939 ± 718 cells.gFW⁻¹) . Abundances of
234 *Gambierdiscus*, *Coolia*, *Amphidinium* and *Sinophysis* genera were constant during both
235 seasons. They never exceeded an average abundance of 30 cells.gFW⁻¹. Abundances of
236 benthic dinoflagellates did not differ between the wet and the dry seasons ($p=0.150$).

237 At Rivière Sens, abundances of benthic dinoflagellates changed with depth during
238 wet season ($p=0.036$). The highest abundances were observed at 7 and 8 m depths ($753 \pm$
239 238 cells. gFW⁻¹) and lowest at 20 m depth (149 ± 82 cells.gFW⁻¹) (Figure 5).
240 *Prorocentrum* spp. dominated the benthic dinoflagellate assemblage. The highest mean
241 abundances were observed during the sampled period at depth of 4 m for *Ostreopsis* spp.
242 (30 ± 51 cells. gFW⁻¹), 5 m for *Sinophysis* spp. (28 ± 30 cells.gFW⁻¹), 7 m for *Sinophysis*
243 spp. (28 ± 30 cells. gFW⁻¹), 8 m for *Prorocentrum* spp. (676 ± 254 cells.gFW⁻¹), 8 - 9 m
244 for *Coolia* spp. (60 ± 42 cells.gFW⁻¹) and 15 m for *Amphidinium* spp. (31 ± 31 cells. gFW⁻¹).
245 ¹). Abundances of benthic dinoflagellates varied with depth also during the dry season
246 ($p=0.003$). The highest abundance was at 4 m ($1\ 850 \pm 656$ cells.gFW⁻¹) and lowest was at
247 20 m depth (26 ± 45 cells.gFW⁻¹) (Fig 5). *Ostreopsis* spp dominated the community of
248 benthic dinoflagellates overall. From 4 to 6 m *Ostreopsis* spp. dominated *Prorocentrum*
249 spp. with respective abundances of 930 ± 433 cells gFW⁻¹ and 619 ± 282 cells.gFW⁻¹
250 whereas from 7 to 9 m *Prorocentrum* spp. dominated *Ostreopsis* spp. with respective
251 abundances of 307 ± 143 cells gFW⁻¹ and 181 ± 91 cells.gFW⁻¹. The highest abundances
252 were observed at depth of 6 m for *Coolia* spp. (59 ± 67), 7 m for *Sinophysis* spp. (21 ± 36),

253 8 m for *Gambierdiscus* spp. (16 ± 14) and 9 m for *Amphidinium* spp. (20 ± 22). The
254 abundances for these genera were low.

255 Abundances of benthic dinoflagellates were similar during the wet and the dry season
256 ($p=0.387$).

257 Preferential depth of *Ostreopsis* spp. and *Prorocentrum* spp. changed according to
258 the dominant genus. *Prorocentrum* spp. present a peak of abundance deeper than
259 *Ostreopsis* spp. when *Prorocentrum* spp. dominated the benthic dinoflagellates community
260 (Gosier during the dry season and Rivière Sens during the wet season). Furthermore, peak
261 abundances of *Prorocentrum* spp. and *Ostreopsis* spp. occurred at the same depth when
262 *Ostreopsis* spp. were dominant in the microalgae community (Gosier during the wet season
263 and Rivière Sens during the dry season). Concerning *Coolia* spp., *Amphidinium* spp. and
264 *Sinophysis* spp., the depth of the peak abundances changed between the seasons at Riviere
265 Sens. Peak abundances of *Coolia* spp. and *Amphidinium* spp. were deeper during the wet
266 season than the dry season at Rivière Sens. The highest abundances of *Coolia* spp. and
267 *Amphidium* spp. were found respectively at 8-9 m depths and 15 m respectively during the
268 wet season while highest abundances of these genera were at 6 m depth and 8 m depth
269 respectively. However, the depth of peak abundance of *Sinophysis* spp. was shallowest
270 during the wet season (5 m depth) than the dry season (8 m depth). *Coolia* spp.,
271 *Amphidinium* spp. and *Sinophysis* spp. have not been found during the dry season at
272 Gosier.

273

274 *Temperature, salinity and benthic dinoflagellates*

275 Highest *Ostreopsis* spp. abundances occurred for the wet season at Gosier with an
276 optimal salinity of 31.3°C and 31.4°C while the highest abundance of *Ostreopsis* spp. was
277 observed at 26.8°C for the wet season at Rivière Sens. *Ostreopsis* spp. abundances

278 occurred in maximal abundance during the dry season when evaporation was maximal and
279 with a water salinity of 36. Highest abundances of *Prorocentrum* spp. were observed
280 during the dry season with a temperature of 27.4°C and a salinity of 35. The highest
281 abundance of *Gambierdiscus* spp. were found at the shallow site (Gosier) during the wet
282 season when seawater temperature was the warmest above 30°C and when salinity was of
283 30.

284 The highest abundances of *Coolia* spp. were found during the warmest season (the wet
285 season), when temperature was included between 29.4°C and 31°C with a salinity of
286 seawater of 30. Highest abundances of *Amphidinium* spp. were found at Gosier and at
287 Rivière Sens during the wet season when temperature and salinity were above 29°C and 30
288 respectively. Highest abundances of *Sinophysis* spp; were found at Riviere Sens, the
289 deepest site when temperature was comprised between 26.8°C and 29.8°C and salinity
290 included 30-36.

291 *Interaction between ecological parameters and benthic dinoflagellates*

292 Relation between environmental parameters measured at both sites (Rivière Sens and
293 Gosier) and benthic dinoflagellates abundances (cells.gFW⁻¹) has been analysed together
294 with a Spearman correlation. None of the studied environmental parameters were
295 significantly linked with total average abundances of benthic toxic dinoflagellates but they
296 influenced several genera independently. The depth, salinity and biomass of *H. stipulacea*
297 (g.m⁻²) were weakly correlated with abundance of benthic dinoflagellates (cells.gFW⁻¹).
298 The depth was negatively correlated with *Ostreopsis* spp. and *Gambierdiscus* abundances
299 while the depth was positively correlated with *Amphidinium* spp.. The salinity was
300 positively correlated with *Ostreopsis* spp. and negatively correlated with *Gambierdiscus*
301 spp. abundances. The temperature was positively correlated with *Gambierdiscus* spp. only

302 (Table 1). Also, a weak positive correlation between *Ostreopsis* spp. abundances and *H.*
303 *stipulacea* biomass was found.

304 Abundances of several genera of benthic dinoflagellates were weakly correlated
305 between them. *Ostreopsis* spp. abundances were correlated with *Prorocentrum* spp.,
306 *Gambierdiscus* spp., and *Amphidinium* spp.. *Prorocentrum* spp. were correlated with
307 *Coolia* spp. *Amphidinium* spp. and *Sinophysis* spp. Only weak positive correlations were
308 found between *Coolia* spp. *Amphidinium* spp. and *Gambierdiscus* spp. and total
309 abundances of benthic dinoflagellates while total abundances were strongly correlated with
310 abundance of *Ostreopsis* spp. and *Prorocentrum* spp..

311 *Density of benthic dinoflagellates*

312 At Gosier the highest abundances of dinoflagellates per square meter were observed
313 at 0 m depth ($6.9 \cdot 10^5 \pm 2.9 \cdot 10^4$ cells.m⁻²) during wet season and at 1.5 m depth ($7.0 \cdot 10^5 \pm$
314 $6.5 \cdot 10^5$ cells.m⁻²) during the dry season (p=0.037) (Figure 6). No difference of total density
315 of benthic dinoflagellates was found between the wet and the dry season (p=0.115).

316 At Rivière Sens the highest abundances of dinoflagellates per square meter were
317 observed at 7 m and 8 m depths ($3.9 \cdot 10^5 \pm 2.0 \cdot 10^5$ cells.m⁻²) during the wet season
318 (p=0.0024) and at 6 m ($2.1 \cdot 10^6 \pm 9.1 \cdot 10^5$ cells.m⁻²) during the dry season (p=0,003). Total
319 densities of benthic dinoflagellates were similar between the wet and the dry season.
320 (p=0.053).

321

322 **Discussion**

323 *Influence of ecological factors*

324 This study examined the depth effect on abundances of epiphytic dinoflagellates.
325 Samples were collected on similar natural substrate at different depths in Guadeloupe at

326 Gosier and Rivière Sens. This approach was possible thanks to the presence of
327 monospecific meadows of *H. stipulacea* along a depth gradient at both sites. However,
328 none area presented a continuous populations of *H. stipulacea* from the surface to 25 m
329 depth. Distribution of dinoflagellates was consequently observed in shallow depth at
330 Gosier and deeper at Riviere Sens.

331 This is a novel study, because no dinoflagellate census has been realized in
332 Guadeloupe so far. Only genera were determined in this study due to morphological
333 identification difficulties leading to determination confusions. Morphogenetic analysis of
334 benthic dinoflagellates present in Guadeloupe and Martinique are under investigation.
335 Furthermore, to our knowledge enumerations of benthic dinoflagellates have never been
336 done on *Halophila stipulacea*,

337 Among the studied parameters, the depth was the principal factor affecting
338 *Ostreopsis* spp. distribution with higher abundances found at the lower depths sampled.
339 Similar trends on depth distribution were also observed in the Pacific Ocean (Richlen and
340 Lobel, 2011) and the Mediterranean Sea (Totti et al., 2010; Cohu et al., 2013) potentially
341 linked with light intensity (Totti et al., 2010). However, this study did not allow to identify
342 separately effects of light intensity and depth on *Ostreopsis* spp. distribution. *Ostreopsis*
343 spp. occurred in maximal abundances with higher salinity in present survey. Indeed, the
344 optimal salinity for growth of *Ostreopsis* spp. in the Caribbean area was 33 (Morton et al.,
345 1992). Also, the temperature was not an ecological factor correlated to abundances of
346 *Ostreopsis* spp. in this study. However, maximal abundances of *Ostreopsis* spp. were
347 included between 26.8°C and 31.4°C in this survey while optimal growth for this genus
348 were 25°C (Morton et al., 1992). The temperature was not found to be contributing to
349 *Ostreopsis* spp. seasonal fluctuations in the Caribbean area (Ballantine et al., 1988;
350 Okolodkov et al., 2007) as in temperate waters (Vila et al., 2001). However, in other

351 studies conducted in the Mediterranean Sea, the highest abundances of *Ostreopsis* spp.
352 were found when surface seawater temperature was the highest (Aligizaki and Nikolaidis,
353 2006; Mangialajo et al., 2008).

354 The depth is the environmental factor with the lowest influence on *Gambierdiscus* spp.
355 abundances. According to different studies, abundances of *Gambierdiscus* were not
356 affected by depth, increased with depth (Richlen and Lobel, 2011) or decreased with
357 depth (Taylor, 1985; Xu et al., 2014). In the present study, abundances were higher in
358 shallow environments. The same trend existed in Pacific Ocean where *Gambierdiscus* spp.
359 abundance at 2–3 m depth exceeded abundances at 10–15 m depth (Xu et al., 2014).
360 Similar distributions were observed in different islands of the Caribbean Sea (Taylor,
361 1985) with a peak of abundance between 0.5 m and 3 m depth. It has been suggested that
362 absence of *Gambierdiscus* spp. in surface water could be explained by decreased salinity
363 due to rainy events (Taylor, 1985). This explanation can be excluded for this study as
364 increased salinity during the dry season at Rivière Sens has not led to increased
365 abundances of *Gambierdiscus* spp.. Furthermore, highest abundances of *Gambierdiscus*
366 spp. were found in this study during the wet season (season with the lowest seawater
367 salinities) and at depths where the salinity was the lowest during this period suggesting
368 seasonal salinities have more impact than salinity variations with depth. Also, the highest
369 abundances of *Gambierdiscus* spp. were found at Gosier during the wet season and when
370 the seawater temperature was the warmest of this study (above 30°C). Optimal growth of
371 *Gambierdiscus* spp. from the Caribbean region was observed at conditions close to the
372 environment conditions with temperature of 29°C and salinity of 30 (Morton et al., 1992).
373 Abundances of *Gambierdiscus* spp. found in this study were particularly low contrary to
374 monitoring conducted in the Caribbean area (Ballantine et al., 1988, 1985; Bomber et al.,
375 1989). Seasonal fluctuations of *Gambiersicus* spp. are still unknown in Guadeloupe.

376 A positive correlation existed between *Coolia* spp. abundances and depth in this study. In
377 the Mediterranean Sea this genus was observed at depths higher than 3 m (Cohu and
378 Lemée, 2012). *Coolia* spp. distribution is often neglected in studies conducted in the
379 tropical areas. Furthermore, among ecological study carried out in tropical areas and
380 focusing on the depth none study has showed distribution of *Coolia* spp.. Only information
381 about low abundances of *Coolia* spp. ($< 1\ 000\ \text{cells.gFW}^{-1}$) have been found in these
382 studies (Delgado et al., 2005; Xu et al., 2014). *Coolia* spp. present in the Caribbean area
383 exhibited optimal growth with a salinity of 33 and a temperature of 29°C (Morton et al.,
384 1992). The results of this ecological study corroborated partially results of this study
385 because highest abundances of *Coolia* spp. were found during the warmest period (the wet
386 season), when temperature was included between 29.4°C and 31°C and with a salinity of
387 seawater of 30. *Coolia* spp. were first reported to synthesize toxins in the early work
388 (Holmes et al., 1995) but none strains examined by Penna et al., (2005) were toxic and
389 Rhodes et al., (2000) have found both toxic and nontoxic strains. The toxicity variation of
390 *Coolia* spp. is difficult to interpreted and cannot be linked with a problem of identification
391 because taxonomic problems have not been reported for this genus (Penna et al., 2005).
392 Effects of *Coolia* spp. on human health are still unknown (Zingone et al., 2006).
393 ~~In fact, despite some initial studies suggested that the species produced cooliatoxin.~~
394 ~~(Holmes et al., 1995), further tests indicated that *Coolia* spp. is not toxic (Delia et al.,~~
395 ~~2015).~~
396 Higher abundances of *Amphidinium* spp. were found in this survey with different
397 temperature and salinity promoting optimal growth of *Amphidinium* spp. Indeed, the
398 ecological study conducted on Caribbean benthic dinoflagellates has found an optimal
399 growth temperature between 26°C and 28°C and a salinity of 34. This genus was found in

400 lowest abundances in Republic of Kiribati (0-12 cells.gFW⁻¹) but distribution of
401 *Amphidinium* spp. has not been studied according to the depth (Xu et al., 2014)
402 *Sinophysis* spp. have been neglected by the ecological study.

403

404 *Species interactions*

405 The *Prorocentrum* spp. peak of abundances was always deeper than the *Ostreopsis*
406 spp. peak of abundance when *Prorocentrum* genus dominated the dinoflagellate
407 community. However peaks of abundance of *Ostreopsis* and *Prorocentrum* genera
408 occurred in surface and at the same depth when *Ostreopsis* spp. dominated the benthic
409 dinoflagellates assemblage. Richlen and Lobel (2011) suggested habitat separation
410 between both genera. Nevertheless, in this study abundances of *Ostreopsis* spp. and
411 *Prorocentrum* spp. were positively correlated, suggesting common preferences and
412 possible competition phenomena and/or allelopathic interactions. The temporal
413 fluctuations of benthic dinoflagellates are still unknown in Guadeloupe. However,
414 monitoring conducted in the Caribbean Sea and the Gulf of Mexico have observed a
415 characteristic dominance of *Prorocentrum* spp. in the benthic dinoflagellate assemblage
416 (Delgado et al., 2005; Okolodkov et al., 2014; Martinez-Cruz et al., 2015; Morton and
417 Faust, 1997). These previous studies support the dominance of *Prorocentrum* spp. at
418 Gosier and at Rivière Sens during the dry and the wet season respectively in this study.
419 Despite the dominance of *Ostreopsis* spp. seems to be unusual in the Caribbean Sea, this
420 prevalence was found in few Caribbean monitoring however none *Prorocentrum* spp.
421 abundance were mentioned (Ballantine et al., 1988). These results sustain dominance of
422 *Ostreopsis* spp. found in this study at Gosier and at Rivière Sens during the wet and the dry
423 season respectively. Furthermore, few studies have been conducted on allelopathic
424 interactions of benthic dinoflagellates (Richlen and Lobel, 2011). *Prorocentrum*,

425 *Ostreopsis*, *Gambierdiscus*, *Coolia*, and *Amphidinium* are known to synthesize
426 allelochemical components inhibiting growth of microalgae (Sugg and VanDolah, 1999;
427 Legrand et al., 2003; Graneli et al., 2008). Assimilation of nutrients and environmental
428 factors affect toxins content of benthic dinoflagellates (Pezzolesi et al., 2012). P-nutrition
429 has been shown to influence toxin production. A rapid P-uptake within few days was found
430 for *Ostreopsis* spp. (Pezzolesi et al., 2014) and *Prorocentrum* spp. (Vanucci et al., 2010)
431 suggesting *Ostreopsis* spp. and *Prorocentrum* spp. could compete. Recently, *Ostreopsis* spp.
432 was found to favour cell attachment of *Prorocentrum* spp. with a positive dose dependent
433 relationship while cell lysis was observed at the same time for *Gambierdiscus* spp.
434 (García-Portela et al., 2016). All these strategies highlight complexity of allelopathic
435 interactions used by benthic dinoflagellates and could explain different distributions of
436 dinoflagellates along the depth

437 *Gambierdiscus* spp., *Amphidinium* spp., *Coolia* spp. and *Sinophysis* spp. were
438 abundances dependent. They co-occurred in low abundances. Positive correlations were
439 also found between *Coolia* spp., *Ostreopsis* spp. and *Prorocentrum* spp., as in the
440 Mediterranean Sea (Cohu and Lemée, 2012).

441 To our knowledge enumerations of benthic dinoflagellates have never been done on
442 *Halophila stipulacea*. In the Caribbean Sea, seagrass species are known to support lower
443 dinoflagellate abundances than macroalgae (Taylor, 1985; Morton and Faust, 1997).
444 However (Okolodkov et al., 2007) found the highest abundance of *Prorocentrum* spp. (31
445 467 cells.gFW⁻¹) on *Thalassia testudinum*, a seagrass, present in the Gulf of Mexico
446 (Okolodkov et al., 2007). This is about 18 times more than the maximum of *Prorocentrum*
447 spp. found in this study. At Belize (Morton and Faust, 1997) the lowest total abundance of
448 benthic dinoflagellates was found on *T. testudinum* and was comparable with average
449 abundances found in Guadeloupe found on *H. stipulacea*.

450 *Substratum and dinoflagellates*

451 Some previous studies have suggested a host preference of benthic *Ostreopsis* spp.
452 depending on the macrophyte morphology with higher abundances on branched thalli
453 (Totti et al., 2010), on Phaeophyceae and Florideophyceae (Monti et al., 2007) and on
454 *Dictyota* sp. (Ballantine et al., 1985). However, more detailed studies on selected seagrass
455 species are still lacking (Martinez-Cruz et al., 2015). In this study, a weak positive
456 correlation was found between *Ostreopsis* spp. abundances (cells.gFW⁻¹) and *H. stipulacea*
457 biomass (g.m⁻²). An ecological study with enumeration of benthic dinoflagellates present
458 on different macrophytes and *H. stipulacea* must be conducted in Guadeloupe in order to
459 confirm a preferential association between *Ostreopsis* spp. and *H. stipulacea*.

460 At Rivière Sens, biomass and leaf surface of *H. stipulacea* increased with depth. A
461 similar pattern was also observed for this species in the Mediterranean (Procaccini et al.,
462 1999). However, availability of space for attachment on macrophyte does not seem to be
463 the main limiting factor for dinoflagellates because during the wet season their maximum
464 abundance was located at 7-8 m depths while leaf surface is maximal at 15-20 m depth. A
465 dense vegetation cover increases available surface colonisable by benthic dinoflagellates
466 but it also decreases light irradiance necessary for the photosynthesis of dinoflagellates
467 limiting their growth.

468 *Halophila stipulacea* is a seagrass grazed by fish (Mariani and Alcoverro, 1999) and
469 turtles (Becking et al., 2014). Presence of dinoflagellates at each depth on *H. stipulacea*
470 must be considered as a risk allowing the entrance of phycotoxins in the food web via
471 herbivorous behaviour regardless of depth. This invasive seagrass would contribute to
472 toxic dinoflagellates growth and persistence of ciguatera fish poisoning in the Caribbean
473 region which is the second area of the world affected by this disease (Chinain et al., 2014).
474 Monitoring of benthic toxic dinoflagellates present on *H. stipulacea* should be set also in

475 Mediterranean Sea to assess capacity of this macrophyte to support toxic benthic
476 dinoflagellates. Until 2007, *H. stipulacea* was too sparse to coexist under canopy of the
477 large native seagrasses of Mediterranean Sea (Williams, 2007). Actually mono-specific
478 meadows of *H. stipulacea* more than 2 000 m² are observed in Mediterranean Sea (Sghaier
479 et al., 2011).

480

481 **Conclusion**

482 Depth was not an environmental factor influencing the total abundance of benthic
483 dinoflagellates however it partially structured the distribution of some dinoflagellates
484 genera suggesting interaction with other parameters. Thereby, monitoring of abundance of
485 benthic dinoflagellates conducted at shallow depths may underestimate the risk due to
486 presence of different benthic dinoflagellates genera. However, this common method seems
487 to be appropriate for the Caribbean area where the main sanitary trouble is due to
488 *Gambierdiercus* spp.. presence which is found in higher abundance at shallow depth. A
489 temporal survey must be conducted at shallow depths in order to know population dynamic
490 of *Gambierdiscus spp.* in Guadeloupe Island.

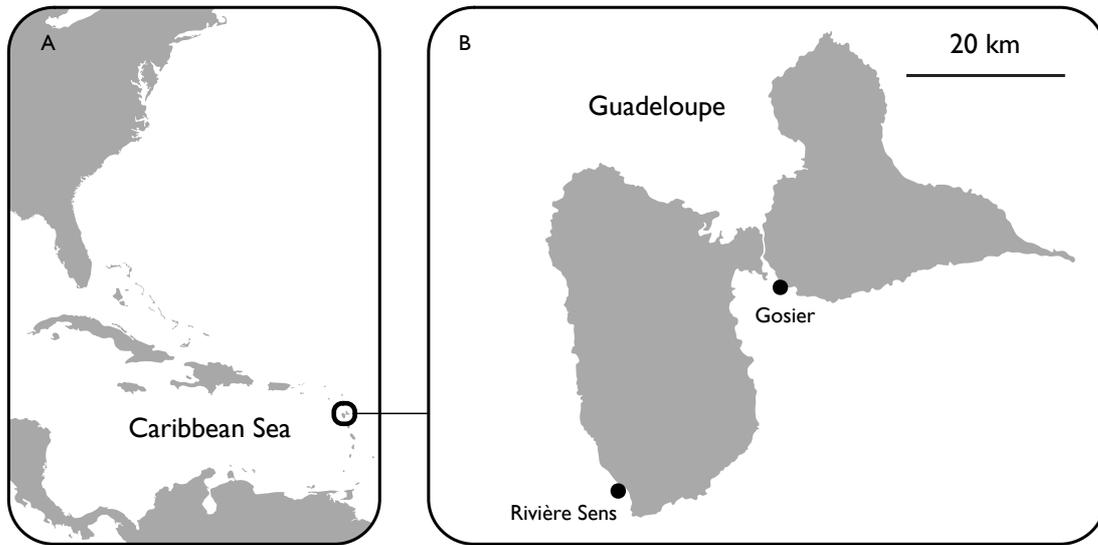
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494 study was partly funded by the PROLITENSAN project (“Fondation de France”) and our
495 group is part of the National French GDR PHYCOTOX (CNRS and Ifremer).

496

Figure and table



497

498 Figure 1: A: Location of Guadeloupe archipelago in the Caribbean Sea, B: Location of

499 Rivière Sens (deep site) and Gosier (shallow site) in Guadeloupe.

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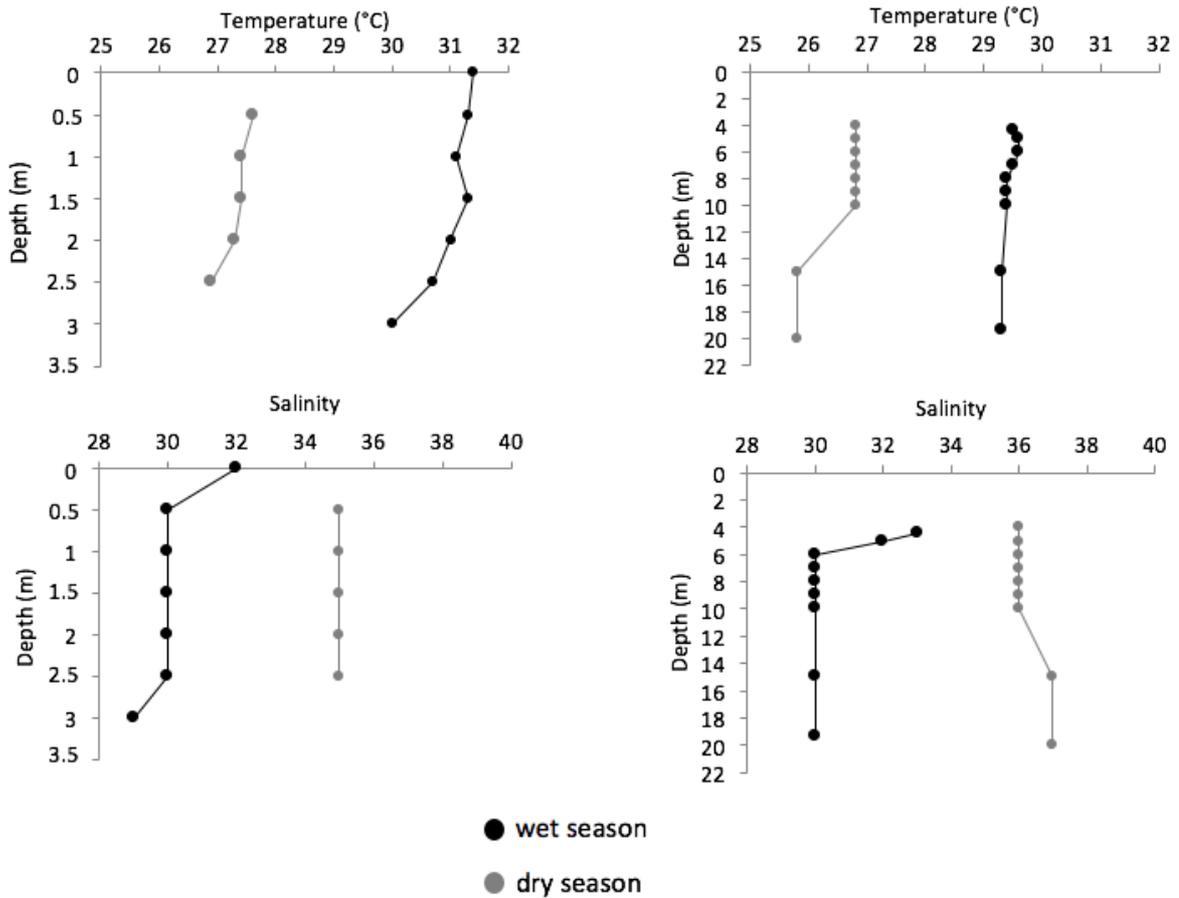
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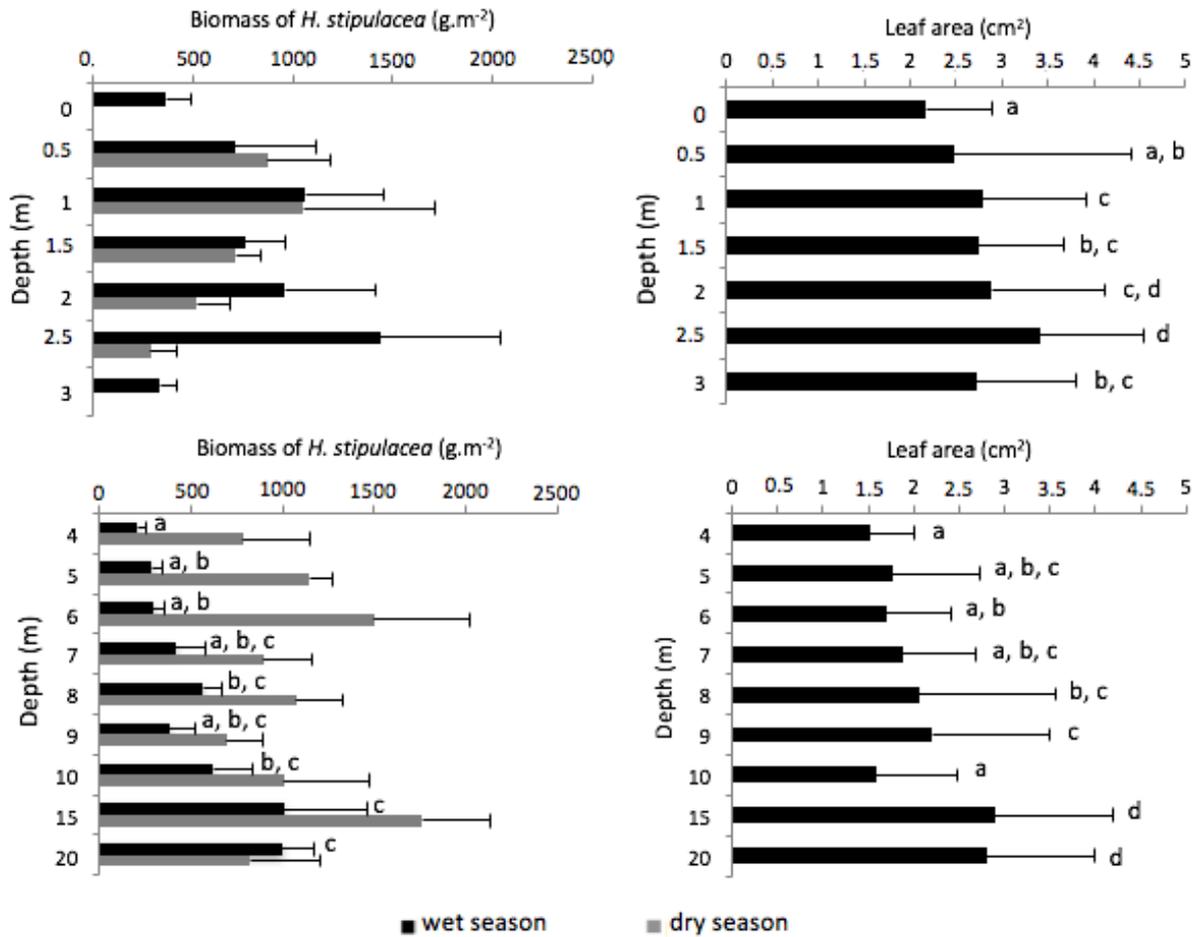


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514 Figure 2. Profiles of temperature and salinity during the wet (black) and the dry (grey)

515 seasons at Gosier (graphs on the left) and Rivière Sens (graphs on the right).

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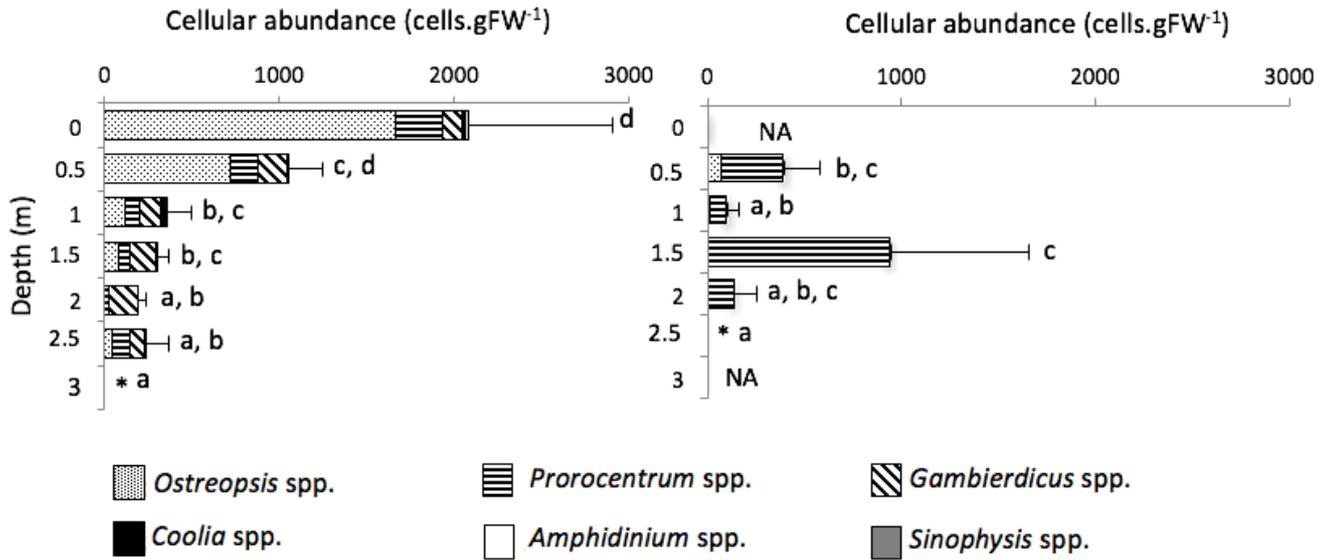
519 Figure 3: Left. Mean *Halophila stipulacea* biomass (left) and leaf surface (cm² per leaf)
 520 (right) (\pm SD, $n = 3$) at different depths at Gosier (above) and Rivière Sens (below) during
 521 the wet (black) and the dry seasons (grey). Significant differences between depths are
 522 indicated with letters (Kruskal Wallis test and Dunn test, $\alpha=0,05$).

523 It should be noticed that, in each station, the trend of *H. stipulacea* exhibits different trends
 524 in distribution with depth in the wet than in the dry season.

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529 Figure 4: Abundance of benthic toxic dinoflagellates with depth during the wet (left) and
 530 the dry (right) season at Gosier. “*” indicates no cell found but depth sampled and “NA”
 531 data no available because no macrophyte was found. Significant differences between
 532 depths are indicated with letters (Kruskal-Wallis test and Dunn test, $\alpha=0,05$).

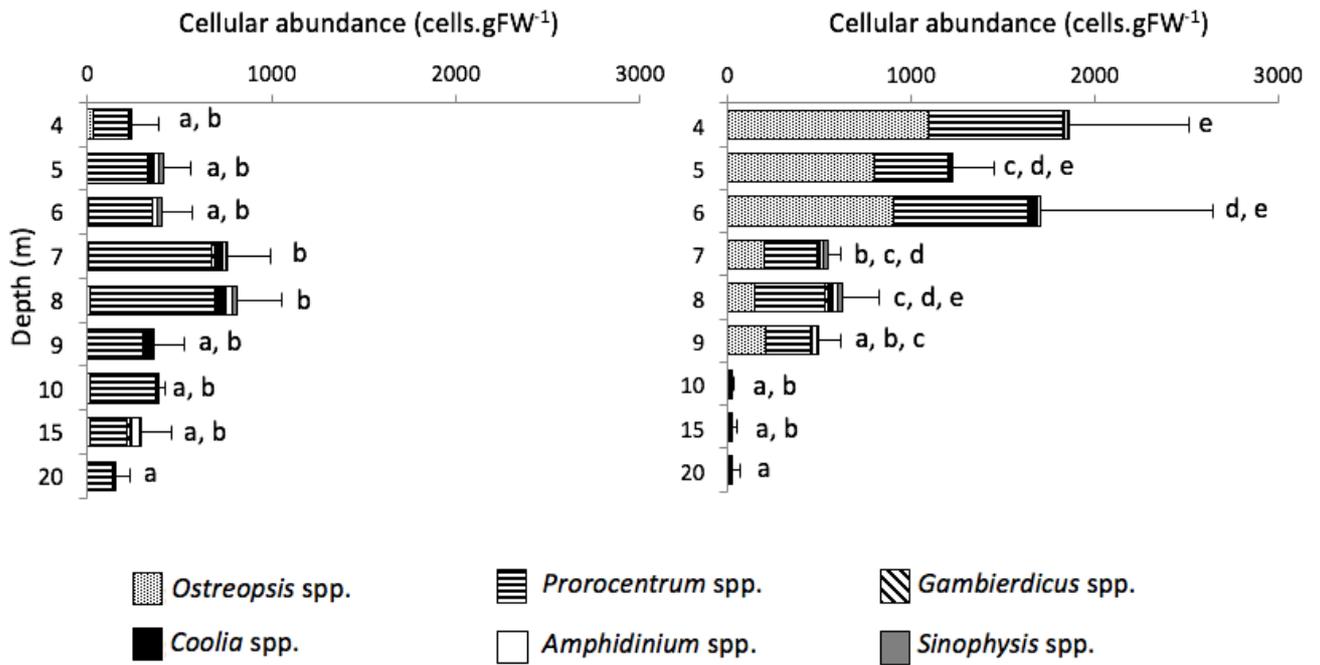
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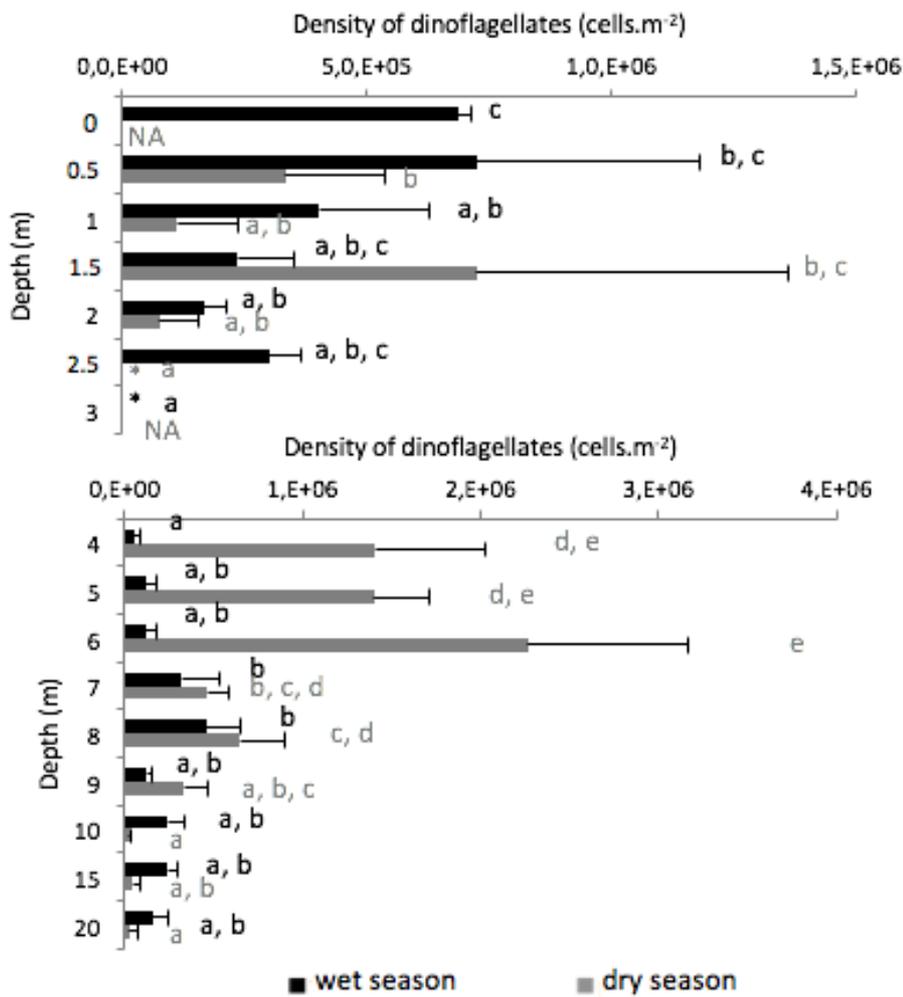
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540 Figure 5: Abundances of benthic toxic dinoflagellates according to depth during the wet

541 (left) and the dry (right) season at Rivière Sens. Significant differences between depths are

542 indicated with letters (Kruskal Wallis test and Dunn test, $\alpha=0,05$).

543



544

545 Figure 6: Mean density of benthic dinoflagellates \pm SD according to depth at Gosier
 546 (above) and at Rivière Sens (below) for the wet (black) and the dry season (grey). “*”
 547 indicates no cell found but depth sampled and “NA” data no available because no
 548 macrophyte was found. Significant differences between depths are indicated with letters
 549 (Kruskal Wallis test and Dunn test, $\alpha=0,05$).

550

551

552 Table 1: Relations between depth (m), temperature (°C), salinity, *H. stipulacea* biomass
 553 (g.m⁻²), total abundances and abundances of *Ostreopsis*, *Prorocentrum*, *Gambierdiscus*,
 554 *Coolia*, *Amphidinium* and *Sinophysis* genera (cells.gFW⁻¹). Coefficient r_s of Spearman,
 555 bold when significant with p<0.05, bold and underlined when significant with p<0.01.

	<i>Ostreopsis</i> spp.	<i>Prorocentrum</i> spp.	<i>Gambierdiscus</i> spp.	<i>Coolia</i> spp.	<i>Amphidinium</i> spp.	<i>Sinophysis</i> spp.	Average abundance
Depth	-0,299	0,015	<u>-0,363</u>	0,260	0,090	0,152	-0,193
Temperature	0,008	-0,046	<u>0,558</u>	-0,064	0,088	0,022	0,096
Salinity	0,210	0,020	<u>-0,388</u>	0,060	-0,093	-0,032	0,044
H.stipulacea Biomass	0,236	-0,107	0,203	0,036	-0,021	-0,182	0,014
<i>Ostreopsis</i> spp.		<u>0,364</u>	0,284	0,037	0,263	0,120	<u>0,695</u>
<i>Prorocentrum</i> spp.			-0,139	0,223	0,220	0,298	<u>0,836</u>
<i>Gambierdiscus</i> spp.				-0,032	0,105	-0,007	0,164
<i>Coolia</i> spp.					0,047	0,094	0,295
<i>Amphidinium</i> spp.						0,248	0,273
<i>Sinophysis</i> spp.							0,242
556 Average abundance							

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