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1 **Distribution of potentially toxic epiphytic dinoflagellates in Saint Martin Island**
2 **(Caribbean Sea, Lesser Antilles)**

3
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13
14 **Abstract:** For the first time, distribution of epiphytic dinoflagellates was studied in Saint Martin
15 Island (Lesser Antilles) during the cyclonic season (between September 1st and 3rd 2015). The
16 present study provides a semi-quantitative analysis because the fresh weight of each macrophyte was
17 estimated around 10 g. The identified genera were: *Ostreopsis*, *Prorocentrum*, *Coolia*, *Amphidinium*,
18 and *Gambierdiscus* in order of decreasing abundance. Highest average abundance values of the
19 genera *Ostreopsis* and *Amphidinium* were hosted by macrophytes of the Phaeophyceae group with
20 ca. 15,000 and 60 cells g⁻¹ respectively. Epiphytic *Coolia* cells were mainly observed on seagrasses
21 with the highest average abundance value of ca. 1000 cells g⁻¹ whereas the genera *Gambierdiscus*
22 and *Prorocentrum* were principally associated with Florideophyceae with the highest average
23 abundances of ca. 70 and 1500 cells g FW⁻¹ respectively. This preliminary study indicates the most
24 relevant locations to survey the biodiversity of potentially toxic epiphytic dinoflagellates in Saint
25 Martin Island.

26

27 **Keywords:** Dinoflagellates, Caribbean Sea, *Ostreopsis*, *Gambierdiscus*, *Prorocentrum*, Ciguatera
28 Fish Poisoning (CFP)

29 **Running title:** Benthic HABs in Saint Martin Island

30

31 **Introduction**

32 Some benthic dinoflagellates are a major concern for public health due to their toxins production.
33 However, few studies focus on the taxonomy of benthic dinoflagellates (Momigliano *et al.* 2013) and
34 the diversity of the microbenthic communities remains largely unexplored especially in the
35 Caribbean Sea (Chomérat *et al.* 2018).

36 In the Caribbean Sea, *Gambierdiscus* species are the causal agent of a worry health issue, the
37 ciguatera fish poisoning (CFP) (Bagnis *et al.* 1980, Litaker *et al.* 2017, Díaz-Asencio *et al.* 2019).
38 The Caribbean Sea presents a CFP incidence rate fluctuating between 12 and 500 cases / 100,000
39 habitants and is consequently the second region of the world most affected by CFP after the Pacific
40 Ocean (Chinain *et al.* 2014). Within the Caribbean Sea, Saint Martin is the most affected island with
41 an estimated CFP incidence rate of 100 cases / 10,000 hab. in 1981 (Tester *et al.* 2010). However,
42 only a single survey focused on the abundances of the *Gambierdiscus* genus on corals in this area
43 (Bourdeau & Bagnis 1989). In another study dealing with the distribution of *Gambierdiscus* spp. in
44 the Gulf of Mexico and the Caribbean Sea, three species have been found in Saint Martin without
45 indications about the colonized substrate (Litaker *et al.* 2017). In both studies, the presence of
46 *Gambierdiscus* on macrophytes was overlooked despite their potential contribution on fluxes of
47 toxin in food web. Herbivorous organisms can be contaminated through selective ingestion of the
48 surficial biofilm containing the toxic epiphytes (Darius *et al.* 2018, Boisnoir *et al.* 2020) or through
49 ingestion of the whole macrophyte covered with toxic epiphyte dinoflagellates (Yasumoto *et al.*
50 1976, Bourdeau & Bagnis 1989, Díaz-Asencio *et al.* 2019).

51 As *Ostreopsis* and *Prorocentrum* are associated with *Gambierdiscus* in tropical areas
52 (Ballantine *et al.* 1988, Besada *et al.* 1982, Faust 2009) they were wrongly related with CFP;
53 however, they are responsible for specific poisonings. In tropical environments, the toxins
54 synthesized by the genus *Ostreopsis* are the causal agent of the palytoxicosis (Alcala *et al.* 1988) and
55 clupeotoxism (Onuma *et al.* 1999, Randall 2005) that occur respectively after the consumption of
56 crustaceans and fish bioaccumulating toxins produced by this genus. The genus *Prorocentrum*, and
57 specifically the species *Prorocentrum lima* is responsible for diarrhetic shellfish poisoning (DSP) not
58 limited to tropical areas (Tripuraneni *et al.* 1997). Among the *Ostreopsis* species morphologically
59 described in the Caribbean Sea (Faust & Morton 1995, Faust 1999) several are known to be toxic
60 (Ukena *et al.* 2001, Scalco *et al.* 2012, Accoroni *et al.* 2017) but the presence of *Ostreopsis* species
61 in this area has not been confirmed by molecular studies (Penna *et al.* 2010). Concerning the genus
62 *Prorocentrum*, 15 species sampled in the Western part of the Caribbean Sea have been described
63 morphologically in the 90's by M.A. Faust (Faust 1990a, Faust 1990b, Faust 1991, Faust 1993a,
64 Faust 1993, Faust 1994, Faust *et al.* 2008) but it is only recently that a taxonomic study conducted in
65 the Eastern Caribbean area confirmed the presence of species known to be toxic (Chomérat *et al.*
66 2018).

67 Distribution of potentially toxic benthic dinoflagellates in the Caribbean justifies special
68 attention to Saint Martin Island. Although CFP incidence rate has not been recently updated for this
69 Island, CFP is still occurring (Boucaud-Maitre *et al.* 2018) despite the regulation of fish consumption
70 by a decree (decree n°2002-1249, <https://bit.ly/2OfU8Ut>). This decree includes 3 different
71 prohibitions that concern fifteen species. Some species (*Caranx bartholomaei*, *Sphyræna*
72 *barracuda*, *Seriola dumerili*, *Seriola rivoliana*) are constantly prohibited from fishing and sale in
73 Saint Martin, Saint Barthelemy and Guadeloupe islands. Other species (*Caranx latus*, *Caranx*
74 *lugubris*, *Caranx ruber*, *Mycteroperca venenosa*, *Mycteroperca tigris*, *Alphestès afer*, *Epinephelus*
75 *morio*, *Gymnothorax funebris*) are prohibited from fishing and sale beyond the parallel 16°5N. The

76 species *Lutjanus buccanella* cannot be sold if its weight exceeds 1 Kg whatever the fishing place.

77 The species *Lutjanus jocu* adds up the two last prohibitions.

78 Genera like *Coolia*, *Amphidinium* and *Sinophysis* need to be considered even if their toxicity to
79 human health is unknown (Botana 2014, Holmes *et al.* 1995). In the framework of a program
80 studying the diversity of benthic dinoflagellates present in the Lesser Antilles, the aim of the present
81 study is to describe the distribution of the benthic dinoflagellates community hosted by macrophytes
82 in Saint Martin Island during the cyclonic period, which extends from June to December in the
83 Caribbean area.

84

85 **Material and methods**

86 *Abundance of benthic dinoflagellates*

87 Samples were collected between September 1st and 3rd, 2015 at 8 sites along the Atlantic and
88 Caribbean coast of Saint Martin Island (fig. 1). All samples were collected between 0.5 and 2 m
89 depth in shore waters.

90 The most abundant benthic macrophytes, which were locally present all year round, as well as
91 the floating *Sargassum* spp., were sampled on each site. Approximately 10 g of macrophyte were
92 carefully sampled with surrounding water in a 250 mL plastic flask avoiding the release of
93 microalgae attached to the macrophyte. Acidic Lugol at 1% (vol/vol) was added in all samples to fix
94 the microalgae and 10 seconds agitation allowed benthic dinoflagellates to detach from the
95 macrophyte. Samples were filtered through a 500 µm mesh sieve (Retsch[®], Ø 100 mm) to separate
96 the macrophyte from the release dinoflagellates. Total seawater volume was measured. Microalgae
97 samples were stored in dark at 4 °C. In total, 42 samples of macrophytes were semi-quantitatively
98 analyzed because the fresh weight of the macrophyte was estimated (10 g).

99 Benthic dinoflagellates were identified at a genus level in order to avoid misidentification
100 based on morphological characters (Hoppenrath *et al.* 2013, Penna *et al.* 2005). Benthic

101 dinoflagellates were counted with a 1 mL Sedgewick Rafter[®] counting cell using a standard light
102 microscope (Leitz, Orthoplan) within one week after sampling. Abundance values and the
103 approximate fresh weight of macrophyte (10 g) allowed the calculation of the number of benthic
104 toxic dinoflagellates per gram of fresh weight of macrophyte (cells g⁻¹).

105 *Temperature and salinity*

106 Temperature and salinity were measured in triplicates at each site in 250 mL seawater samples
107 collected close to macrophytes (between 0.5 and 2 m depth).

108 Temperature was measured using a Hanna[®] thermometer and salinity by a Master-S/MilliM
109 ATAGO[®] manual refractometer.

110 *Data analysis*

111 The descriptive analytical values of temperature and salinity are presented as mean ± standard
112 error (SE). Due to a semi-quantitative approach, only the mean is presented for the abundance values
113 of benthic dinoflagellates.

114

115 **Results**

116 *Temperature and salinity*

117 The temperature of the seawater fluctuated between 29.7 ± 0.0 °C and 30.8 ± 0.0 °C (n=3)
118 and the salinity varied between 35.0 ± 0.0 and 40.0 ± 0.0 during the sampling at the different sites at
119 Saint Martin Island. Generally, the average temperature of the seawater was 30.3 ± 0.1 °C and the
120 average salinity was 36.0 ± 0.6 (n=8) during the period study. (Table 1).

121 *Distribution of benthic dinoflagellates*

122 The genera *Ostreopsis*, *Prorocentrum* and *Coolia* were absent at Orient Bay. The genus
123 *Amphidinium* was absent at Lay Bay, Dawn Beach and Orient Bay. The genus *Gambierdiscus* was
124 present only at Plum bay and Simpson Lagoon Bay.

125 The genus *Ostreopsis* was observed mostly on Phaeophyceae and with decreasing
126 abundances on Ulvophyceae, seagrasses, and Florideophyceae. The highest average abundance was
127 found on benthic *Sargassum* spp. at Dawn Beach. Indeed, this macrophyte hosted on average 15,000
128 cells g⁻¹ (fig. 2).

129 Observed *Prorocentrum* were mostly on Florideophyceae with an average abundance of 1500
130 cells g⁻¹ at Simpson Lagoon Bay. This genus was found to a lesser extent on Phaeophyceae,
131 seagrasses, and Ulvophyceae.

132 The genus *Coolia* was found preferentially on seagrasses than on Phaeophyceae and
133 Ulvophyceae and Florideophyceae. This genus was hosted with the highest abundance of ca. 1000
134 cells g⁻¹ on the seagrass *Thalassia testudinum* Koenig, 1805 collected at Nettle Bay.

135 The genus *Amphidinium* was found with the highest average abundance on Phaeophyceae,
136 seagrasses, Florideophyceae and Ulvophyceae. The highest average abundance of *Amphidinium* cells
137 has been observed at Friar's Bay on the Phaeophyceae, *Padina* spp., with ca. 60 cells g⁻¹.

138 The genus *Gambierdiscus* was found only at Simpson Bay Lagoon (on average ca. 60 cells
139 g⁻¹) and Plum Bay (on average ca. 20 cells g⁻¹). This genus was mainly associated with
140 *Florideophyceae* and to a lower extent to seagrasses. The highest average abundance of this genus
141 was found on the Florideophyceae, *Gracilaria* spp. collected at Simpson Bay Lagoon (on average ca.
142 70 cells g⁻¹). At Simpson Bay Lagoon up to 125 cells g⁻¹ were found in a sample. None
143 *Gambierdiscus* cell was observed on Phaeophyceae and Ulvophyceae.

144

145 **Discussion**

146 *Temperature and salinity*

147 The present study was set up during the cyclonic period in the Caribbean Sea. This period is
148 characterized by a high seawater temperature (above 28 °C) and a low salinity (below 35) due to
149 heavy rainfall (Ballantine *et al.* 1988, Delgado *et al.* 2006, Boisnoir *et al.* 2018, Boisnoir *et al.*

2019a, Arbeláez M. *et al.* 2020). Temperature and salinity measured in Saint Martin Island were above means recorded during ecological studies conducted on benthic dinoflagellates in the Caribbean area (Ballantine *et al.* 1988, Delgado *et al.* 2006, Boisnoir *et al.* 2018, Boisnoir *et al.* 2019a, Arbeláez M. *et al.* 2020). These results suggest low rainfall and high light irradiance before and during the period sampling. In the Caribbean area, the wet season is watched because this period is supposed to promote the occurrence of high abundance values of benthic dinoflagellates (Ballantine *et al.* 1988) and to be related with high CFP cases (Tosteson 2004) due to long period with high seawater temperature (Tester *et al.* 2010, Tosteson 2004). However, these trends were not always found (de Fouw *et al.* 2001, Carlson & Tindall 1985). Furthermore, the relation between abundance values of *Gambierdiscus* cells and environmental conditions are complex and must consider parameters such as the salinity (Ballantine *et al.* 1988), light irradiance (Morton *et al.* 1992), substrate preferences (Lobel *et al.* 1988, Boisnoir *et al.* 2019a), and nutrients (Morton & Faust 1997, Delgado *et al.* 2006, Irola-Sansores *et al.* 2018).

163

164 *Benthic dinoflagellates community*

165 The species found in Saint Martin Island confirm that the benthic dinoflagellates community
166 composition is in agreement with other studies performed in the Caribbean Sea (Morton & Faust
167 1997, Delgado *et al.* 2006, Irola-Sansores *et al.* 2018, Boisnoir *et al.* 2019a). In the present study, the
168 abundance values of benthic dinoflagellates have to be considered with caution as samples were
169 semi-quantitatively analyzed (the fresh weight of macrophyte was estimated around 10 g). The
170 present study provides order of magnitude and not usual quantitative parameters such as the mean
171 and associated standard error to appreciate the global distribution of benthic dinoflagellates in Saint
172 Martin Island during the cyclonic season.

173 Potentially toxic epiphytic *Ostreopsis*, *Prorocentrum* and *Gambierdiscus* genera
174 accompanied by the genera *Coolia* and *Amphidinium* were found in Saint Martin Island for the first

175 time in the present study. The genus *Sinophysis* was not found in Saint Martin although this genus
176 was previously observed in the benthic dinoflagellate community of other areas of the Caribbean Sea
177 (Faust 1993b, Chomérat 2016, Boisnoir *et al.* 2019a).

178 The abundance densities of the genus *Ostreopsis* were lower in Cuba and Martinique islands
179 than abundances values found in the present study. Indeed, during the cyclonic period less than 1500
180 cells g⁻¹ were found in both islands (Delgado *et al.* 2006, Boisnoir *et al.* 2019a) while densities
181 reached 15,000 cells g⁻¹ in the present study. Similar abundances of *Ostreopsis* spp. were found in
182 Puerto-Rico (Ballantine *et al.* 1988) and at Puerto Morelos along the Caribbean coast of Mexico
183 (Irola-Sansores *et al.* 2018) where average cell densities reached respectively 16,000 cells g⁻¹ and
184 13,000 cells g⁻¹ during the wet season (Ballantine *et al.* 1988, Irola-Sansores *et al.* 2018). However,
185 up to 100,000 cells g⁻¹ were found at Bois Jolan in Guadeloupe during the wet season (Boisnoir *et*
186 *al.* 2019). The dominance of the genus *Ostreopsis* was reported along the Caribbean coast of Mexico
187 (Irola-Sansores *et al.* 2018), Puerto-Rico Island (Ballantine *et al.* 1988) and Guadeloupe (Boisnoir *et*
188 *al.* 2019a, Boisnoir *et al.* 2019a). In the present study, this trend must be interpreted with caution.
189 Indeed, the dominance of the genus *Ostreopsis* was recorded in Martinique Island between January
190 and February 2014 (Boisnoir *et al.* 2019a) but it was not observed during a seasonal monitoring
191 settled during 18 months (Boisnoir *et al.* 2019a) suggesting that the dominance of *Ostreopsis* can be
192 occasional. In the present study, highest abundances of the genus *Ostreopsis* were found on benthic
193 *Sargassum* spp. while none cells were found on pelagic *Sargassum* spp. In other studies carried out
194 in the Gulf of Mexico and the Caribbean Sea, less than 200 cells g⁻¹ were recorded on pelagic
195 *Sargassum* spp. (Bomber *et al.* 1988, Boisnoir *et al.* 2019a). These results suggest that benthic
196 *Sargassum* spp. should contribute to the potential toxic risk of benthic dinoflagellates contrarily to
197 pelagic *Sargassum* spp. However, pelagic *Sargassum* spp. can be an important vector for exchanging
198 microalgae populations (Kim *et al.* 2019). Arrival of toxic benthic dinoflagellates species has been
199 observed in southeast of Australia after long distances on drifting macrophytes and plastic debris

200 (Larsson *et al.* 2018). The dinoflagellate populations can be harbored by pelagic *Sargassum* and
201 homogenized between the West Indies, Gulf of Mexico and the African coasts (Boisnoir *et al.*
202 2019a).

203 The genus *Prorocentrum* is usually dominant in ecological studies settled in the Gulf of
204 Mexico (Okolodkov *et al.* 2007, Martínez-Cruz *et al.* 2015), and in the Caribbean Sea (Delgado *et al.*
205 2006, Morton & Faust 1997, Arbeláez M. *et al.* 2020) but this dominance was not observed in Saint
206 Martin Island. As in the present study, low abundance values of *Prorocentrum* were found in Cuba,
207 Guadeloupe and Martinique where less than 2000 cells g⁻¹ were found during the wet season
208 (Delgado *et al.* 2006, Boisnoir *et al.* 2019a). However, abundance values reached more than 20,000
209 along the Caribbean coast of Colombia during the rainy season (Arbeláez M. *et al.* 2020). During the
210 rainy season in the Caribbean Sea, the dominance of *Prorocentrum* genus seems to occur with fewer
211 cells than when the benthic dinoflagellates community is dominated by *Ostreopsis*.

212 The presence of the genus *Gambierdiscus* at only 2 sites in Saint Martin Island is surprising
213 because this Island is located in the high CFP prevalence (Olsen *et al.* 1984). Furthermore, this genus
214 was previously observed at 8 sites (9 sites in total) on undamaged corals, *Acropora palmata*
215 (Bourdeau & Bagnis 1989). In this previous study, *Gambierdiscus* would be associated with
216 maximal abundance values close to 20 cells g⁻¹ of macrophyte fresh weight (Bourdeau & Bagnis
217 1989) lower than in the present study. The increase of abundance values of *Gambierdiscus* spp. can
218 be explained with the probable increase of anthropic pressures near the coral reefs of Saint Martin
219 Island (Chinain *et al.* 2010, Hoegh-Guldberg 1999) between the study of Bourdeau and Bagnis
220 (1989) and the present study. Anthropic disturbances such as dredging and construction activities
221 near coral reefs often involve a phase shift from coral reefs ecosystem to macrophytes (Hoegh-
222 Guldberg 1999). Hence, this new ecosystem with abundant macrophytes provides additional and
223 ideal substrate for the attachment of benthic dinoflagellates responsible for the CFP (Chinain *et al.*
224 2010, Rongo & van Woosik 2011, Morrison *et al.* 2008).

225 Usually the bloom formation threshold for this genus is 1000 cells g^{-1} as at such cell densities the
226 amount of toxins begins to substantially accumulate in the food web (Litaker *et al.* 2010). However,
227 in some areas as Saint Barthelemy, Guadeloupe, Martinique, Caribbean coast of Colombia and
228 Mexico, abundance values of *Gambierdiscus* have never been found to exceed 1000 cells g^{-1}
229 (Boisnoir *et al.* 2019a, Boisnoir *et al.* 2018, Boisnoir *et al.* 2019b, Arbeláez M. *et al.* 2020, Irola-
230 Sansores *et al.* 2018, Lobel *et al.* 1988) and CFP cases occurred (Boucaud-Maitre *et al.* 2018, Tester
231 *et al.* 2010, Gaitán 2007, Núñez-Vázquez *et al.* 2019) suggesting that the amount of toxins begins to
232 affect the food web at a lower cell densities in some areas. It appears that the bloom threshold needs
233 to be established regionally in the Caribbean Sea (Boisnoir *et al.* 2019b). Saint Martin could be a part
234 of islands of the Lesser Antilles where abundance values of *Gambierdiscus* never exceed 1000 cells
235 g^{-1} and where CFP still occur. The bloom formation threshold of 50 cells g^{-1} proposed by Boisnoir
236 *et al.* (2019a) could be suitable for Saint Martin Island. A seasonal monitoring is necessary to know
237 if abundance values of *Gambierdiscus* can exceed or not 1000 cells g^{-1} in order to determine if
238 samples were collected during a bloom at Simpson Lagoon Bay.

239 The genera *Coolia* and *Amphidinium* are rarely mentioned in ecological studies carried out in
240 the Caribbean basin. The low abundances found in this present study similar to abundances observed
241 in Guadeloupe and Martinique (Boisnoir *et al.* 2019a).

242 *Spatial distribution*

243 Due to the high dinoflagellate abundance, the sites of Dawn Beach, Nettle Bay, Friar's Bay,
244 Simpson Lagoon Bay and Plum Bay are interesting to assess the diversity of benthic dinoflagellates
245 in Saint Martin Island. Before to be excluded, Orient Bay needs to be explored one more time without
246 pelagic *Sargassum* grounding.

247

248 **Conclusion**

249 Potentially toxic epiphytic dinoflagellates including the genus *Ostreopsis*, *Prorocentrum*, and
250 *Gambierdiscus* were found for the first time in Saint Martin Island. These genera were accompanied
251 by the non-toxic genera *Coolia* and *Amphidinium*. The species found in Saint Martin Island confirm
252 that the benthic dinoflagellates community composition is in agreement with other studies performed
253 in the Caribbean Sea. The present study revealed different distributions for each genus highlighting
254 stations that will have to be explored to assess the diversity of benthic dinoflagellates species present
255 in Saint Martin Island. In order to study the diversity of benthic dinoflagellate species present in the
256 Caribbean area, it is now necessary to define morpho-genetically the species previously described
257 according to morphological criteria.

258

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261

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Tables

421 Table 1: Temperature (°C), salinity and macrophytes collected in Saint Martin Island.
 422 Temperature and salinity were recorded in triplicates and macrophytes were sampled three times at
 423 each site.

Date	Station	Macrophyte	Temperature (°C)	Salinity
09/01/2015	Grandes Cayes	<i>Thalassia testudinum</i> Koenig, 1805 Benthic <i>Sargassum</i> spp.	30.2 ± 0.0	35 ± 0.0
09/01/2015	Friar's Bay	<i>Padina</i> spp. <i>Laurencia</i> complex	30.0 ± 0.0	35 ± 0.0
09/01/2015	Nettle Bay	<i>Thalassia testudinum</i> Koenig, 1805	30.5 ± 0.0	35 ± 0.0
09/02/2015	Plum Bay	<i>Gracilaria</i> spp. <i>Halimeda</i> spp.	30.8 ± 0.0	35 ± 0.0
09/02/2015	Simpson Lagoon Bay	<i>Halophila stipulaceae</i> Forsskål (Ascherson), 1967 <i>Gracilaria</i> spp.	30.3 ± 0.0	36 ± 0.0
09/02/2015	Lay Bay	<i>Dasycladis</i> spp. <i>Gracilaria</i> spp.	30.5 ± 0.0	35 ± 0.0
09/03/2015	Orient Bay	Pelagic <i>Sargassum</i> spp.	29.7 ± 0.0	40 ± 0.0
09/03/2015	Dawn Beach	Benthic <i>Sargassum</i> spp. <i>Udotea</i> spp.	30.4 ± 0.0	37 ± 0.0

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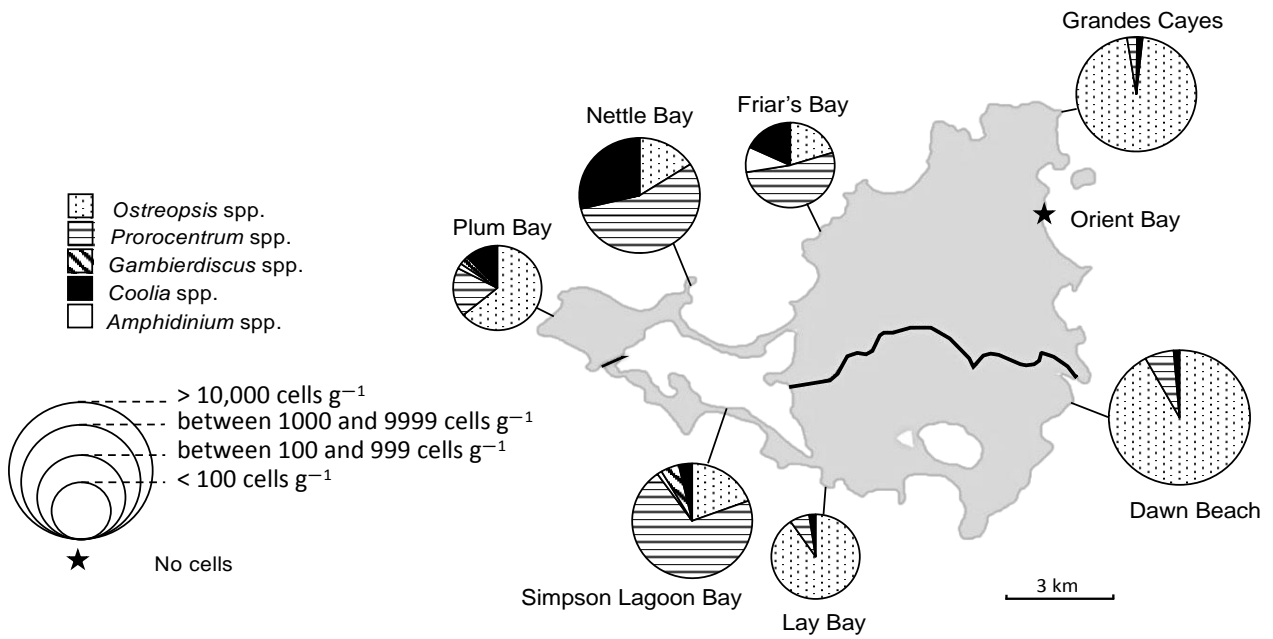
426 **Figure legends**

427 Fig. 1: Distribution of potentially toxic benthic dinoflagellates abundances (cells g⁻¹) in Saint
 428 Martin Island.

429 Fig. 2: Distribution of potentially toxic benthic dinoflagellates abundances (cells g⁻¹) on A.
 430 Florideophyceae, B. Phaeophyceae, C. Ulvophyceae and D. seagrasses in Saint Martin Island.

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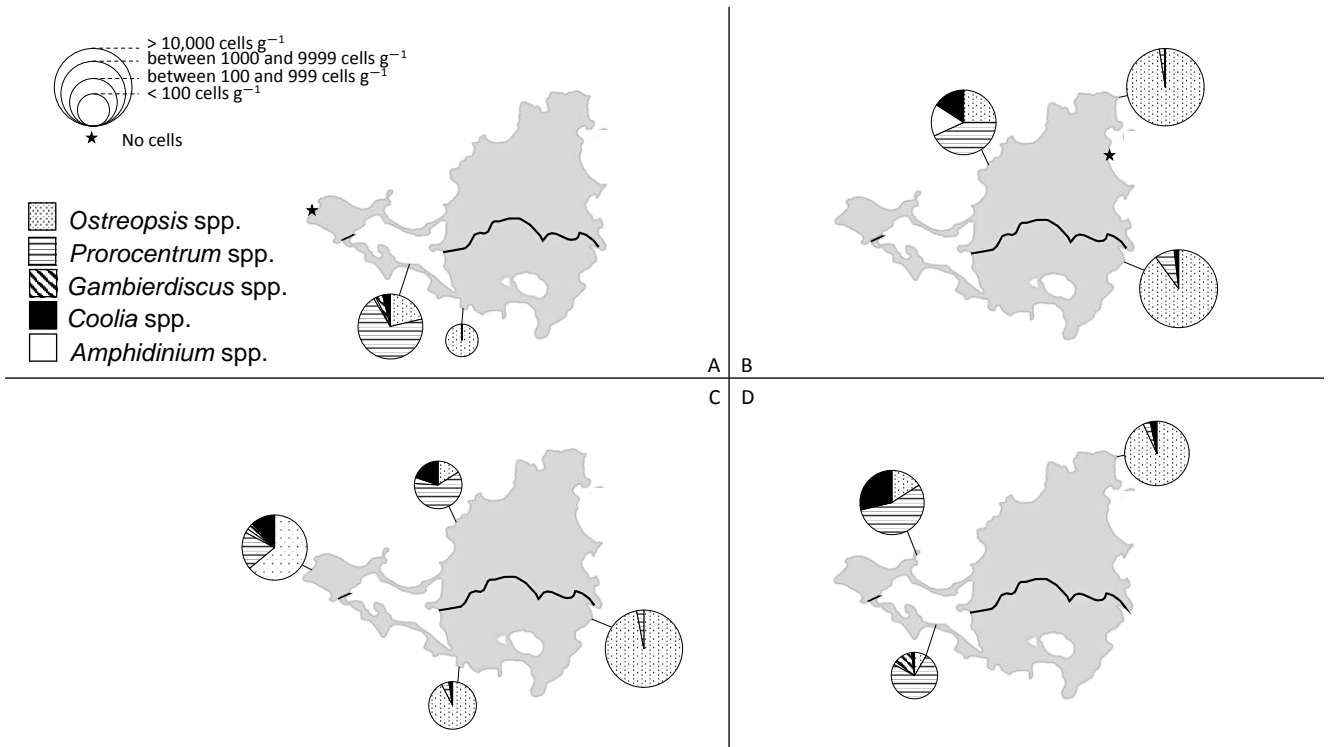
432 **Figures**



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434 Fig. 1: Distribution of potentially toxic benthic dinoflagellates abundances (cells g⁻¹) in Saint
 435 Martin Island.

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 439 Florideophyceae, B. Phaeophyceae, C. Ulvophyceae and D. seagrasses in Saint Martin Island.