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

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

Running title: Benthic HABs in Saint Martin Island

Introduction

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

In the Caribbean Sea, Gambierdiscus species are the causal agent of a worry health issue, the ciguatera fish poisoning (CFP) (Bagnis et al. 1980, Litaker et al. 2017, Díaz-Asencio et al. 2019). The Caribbean Sea presents a CFP incidence rate fluctuating between 12 and 500 cases / 100,000 habitants and is consequently the second region of the world most affected by CFP after the Pacific Ocean (Chinain et al. 2014). Within the Caribbean Sea, Saint Martin is the most affected island with an estimated CFP incidence rate of 100 cases / 10,000 hab. in 1981 (Tester et al. 2010). However, only a single survey focused on the abundances of the Gambierdiscus genus on corals in this area (Bourdeau & Bagnis 1989). In another study dealing with the distribution of Gambierdiscus spp. in the Gulf of Mexico and the Caribbean Sea, three species have been found in Saint Martin without indications about the colonized substrate (Litaker et al. 2017). In both studies, the presence of Gambierdiscus on macrophytes was overlooked despite their potential contribution on fluxes of toxin in food web. Herbivorous organisms can be contaminated through selective ingestion of the surficial biofilm containing the toxic epiphytes (Darius et al. 2018, Boisnoir et al. 2020) or through ingestion of the whole macrophyte covered with toxic epiphyte dinoflagellates (Yasumoto et al. 1976, Bourdeau & Bagnis 1989, Díaz-Asencio et al. 2019).
As *Ostreopsis* and *Prorocentrum* are associated with *Gambierdiscus* in tropical areas (Ballantine *et al.* 1988, Besada *et al.* 1982, Faust 2009) they were wrongly related with CFP; however, they are responsible for specific poisonings. In tropical environments, the toxins synthesized by the genus *Ostreopsis* are the causal agent of palytoxicosis (Alcala *et al.* 1988) and clupeotoxism (Onuma *et al.* 1999, Randall 2005) that occur respectively after the consumption of crustaceans and fish bioaccumulating toxins produced by this genus. The genus *Prorocentrum*, and specifically the species *Prorocentrum lima* is responsible for diarrhetic shellfish poisoning (DSP) not limited to tropical areas (Tripuraneni *et al.* 1997). Among the *Ostreopsis* species morphologically described in the Caribbean Sea (Faust & Morton 1995, Faust 1999) several are known to be toxic (Ukena *et al.* 2001, Scalco *et al.* 2012, Accoroni *et al.* 2017) but the presence of *Ostreopsis* species in this area has not been confirmed by molecular studies (Penna *et al.* 2010). Concerning the genus *Prorocentrum*, 15 species sampled in the Western part of the Caribbean Sea have been described morphologically in the 90’s by M.A. Faust (Faust 1990a, Faust 1990b, Faust 1991, Faust 1993a, Faust 1993, Faust 1994, Faust *et al.* 2008) but it is only recently that a taxonomic study conducted in the Eastern Caribbean area confirmed the presence of species known to be toxic (Chomérat *et al.* 2018).

Distribution of potentially toxic benthic dinoflagellates in the Caribbean justifies special attention to Saint Martin Island. Although CFP incidence rate has not been recently updated for this Island, CFP is still occurring (Boucaud-Maitre *et al.* 2018) despite the regulation of fish consumption by a decree (decree n°2002-1249, https://bit.ly/2OfU8Ut). This decree includes 3 different prohibitions that concern fifteen species. Some species (*Caranx bartholomaei*, *Sphyraena barracuda*, *Seriola dumerili*, *Seriola rivoliana*) are constantly prohibited from fishing and sale in Saint Martin, Saint Barthelemy and Guadeloupe islands. Other species (*Caranx latus*, *Caranx lugubris*, *Caranx ruber*, *Mycteroperca venenosa*, *Mycteroperca tigris*, *Alphestes afer*, *Epinephelus morio*, *Gymnothorax funebris*) are prohibited from fishing and sale beyond the parallel 16°5N. The
species *Lutjanus buccanella* cannot be sold if its weight exceeds 1 Kg whatever the fishing place.

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

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

**Material and methods**

*Abundance of benthic dinoflagellates*

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

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

Benthic dinoflagellates were identified at a genus level in order to avoid misidentification based on morphological characters (Hoppenrath *et al.* 2013, Penna *et al.* 2005). Benthic
Dinoflagellates were counted with a 1 mL Sedgewick Rafter© counting cell using a standard light microscope (Leitz, Orthoplan) within one week after sampling. Abundance values and the approximate fresh weight of macrophyte (10 g) allowed the calculation of the number of benthic toxic dinoflagellates per gram of fresh weight of macrophyte (cells g⁻¹).

Temperature and salinity

Temperature and salinity were measured in triplicates at each site in 250 mL seawater samples collected close to macrophytes (between 0.5 and 2 m depth). Temperature was measured using a Hanna© thermometer and salinity by a Master-S/MilliM ATAGO© manual refractometer.

Data analysis

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

Results

Temperature and salinity

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

Distribution of benthic dinoflagellates

The genera Ostreopsis, Prorocentrum and Coolia were absent at Orient Bay. The genus Amphidinium was absent at Lay Bay, Dawn Beach and Orient Bay. The genus Gambierdiscus was present only at Plum bay and Simpson Lagoon Bay.
The genus *Ostreopsis* was observed mostly on Phaeophyceae and with decreasing abundances on Ulvophyceae, seagrasses, and Florideophyceae. The highest average abundance was found on benthic *Sargassum* spp. at Dawn Beach. Indeed, this macrophyte hosted on average 15,000 cells g$^{-1}$ (fig. 2).

Observed *Prorocentrum* were mostly on Florideophyceae with an average abundance of 1500 cells g$^{-1}$ at Simpson Lagoon Bay. This genus was found to a lesser extent on Phaeophyceae, seagrasses, and Ulvophyceae.

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

The genus *Amphidinium* was found with the highest average abundance on Phaeophyceae, seagrasses, Florideophyceae and Ulvophyceae. The highest average abundance of *Amphidinium* cells has been observed at Friar’s Bay on the Phaeophyceae, *Padina* spp., with ca. 60 cells g$^{-1}$.

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

**Discussion**

**Temperature and salinity**

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

Benthic dinoflagellates community

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

Potentially toxic epiphytic Ostreopsis, Prorocentrum and Gambierdiscus genera accompanied by the genera Coolia and Amphidinium were found in Saint Martin Island for the first
time in the present study. The genus *Sinophysis* was not found in Saint Martin although this genus was previously observed in the benthic dinoflagellate community of other areas of the Caribbean Sea (Faust 1993b, Chomérat 2016, Boisnoir et al. 2019a).

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

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

The presence of the genus *Gambierdiscus* at only 2 sites in Saint Martin Island is surprising because this Island is located in the high CFP prevalence (Olsen et al. 1984). Furthermore, this genus was previously observed at 8 sites (9 sites in total) on undamaged corals, *Acropora palmata* (Bourdeau & Bagnis 1989). In this previous study, *Gambierdiscus* would be associated with maximal abundance values close to 20 cells g\(^{-1}\) of macrophyte fresh weight (Bourdeau & Bagnis 1989) lower than in the present study. The increase of abundance values of *Gambierdiscus* spp. can be explained with the probable increase of anthropic pressures near the coral reefs of Saint Martin Island (Chinain et al. 2010, Hoegh-Guldberg 1999) between the study of Bourdeau and Bagnis (1989) and the present study. Anthropic disturbances such as dredging and construction activities near coral reefs often involve a phase shift from coral reefs ecosystem to macrophytes (Hoegh-Guldberg 1999). Hence, this new ecosystem with abundant macrophytes provides additional and ideal substrate for the attachment of benthic dinoflagellates responsible for the CFP (Chinain et al. 2010, Rongo & van Woesik 2011, Morrison et al. 2008).
Usually the bloom formation threshold for this genus is 1000 cells g\(^{-1}\) as at such cell densities the amount of toxins begins to substantially accumulate in the food web (Litaker et al. 2010). However, in some areas as Saint Barthelemy, Guadeloupe, Martinique, Caribbean cost of Colombia and Mexico, abundance values of *Gambierdiscus* have never been found to exceed 1000 cells g\(^{-1}\) (Boisnoir et al. 2019a, Boisnoir et al. 2018, Boisnoir et al. 2019b, Arbeláez M. et al. 2020, Irola-Sansores et al. 2018, Lobel et al. 1988) and CFP cases occurred (Boucaud-Maitre et al. 2018, Tester et al. 2010, Gaitán 2007, Núñez-Vázquez et al. 2019) suggesting that the amount of toxins begins to affect the food web at a lower cell densities in some areas. It appears that the bloom threshold needs to be established regionally in the Caribbean Sea (Boisnoir et al. 2019b). Saint Martin could be a part of islands of the Lesser Antilles where abundance values of *Gambierdiscus* never exceed 1000 cells g\(^{-1}\) and where CFP still occur. The bloom formation threshold of 50 cells g\(^{-1}\) proposed by Boisnoir et al. (2019a) could be suitable for Saint Martin Island. A seasonal monitoring is necessary to know if abundance values of *Gambierdiscus* can exceed or not 1000 cells g\(^{-1}\) in order to determine if samples were collected during a bloom at Simpson Lagoon Bay.

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

*Spatial distribution*

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

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

**Acknowledgments**

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Principal water

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Toxicity responses of Mediterranean

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Structure 

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Tables
Table 1: Temperature (°C), salinity and macrophytes collected in Saint Martin Island.

Temperature and salinity were recorded in triplicates and macrophytes were sampled three times at each site.

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

**Figure legends**

Fig. 1: Distribution of potentially toxic benthic dinoflagellates abundances (cells g$^{-1}$) in Saint Martin Island.

Fig. 2: Distribution of potentially toxic benthic dinoflagellates abundances (cells g$^{-1}$) on A. Florideophyceae, B. Phaeophyceae, C. Ulvophyceae and D. seagrasses in Saint Martin Island.

**Figures**
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