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Aurélie Boisnoir, Pierre-Yves Pascal, Sophie Marro and Rodolphe Lémée*

First spatial distribution of potentially toxic benthic dinoflagellates in the Lesser Antilles (Guadeloupe and Martinique), Caribbean Sea

Abstract: For the first time, distribution and abundances (cells per gram of fresh macrophyte weight) of potentially toxic benthic dinoflagellates were studied around Guadeloupe (20 sites) and Martinique (six sites) islands (Lesser Antilles, Caribbean Sea). Benthic dinoflagellates were identified at the genus level and cell counts were undertaken on different host species of macroalgae and seagrasses. Abundance values of potentially toxic benthic dinoflagellates were one order of magnitude higher in Guadeloupe than in Martinique. The highest abundances of benthic dinoflagellates were found in the northern part of Guadeloupe Island, while their distribution was more homogeneous in Martinique. *Ostreopsis* was the dominant genus in Guadeloupe and Martinique. Regarding biotic substrate preferences, Phaeophyceae hosted the highest total abundances of benthic dinoflagellates on both islands, while the lowest total abundances were observed on Ulvophyceae in Guadeloupe and Florideophyceae in Martinique. The genus *Gambierdiscus*, known as the causal agent of the ciguatera fish poisoning (CFP), developed on all macrophyte groups on both islands without showing any preferences towards biotic substrates. The presence of this potentially harmful dinoflagellate genus in both islands could explain the existence of local cases of CFP in Guadeloupe and Martinique islands.

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Introduction

Several species of benthic dinoflagellates can generate harmful algal blooms (HABs), sometimes leading to poisoning through the production of toxins (Yasumoto et al. 1987, Holmes and Teo 2002). These phycotoxins synthesized by benthic dinoflagellates can cause ecological damage with significant mass mortalities of marine organisms (Shears and Ross 2009) as well as severe seafood poisoning through the consumption of contaminated fish products by humans (Valdiglesias et al. 2013). Several *Ostreopsis* species are able to synthesize palytoxins which are probably the cause of palytoxicosis and clupeotoxism revealed after consuming contaminated fish and crustaceans in tropical areas (Alcala et al. 1988, Randall 2005). Most species belonging to the genus *Gambierdiscus* Adachi et Fukuyo are described as the causal agent of ciguatera fish poisoning (CFP). Species of this genus synthesize ciguatoxins (Bagnis 1981, Yasumoto et al. 1987) that accumulate within different levels of the food chain (Ledreux et al. 2014) and make the consumption of some carnivorous fish more dangerous for the human population compared to other marine organisms at lower levels of the food chain (Pérez-Arellano et al. 2005). CFP is the most widespread non-bacterial food poisoning associated with the consumption of tropical fish (Tester et al. 2009) and impacts several islands of the Pacific Ocean (incidence rate (IR): 18,000 cases/100,000 inhabitants), Indian Ocean (IR < 10,000 cases/100,000 inhabitants) and Caribbean Basin (IR: 12–500 cases/100,000 inhabitants) (Chinain et al. 2014). Some benthic species of the genus *Prorocentrum*

Ehrenberg can cause illness in humans through diarrhetic shellfish poisoning (DSP) due to the presence of okadaic acid in seafood (Tripuraneni et al. 1997, Lee et al. 2016). Several species of *Coolia* Meunier and *Amphidinium* Claparède et Lachmann are able to synthesize toxins which affect marine life, but the bioaccumulation of these toxins through the marine food chain has not yet been proven (Holmes et al. 1995, Botana 2014, Ben-Gharbia et al. 2016). To our knowledge, the implications of *Coolia*, *Amphidinium* and *Sinophysis* species for human health have not been reported nor proven. Moreover, these genera are often found associated with the other genera listed above, making the relation to toxicity difficult to establish.

CFP is considered the most significant health hazard due to tropical fish consumption apart from bacterial contamination. The survey of CFP and associated poisoning can be performed using three main methods: (i) epidemiological studies, (ii) assessment of toxins in organisms at different trophic levels, and (iii) abundance measurement and distribution of microalgae responsible for poisoning. In the Caribbean, currently available data suggest that there is a wide variability regarding the attention given to CFP (Tester et al. 2009). Monitoring and/or ecological studies involving the distribution of benthic dinoflagellates have already been undertaken in the Gulf of Mexico (Okolodkov et al. 2007, 2014), the Florida Keys (Norris et al. 1985) and in Caribbean islands such as the Bahamas Archipelago (Bomber et al. 1988), Cuba (Delgado et al. 2005), Virgin Islands (Carlson and Tindall 1985) and Puerto Rico (Ballantine et al. 1985, 1988). Toxin identification studies associated with CFP in marine organisms have also been performed in the northern part of the Lesser Antilles, in St Martin and St Barthélemy (Bagnis 1981, Bourdeau and Bagnis 1989). Since 2002, a decree prohibits the fishing and commercialization of several fish species in both islands. Epidemiological studies dating from 1996 to 2006 revealed that the highest incidence rates of CFP were reported in Antigua–Barbuda and Montserrat with 34 and 59 cases per 10,000 inhabitants per year, respectively (Tester et al. 2010).

The territories north of Martinique presented a higher prevalence of ciguatera compared to the south (Olsen et al. 1984), so that Guadeloupe and Martinique are respectively inside and outside of the high prevalence area. For both islands, all CFP cases were reported to the Regional Health Agency thereby triggering epidemiological surveys, which showed that the frequency of CFP outbreaks was stable in Guadeloupe between 2013 and 2016 (Boucaud-Maitre et al. 2018), while CFP cases decreased in Martinique between 1998 and 2007 (Rosine et al. 2008). Despite the presence of CFP, no data relative to the abundance of benthic dinoflagellates are available in those two islands. Such ecological studies are now needed, focusing not only on *Gambierdiscus* species, but also on other potentially toxic genera, such as *Ostreopsis* Schmidt, *Prorocentrum*, *Coolia*, and *Amphidinium*.

Species of *Ostreopsis* and *Gambierdiscus* are particularly harmful to human populations and interest in studying these dinoflagellates has revived since the discovery of cryptic species (Richlen et al. 2008, Parsons et al. 2012, Nishimura et al. 2013, Penna et al. 2014). *Ostreopsis siamensis* Schmidt and *Gambierdiscus toxicus* Adachi et Fukuyo are the type species generally described for these genera. Over several years, the observations of morphological features by light microscopy have led to misidentifications (Litaker et al. 2009, Parsons et al. 2012). By using more recent molecular techniques and scanning electron microscopy (SEM) observations, more than a dozen *Ostreopsis* and *Gambierdiscus* species have been identified, and several of them are known to be toxic (Litaker et al. 2010, Accoroni et al. 2016, Rhodes et al. 2017). Using SEM and molecular tools proved to be useful and mandatory in studying the distribution, the diversity and the description of benthic dinoflagellates. However, such techniques are time-consuming and not compatible with large-scale ecological studies involving a large number of samples. On the other hand, the use of quantitative polymerase chain reaction (qPCR) seems promising for ecological studies (Smith et al. 2017) but needs to be improved and specific primers need to be designed for all the species potentially present.

In the framework of a large program aiming to study the diversity, ecology and impact of toxic benthic dinoflagellates in the Lesser Antilles (Guadeloupe and Martinique islands), the first steps, developed for this work, were (i) to describe the distribution and abundances of benthic dinoflagellates at the genus level and (ii) to identify potential affinities between ecological variables and biotic substrates hosting specific harmful dinoflagellate genera.

Materials and methods

Sampling

Samples were collected between 26 January and 15 February 2014 in the Lesser Antilles, off the Caribbean and Atlantic coasts of Guadeloupe (20 stations) and Martinique (six stations) islands (Figure 1). The northern part of Martinique was not explored due to harsh environmental conditions (swell, steep coast) which made sampling difficult. At each station, macrophyte species with the highest spatial coverage were collected by snorkeling (Table 1).

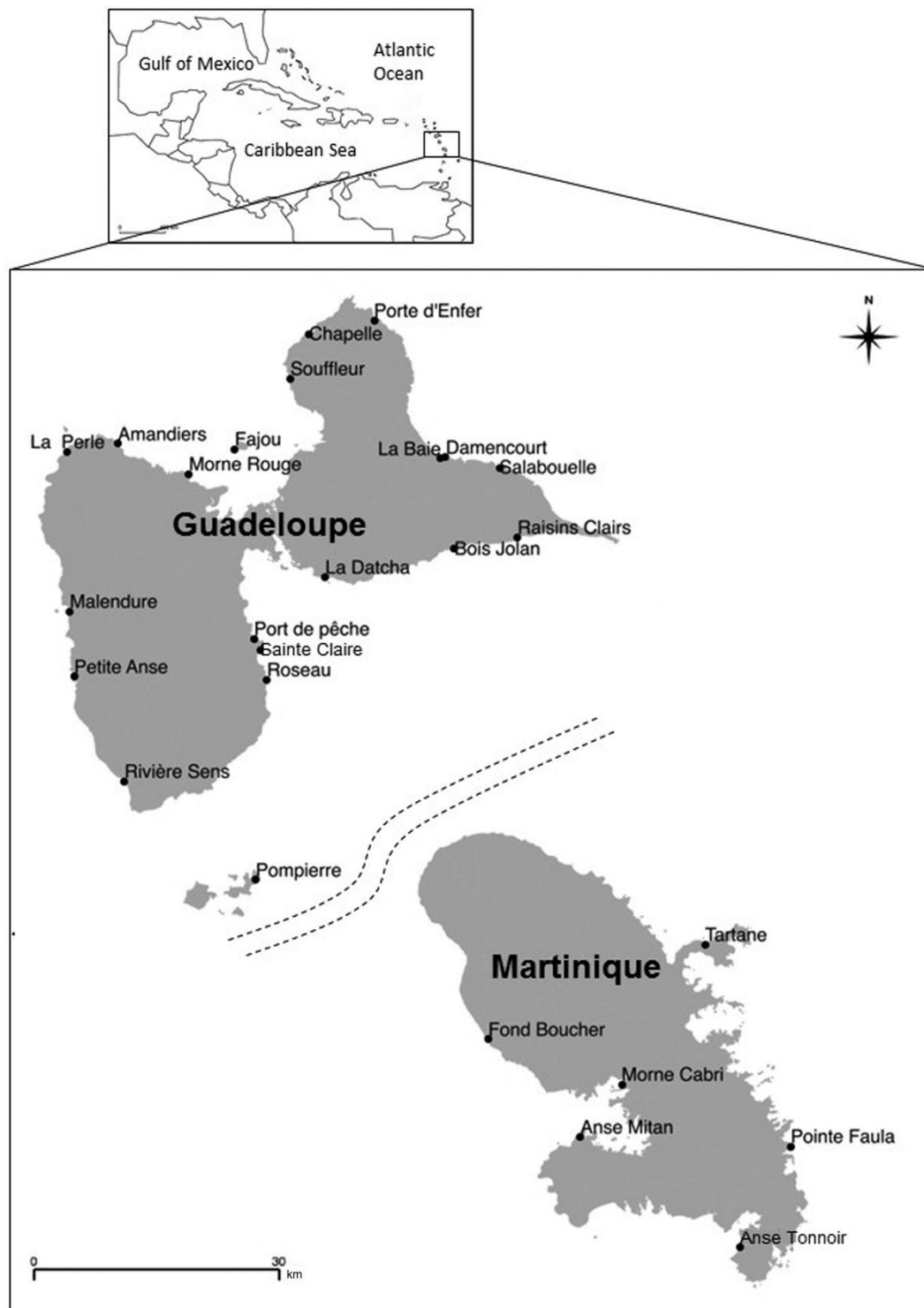


Figure 1: Map of Guadeloupe and Martinique with sampling sites. Dominique is not shown between islands.

Macrophytes were sampled referring to the protocol used to evaluate the abundance of *Ostreopsis cf. ovata* Fukuyo in the Mediterranean Sea (Cohu et al. 2011, 2013). All macrophytes were collected with care to avoid the loss of epiphytic cells at a water depth varying between 0.5 and 3 m. Macrophytes were sampled with their surrounding seawater in 250-ml plastic flasks and immediately fixed with acidic Lugol (1% vol/vol). Each macrophyte was vigorously shaken during 10 s and rinsed twice with 100 ml of 0.2- μ m filtered seawater to collect a maximum of epiphytic dinoflagellates. The macroalgal biomass was separated from the seawater sample by using a 500 μ m-meshed sieve (Retsch®, \varnothing 100 mm). The biomass was then dried with absorbent paper and weighed (OHAUS®, Adventurer Pro Precision Balance, \pm 0.1 g) to estimate the fresh weight. A total of 161 samples were collected and analyzed.

Table 1: Locations of sampling stations and number of samples of Ulvophyceae, Phaeophyceae, Florideophyceae and seagrasses collected in Guadeloupe and Martinique.

Sites	Orientation	Latitude	Longitude	Ulvophyceae	Phaeophyceae	Florideophyceae	Seagrasses
Guadeloupe							
Souffleur	Caribbean	16°25'22.7"N	61°32'00.2"W	0	5	3	2
Chapelle	Caribbean	16°28'17.9"N	61°30'46.2"W	2	4	3	0
Porte d'Enfer	Atlantic	16°29'11.8"N	61°26'29.9"W	4	2	2	0
Damencourt	Atlantic	16°20'13.5"N	61°21'48.9"W	0	4	1	0
La Baie	Atlantic	16°20'07.0"N	61°22'02.4"W	2	0	2	0
Salabouelle	Atlantic	16°19'30.3"N	61°18'14.4"W	2	2	1	1
Raisins Clairs	Atlantic	16°14'54.9"N	61°17'02.9"W	2	6	3	1
Bois Jolan	Atlantic	16°14'08.2"N	61°21'11.7"W	5	0	2	2
La Datcha	Atlantic	16°12'17.3"N	61°29'41.9"W	0	1	3	2
Port de pêche	Atlantic	16°08'14.1"N	61°34'23.7"W	1	0	1	0
Sainte Claire	Atlantic	16°07'29.0"N	61°33'59.0"W	2	0	0	3
Roseau	Atlantic	16°05'32.3"N	61°33'32.3"W	5	0	1	1
Pompierre	Atlantic	15°52'20.1"N	61°34'16.8"W	1	0	1	1
Rivière Sens	Caribbean	15°58'50.4"N	61°42'55.1"W	0	1	2	0
Petite Anse	Caribbean	16°05'43.1"N	61°46'14.7"W	0	1	0	1
Malendure	Caribbean	16°07'50.2"N	61°46'11.6"W	1	1	2	1
La Perle	Caribbean	16°20'33.1"N	61°46'40.0"W	0	2	2	0
Amandiers	Mangrove	16°21'04.3"N	61°43'21.4"W	6	0	2	1
Morne Rouge	Mangrove	16°19'05.5"N	61°38'40.6"W	1	0	0	0
Fajou	Mangrove	16°20'44.4"N	61°35'39.5"W	0	1	0	0
Martinique							
Tartane	Atlantic	14°45'30.0"N	60°55'15.8"W	4	0	1	1
Pointe Faula	Atlantic	14°32'27.6"N	60°49'43.7"W	6	4	0	3
Anse Tonnoir	Caribbean	14°25'59.0"N	60°53'00.0"W	4	1	3	1
Anse Mitan	Caribbean	14°33'03.6"N	61°03'17.0"W	4	2	4	0
Morne Cabri	Mangrove	14°36'20.5"N	61°01'17.8"W	0	1	0	1
Fond Boucher	Caribbean	14°39'22.6"N	61°09'15.6"W	1	4	3	2

Dinoflagellate cell abundances

Epiphytic cell abundance measurements (cells per gram of fresh macrophyte weight) were performed by cell counts using 1-ml Sedgewick-Rafter cells® with a standard light microscope in brightfield optics at 20 \times magnification (Zeiss, Axio Lab.A1). The number of cells per gram of fresh weight (cells g⁻¹) was calculated considering the amount of water present for each sample and the weight of the fresh macrophyte. Cell counts were undertaken for *Ostreopsis*, *Prorocentrum*, *Gambierdiscus*, *Coolia* and *Amphidinium* genera. *Gambierdiscus* and *Fukuyoa* Gómez, Qiu, Lopes et Lin were difficult to differentiate by light microscopy, therefore the latter when present, were counted as *Gambierdiscus*. The genus *Sinophysis* was not considered in this study.

Phylogenetic and morphological categories of macrophytes

Macrophytes were identified to the genus level (Table 2). When the genus determination was uncertain, the word "complex" was added. Macrophytes were classified into Ulvophyceae, Phaeophyceae,

Florideophyceae and seagrass groups in order to evaluate a potential relationship between benthic dinoflagellates and these phylogenetic groups of macrophytes. Morphological characteristics of macrophytes were described as blade-, cylindrical or non-ramified.

Environmental parameters

Sampling sites were classified into three categories: Atlantic coast, Caribbean coast, and mangrove environment.

Table 2: Number of samples collected for each macrophyte genus in Guadeloupe and Martinique.

	Guadeloupe	Martinique	Morphology
Ulvophyceae	34	19	
<i>Bryopsis</i> spp.	3	1	CR
<i>Caulerpa</i> spp.	11	10	BR/CR
<i>Chaetomorpha</i> spp.	2	2	NR
<i>Halimeda</i> spp.	6	0	BR
<i>Penicillus</i> spp.	2	2	CR
<i>Udotea</i> spp.	3	4	NR
<i>Ulva</i> spp.	5	0	NR
<i>Valonia</i> spp.	2	0	CR
Phaeophyceae	31	12	
<i>Dictyopteris</i> spp.	1	0	CR
<i>Dictyota</i> spp.	18	8	BR
<i>Padina</i> spp.	5	3	NR
<i>Sargassum</i> spp.	6	1	BR
<i>Turbinaria</i> spp.	1	0	BR
Florideophyceae	30	10	
<i>Acanthophora</i> spp.	6	5	BR
<i>Amphiroa</i> spp.	2	0	CR
<i>Asparagopsis</i> spp.	1	0	CR
<i>Bryothamnion</i> spp.	3	0	BR
<i>Ceramium</i> spp.	2	1	CR
<i>Digenea simplex</i> (Wulfen) C. Agardh	1	0	CR
<i>Galaxaura</i> complex	11	2	CR
<i>Jania</i> spp.	1	1	CR
<i>Laurencia</i> complex	1	0	BR
<i>Liagora</i> complex	0	1	CR
<i>Palisada perforata</i> (Bory) Nam	2	0	CR
Seagrass	16	8	
<i>Halodule</i> spp.	0	1	NR
<i>Halophila</i> spp.	2	4	NR
<i>Syringodium filiforme</i> Kützing	5	2	NR
<i>Thalassia testudinum</i> König	9	1	NR

The thallus morphology is indicated as follows: NR, Non-ramified; CR, cylindrical-ramified; BR, blade-ramified.

The orientation of the bays was determined by a perpendicular line passing through the bottom of the bay and using the four cardinal points. The slope of the continental shelf (the zone where macrophytes were collected) of each site was estimated and classified in three categories: low (<10°), average (between 10° and 30°) and strong (>30°) slope. The official National Institute of Geography (<http://www.ign.fr/>, last visit: 28 August 2017) provided the necessary data to determine the freshwater input as close as 200 m from each sampling site. Water transparency was also noted qualitatively and sampling sites were distinguished in two categories: clear and turbid waters.

Another factor considered in this study was the presence of chlordecone, a toxic, persistent, organochlorine insecticide used between 1972 and 1993 in French Caribbean islands. Driven by the water cycle, this terrestrial pollutant is progressively transferred to aquatic ecosystems (Coat et al. 2011). In a regional ordinance (2005; <http://bit.ly/2uCCVs8>, last visit: 26 August 2017) which prohibits fishing, consumption and marketing of organisms originating from Guadeloupe and Martinique, the littoral is divided into three categories: area with a total fishing ban, area with a fishing ban only on some species, and a no ban area. Sampling sites in this present study were localized within these three different areas linked to chlordecone levels.

Data analysis

The geographical mapping of benthic dinoflagellate abundances was made by using Qgis 2.10 PISA software© and statistical analyses were performed with XLSTAT software©. Cell abundances of dinoflagellates in Guadeloupe and Martinique did not follow a normal distribution, therefore non-parametric statistical analyses were used. Kruskal-Wallis test was used to compare the distribution of each dinoflagellate genus (i) between Guadeloupe and Martinique, (ii) according to macrophyte hosts of different groups and morphology, and (iii) according to sampling sites and environmental parameters. All descriptive analyses are presented as the mean. Multiple pairwise comparisons using the Conover-Iman Test were performed after Kruskal-Wallis test to compare the results from each treatment.

Results

Macrophytes

A total of 111 macrophyte samples were collected in Guadeloupe and 49 in Martinique (Table 2). In both islands, the Ulvophyceae was the dominant host group, followed by the Phaeophyceae, Florideophyceae, and seagrasses. Blade-ramified macrophytes dominated the seagrass group in both islands. In Guadeloupe, seagrasses were more cylindrically ramified than non-ramified, in contrast to Martinique. Twenty-six macrophyte genera were identified in Guadeloupe and 17 in Martinique. The macrophyte diversity was greater within the Florideophyceae and Ulvophyceae groups for both islands compared to seagrasses and Phaeophyceae. Phaeophyceae were more diversified than seagrasses in Guadeloupe and the opposite was observed in Martinique.

In each group of macrophytes, one genus dominated widely. Ulvophyceae were dominated by the genus *Caulerpa* Lamouroux, Phaeophyceae by the genus *Dictyota* Lamouroux and Florideophyceae by the *Galaxaura* complex. For the seagrass group, *Thalassia* Banks ex König was the dominant genus in Guadeloupe while *Halophila* Du Petit-Thouars predominated in Martinique.

Cell abundances of benthic dinoflagellates

Abundances of benthic dinoflagellates in Guadeloupe ranged between 0 and 55,910 cells g⁻¹ and were one order of magnitude higher than in Martinique ($p = 0.018$). The genus *Ostreopsis* was dominant in both islands. Cell abundances of *Ostreopsis* ($p < 0.001$) and *Coolia* ($p = 0.001$) were significantly different between the two islands, while *Prorocentrum*, *Gambierdiscus* and *Amphidinium* abundances were similar ($p > 0.05$; Table 3).

Table 3: Results of Kruskal-Wallis tests comparing abundance of five potentially toxic benthic dinoflagellate genera between Guadeloupe and Martinique.

	p-Value
<i>Ostreopsis</i>	<0.001 ^a
<i>Prorocentrum</i>	0.327
<i>Gambierdiscus</i>	0.368
<i>Amphidinium</i>	0.115
<i>Coolia</i>	0.001 ^a
Total	0.018 ^a

^aIndicates p-value significant at $\alpha = 0.05$.

In Guadeloupe, abundances of *Ostreopsis* averaged 2403 cells g⁻¹, *Prorocentrum* averaged 1177 cells g⁻¹, and *Coolia* and *Amphidinium* averaged 212 cells g⁻¹ and 14 cells g⁻¹, respectively. The genus *Gambierdiscus* was estimated with an average abundance of 6 cells g⁻¹ overall macrophytes. Significant positive correlations were found between *Ostreopsis*, *Prorocentrum*, *Amphidinium* and *Coolia* genera (Table 4).

Table 4: Coefficient r_s of Spearman rank correlation for abundances among five potentially toxic benthic dinoflagellates in Guadeloupe and Martinique islands.

	<i>Prorocentrum</i>	<i>Gambierdiscus</i>	<i>Coolia</i>	<i>Amphidinium</i>
Guadeloupe				
<i>Ostreopsis</i>	0.583 ^c	<0.001	0.260 ^c	0.081 ^a
<i>Prorocentrum</i>		0.025	0.253 ^c	0.120 ^b
<i>Gambierdiscus</i>			0.017	<0.001
<i>Coolia</i>				0.191 ^c
Martinique				
<i>Ostreopsis</i>	0.036	0.090 ^a	0.031	0.003
<i>Prorocentrum</i>		0.006	0.009	0.023
<i>Gambierdiscus</i>			0.018	0.014
<i>Coolia</i>				0.002

^ap < 0.05, ^bp < 0.001, ^cp < 0.0001.

In Martinique, *Ostreopsis* abundances reached an average of 260 cells g⁻¹, *Prorocentrum* reached 247 cells g⁻¹, while *Coolia* and *Amphidinium* averaged 10 cells g⁻¹ and 4 cells g⁻¹, respectively. The abundance of the genus *Gambierdiscus* was estimated with an average of 6 cells g⁻¹ overall macrophytes. A significant positive correlation existed between *Ostreopsis* and *Gambierdiscus* (Table 4).

In Guadeloupe, the highest average abundances of potentially toxic benthic dinoflagellates were found at Chapelle (18,180 cells g⁻¹, n = 9), Souffleur (10,753 cells g⁻¹, n = 10) and Fajou (14,167 cells g⁻¹, n = 1) sampling sites (Conover-Iman Test, p < 0.001, Figure 2). The genus *Ostreopsis* was found with the highest abundances (p < 0.001) at these three sites, varying between 3408 and 14,751 cells g⁻¹. Souffleur was the site with the highest abundance of *Prorocentrum* (5823 cells g⁻¹, n = 10, p < 0.001). Rivière Sens, Pompierre and Morne Rouge had the highest abundances of *Gambierdiscus* (p < 0.001), ranging between 54 and 105 cells g⁻¹ (Figure 3).

In Martinique, abundances of benthic dinoflagellates were significantly higher at Fond Boucher (1134 cells g⁻¹) and Morne Cabri (2543 cells g⁻¹) compared to other sites. The highest cell abundance of *Ostreopsis* (1049 cells g⁻¹, p = 0.001) was recorded in Fond Boucher, which significantly differed from the other sampling sites. Cell abundances of *Gambierdiscus*, *Amphidinium*, and *Coolia* were comparable between all sampling sites in Martinique, with the lowest abundances of benthic dinoflagellates recorded at Tartane.

Relationships between benthic dinoflagellates and macrophytes

Macrophyte groups hosted different abundances of potentially toxic benthic dinoflagellates. In Guadeloupe, the total cell abundances of benthic dinoflagellates were significantly higher on seagrasses (5098 cells g⁻¹) and on Phaeophyceae (6343 cells g⁻¹), than on Ulvophyceae

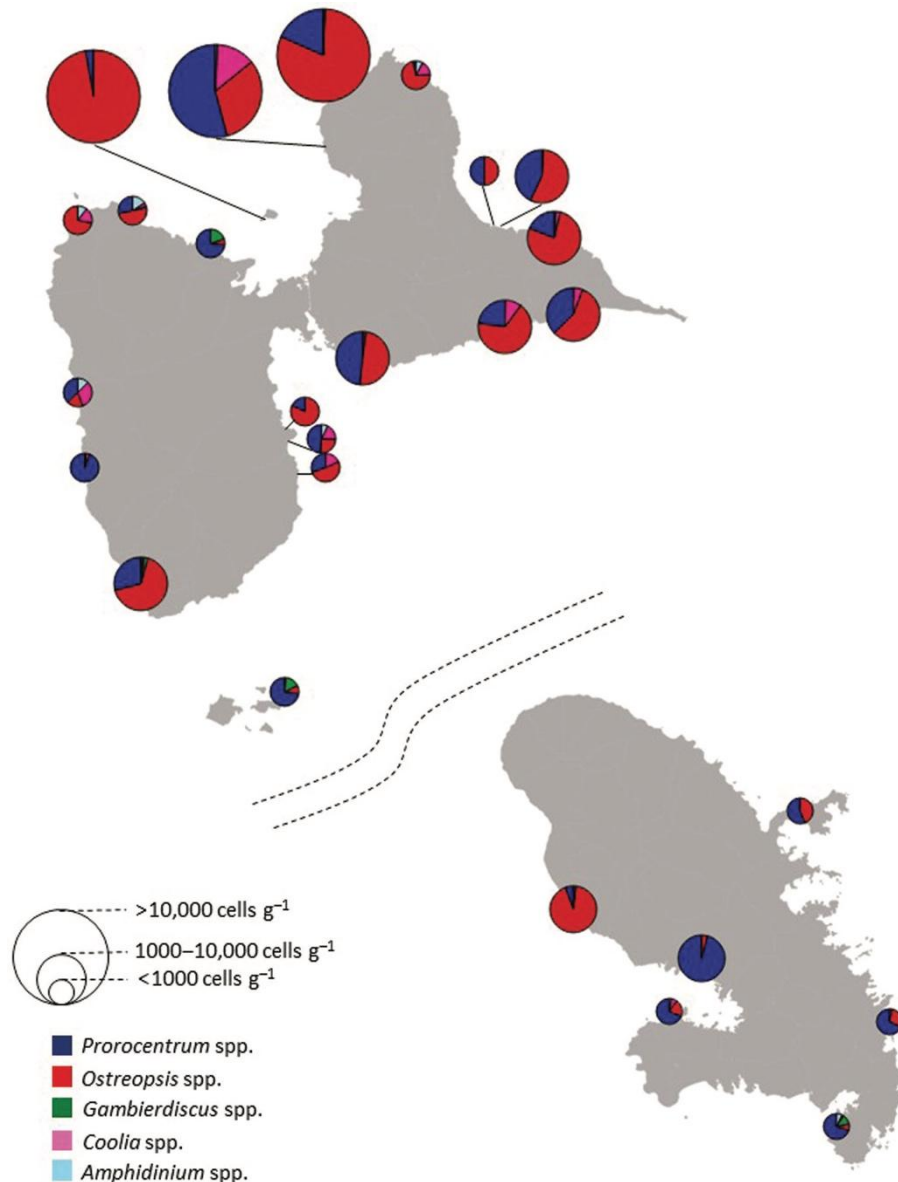


Figure 2: Average abundances (cells g⁻¹) of five potentially toxic benthic dinoflagellates in Guadeloupe (above) and Martinique (below).

(424 cells g⁻¹, $p < 0.001$; Figure 4). *Ostreopsis* and *Coolia* abundances were found with highest values on Phaeophyceae ($p < 0.001$) whilst the genus *Prorocentrum* was found with the highest abundances on several groups of macrophytes such as Phaeophyceae, seagrasses, and Florideophyceae ($p < 0.001$). *Amphidinium* and *Gambierdiscus* cell abundances reached similar levels on all macrophyte groups ($p > 0.05$; Figure 5). The total abundance of dinoflagellates was not related to the morphology of the macrophytes. Indeed, no significant differences were found between the abundance values for benthic dinoflagellates on different morphologies of macrophytes ($p = 0.074$). However, when considering the different dinoflagellate

genera independently, the results showed that *Prorocentrum* and *Ostreopsis* had the highest cell abundances on the blade-ramified substratum ($p < 0.04$; Figure 5A).

In Martinique, the highest abundance of dinoflagellates was found on Phaeophyceae (975 cells g^{-1}) and Florideophyceae (547 cells g^{-1}), while the lowest abundance was reported on Ulvophyceae (151 cells g^{-1} , $p = 0.001$; Figure 6). *Ostreopsis* had the highest cell abundances on the Phaeophyceae with, on average, 712 cells g^{-1} ($p < 0.0003$). *Prorocentrum*, *Gambierdiscus*, *Amphidinium* and *Coolia* had similar abundance levels on the different macrophyte groups (Figure 5B). Blade-ramified macrophytes hosted significantly more potentially toxic benthic

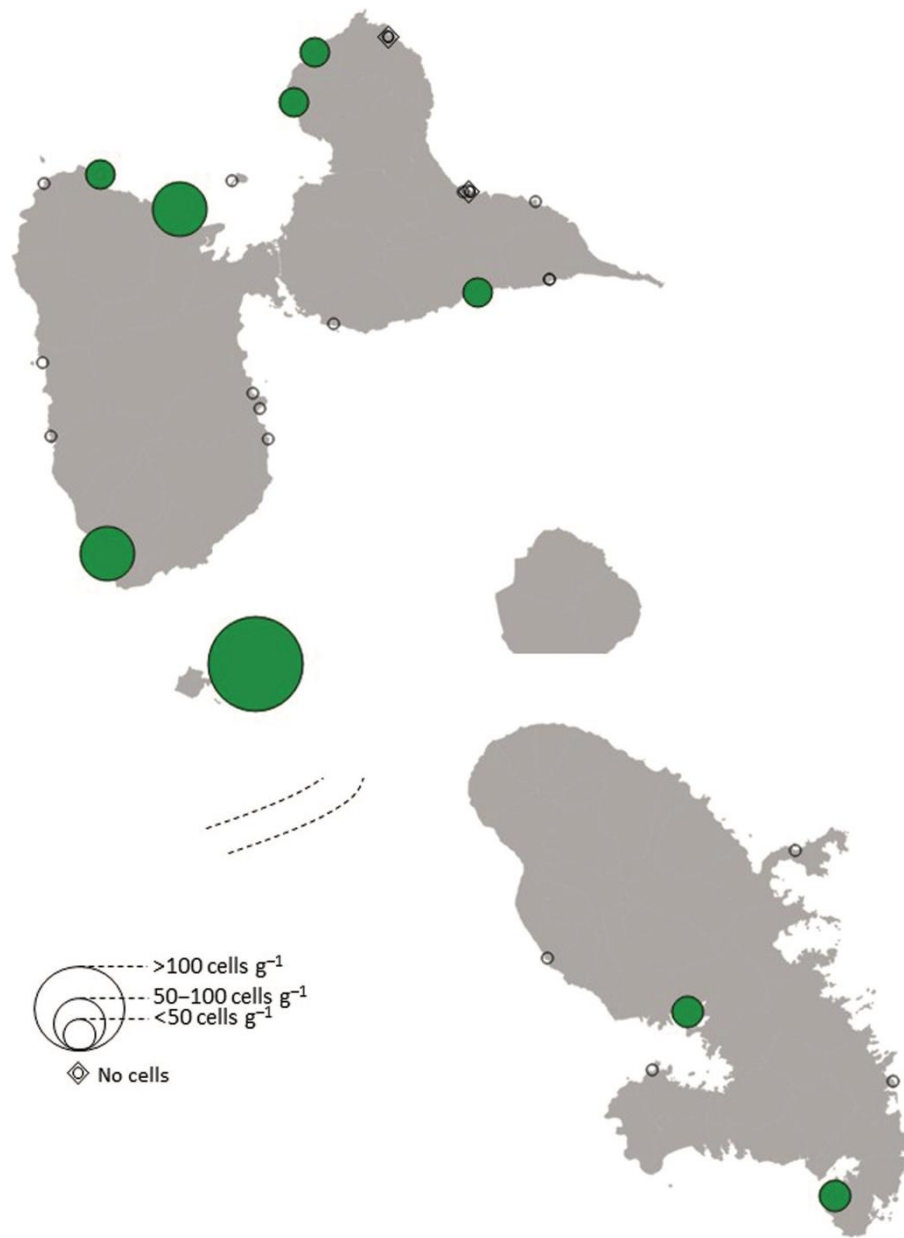


Figure 3: Average abundances (cells g^{-1}) of the benthic dinoflagellate *Gambierdiscus* in Guadeloupe (above) and Martinique (below).

dinoflagellates compared to cylindrical and non-ramified macrophytes ($p = 0.012$; Figure 5B). *Ostreopsis* was found with higher cell abundances on blade-ramified macrophytes than on non-ramified macrophytes ($p = 0.015$).

Relationship between benthic dinoflagellates and ecological parameters

In Guadeloupe, potentially toxic benthic dinoflagellates were more abundant on the Caribbean coast (8469 cells g⁻¹)

than on the Atlantic coast (1918 cells g⁻¹) or in mangrove environments (1371 cells g⁻¹; $p = 0.001$; Figure 2). Sampling sites with turbid water presented lower abundances of benthic dinoflagellates (797 cells g⁻¹) than sites with clear water (5718 cells g⁻¹; $p = 0.009$). The identified zones contaminated by chlordecone had the lowest abundances of dinoflagellates (509 cells g⁻¹ $p = 0.034$), whereas around 4494 cells g⁻¹ were found in areas not contaminated. The occurrence of freshwater inflow within 200 m of the sampling site did not affect the abundance of epibenthic dinoflagellates ($p > 0.934$). In Martinique, no environmental parameters considered in the present study affected the abundance of benthic dinoflagellates ($p > 0.05$).

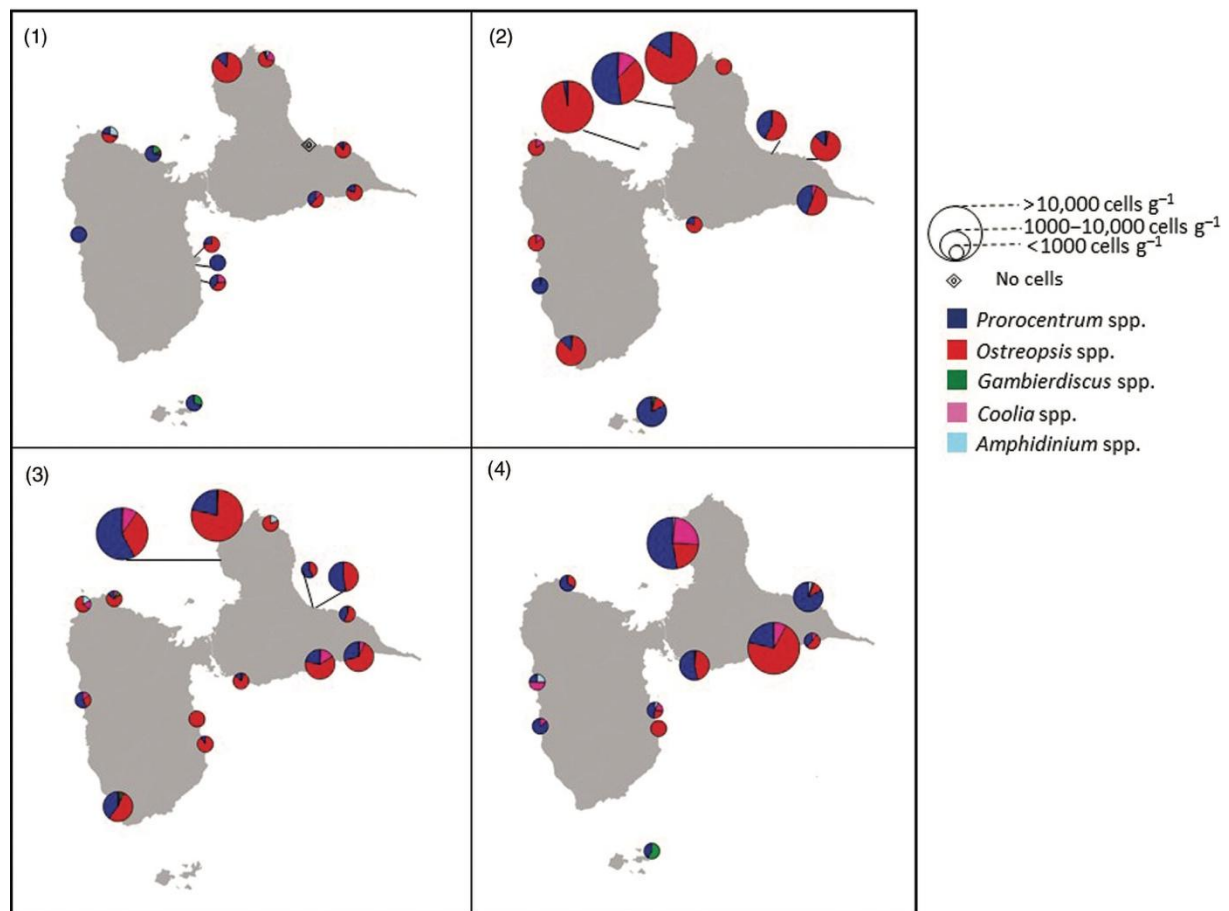


Figure 4: Average abundances (cells g⁻¹) of five potentially toxic benthic dinoflagellates on (1) Ulvophyceae, (2) Phaeophyceae, (3) Florideophyceae and (4) Seagrasses in Guadeloupe. No mark appears when a macrophyte group was not found at a station.

Discussion

This work is the first exploratory study focusing on the distribution and abundance of potentially toxic benthic dinoflagellates in Guadeloupe and Martinique. Species of the genera *Ostreopsis*, *Prorocentrum*, *Gambierdiscus*, *Coolia*, and *Amphidinium* were identified in all sampling sites. Differences in abundance and distribution emerged between both islands. Results also showed that dinoflagellate genera had preferences for certain biological substrates as well as relationships with environmental parameters. Similar studies have been undertaken in other Caribbean islands, but cell abundances were not always expressed in the same units (Carlson and Tindall 1985) and differed considering the sampling period and the macrophytes used as hosts.

Dinoflagellate abundances and affinities with macrophytes

Ostreopsis and *Prorocentrum* were the most abundant genera of potentially toxic epibenthic dinoflagellates in Guadeloupe and Martinique. Cell abundances of *Ostreopsis* were comparable to previous studies undertaken in the Caribbean basin (Ballantine et al. 1985, 1988, Okolodkov et al. 2014). In this study, *Ostreopsis* abundances varied between 963 and 5677 cells g⁻¹ on the same macrophyte in Guadeloupe and in Martinique. A previous study carried out in Cuba, between January 2000 and 2002, showed that the genus *Prorocentrum* reached an average abundance of 50 cells g⁻¹ for several combined macrophytes (Delgado et al. 2005), which is respectively, 23 to 5 times less than the cell abundances recorded in Guadeloupe and Martinique.

(A) Guadeloupe

Genera	Pairwise comparisons	p-Value	Genera	Pairwise comparisons	p-Value
<i>Ostreopsis</i>	Ulv Sea Flo Pha	p < 0.0001	<i>Ostreopsis</i>	NR CR BR	p = 0.002
<i>Prorocentrum</i>	Ulv Flo Sea Pha	p < 0.0001	<i>Prorocentrum</i>	CR NR BR	p = 0.037
<i>Gambierdiscus</i>	Ulv Sea Pha Flo	p = 0.211	<i>Gambierdiscus</i>	NR BR CR	p = 0.142
<i>Amphidinium</i>	Ulv Pha Sea Flo	p = 0.182	<i>Amphidinium</i>	NR CR BR	p = 0.298
<i>Coolia</i>	Ulv Sea Flo Pha	p = 0.029	<i>Coolia</i>	CR NR BR	p = 0.466
Total	Ulv Flo Sea Pha	p = 0.0001	Total	CR NR BR	p = 0.074

(B) Martinique

Genera	Pairwise comparisons	p-Value	Genera	Pairwise comparisons	p-Value
<i>Ostreopsis</i>	Ulv Sea Pha Flo	p < 0.003	<i>Ostreopsis</i>	NR CR BR	p = 0.002
<i>Prorocentrum</i>	Ulv Sea Flo Pha	p = 0.18	<i>Prorocentrum</i>	CR NR BR	p = 0.037
<i>Gambierdiscus</i>	Ulv Pha Sea Flo	p = 0.140	<i>Gambierdiscus</i>	NR BR CR	p = 0.423
<i>Amphidinium</i>	Pha Sea Ulv Flo	p = 0.288	<i>Amphidinium</i>	NR BR CR	p = 0.328
<i>Coolia</i>	Sea Ulv Pha Flo	p = 0.0326	<i>Coolia</i>	BR CR NR	p = 0.112
Total	Ulv Sea Flo Pha	p = 0.001	Total	CR NR BR	p = 0.012

Figure 5: Multiple pairwise comparisons using the Conover-Iman procedure and p-value of Kruskal-Wallis test ($\alpha = 0.05$) for comparison of phylogenetic macrophyte groups (left) and macrophyte morphology groups (right) for each genus of the five potentially toxic benthic dinoflagellates in (A) Guadeloupe (n = 111) and (B) Martinique (n = 50). Ulv, Ulvophyceae; Sea, Seagrasses; Flo, Florideophyceae; Pha, Phaeophyceae; NR, non-ramified; CR, cylindrical-ramified; BR, bladeramified. Significant p-values are in bold. The groups and morphology of macrophytes are listed in order of benthic dinoflagellates mean abundances values, with lowest on left and highest on right.

In Guadeloupe, *Prorocentrum* and *Ostreopsis* were identified on the same biological substrates and with the highest abundance values on blade-ramified macrophytes. *Ostreopsis* was found preferentially on Phaeophyceae alone, while *Prorocentrum* was found preferentially on Phaeophyceae, Florideophyceae, and seagrasses. In Martinique, *Ostreopsis* was found in high abundances on blade-ramified macrophytes and preferentially on Florideophyceae, while the abundance of *Prorocentrum* was not affected by macrophyte morphology. Affinities of *Prorocentrum* and *Ostreopsis* species for macrophytes differed in other Caribbean studies. In Cuba, *Prorocentrum* was preferentially observed on Phaeophyceae and Ulvophyceae (Delgado et al. 2005) while *Ostreopsis* did not demonstrate any preference for either host (Ballantine et al. 1985).

Average abundance values of *Gambierdiscus* were similar between Guadeloupe and Martinique, but 10 times lower than abundances reported from Puerto Rico (between 100 and 1250 cells g⁻¹; Ballantine et al. 1988). *Gambierdiscus* seemed able to settle on all macrophytes and showed no preference for a specific group nor morphology, as was previously reported (Saint Martin et al. 1988). However in Cuba,

Gambierdiscus prevailed on Phaeophyceae, Florideophyceae and then Ulvophyceae (Delgado et al. 2005), and in Puerto Rico, *Dictyota* was a better host than the seagrass *Thalassia testudinum* König (Ballantine et al. 1985).

In this study, *Amphidinium* and *Coolia* were present at very low cell abundances. Such results coincided with previous studies reporting low abundances during different sampling periods in the Gulf of Mexico (Okolodkov et al. 2007).

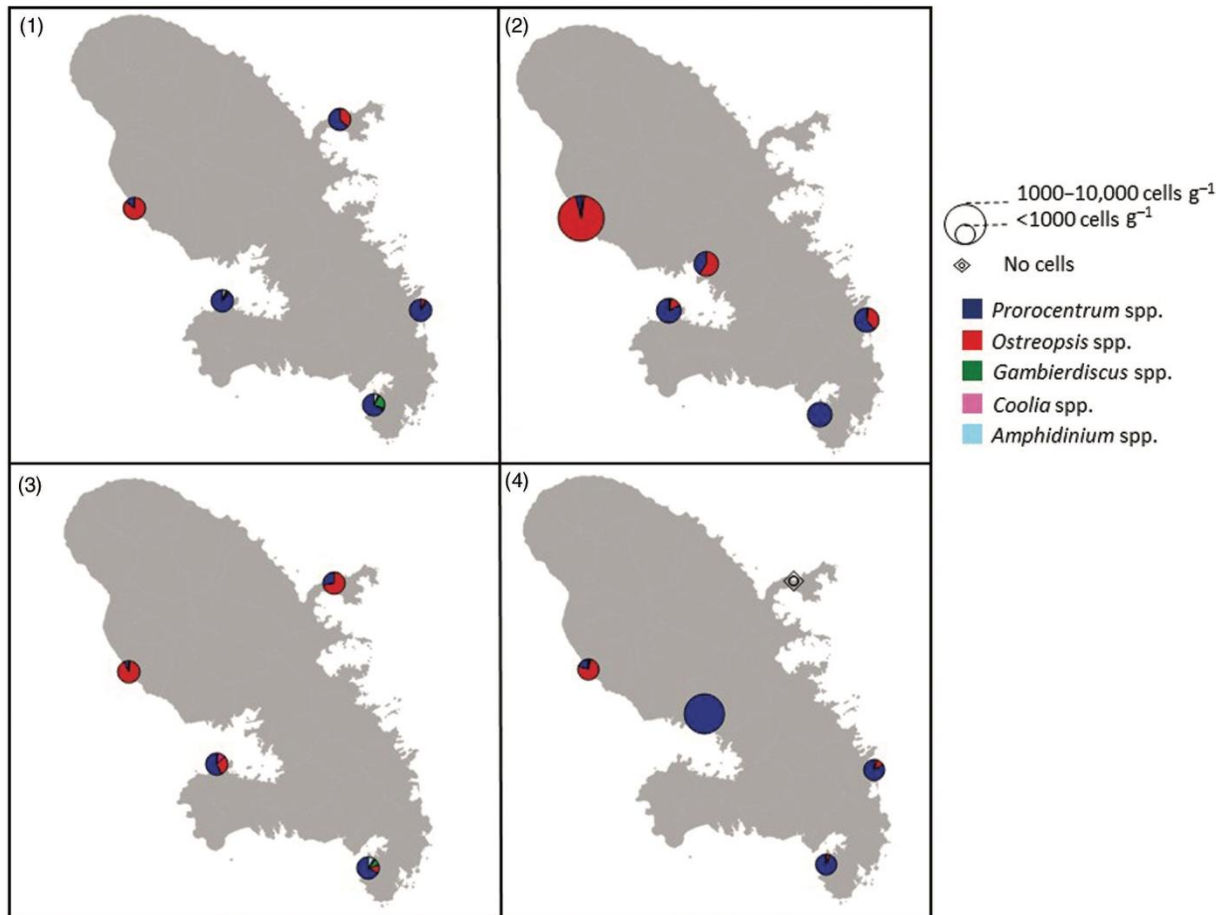


Figure 6: Average abundances (cells g⁻¹) of five potentially toxic benthic dinoflagellates on (1) Ulvophyceae, (2) Phaeophyceae, (3) Florideophyceae and (4) Seagrasses in Martinique island.

Dinoflagellate abundance and environmental variables

In this study, benthic dinoflagellates were more abundant in Guadeloupe (particularly on the northern coast) than in Martinique. This pattern does not appear to be linked to temperature, as the maximum (September) and minimum (February) sea surface temperatures were similar between both islands (see maps of sea surface temperature across the Caribbean in Tester et al. 2010). Nevertheless, this pattern must be confirmed by seasonal variation studies. Different periodic patterns of benthic dinoflagellate abundance have already been highlighted in the Caribbean (Ballantine et al. 1985, Okolodkov et al. 2014), but the origin of those fluctuations varied and was not consistent (e.g. temperature, nutrient inputs, seasonal occurrence of main macrophytes). In Guadeloupe, benthic dinoflagellates were more abundant on the Caribbean coast than on the Atlantic coast and in mangroves.

The inflow of freshwater relatively close to each station (up to 200 m) did not affect the total abundance of benthic dinoflagellates in Guadeloupe and Martinique.

As for marine areas exposed to chlordecone inputs, benthic dinoflagellates showed lower abundance levels in Guadeloupe sampling sites with a total fishing ban compared to sites without contamination,

while this trend was not observed in Martinique, probably because the sampling sites were mainly outside the fishing banned areas.

Ciguatera and *Gambierdiscus*

The genus *Gambierdiscus* is the causal agent of CFP and the Caribbean has the second highest incidence rate in the world (Chinain et al. 2014). In this study, *Gambierdiscus* was found in Guadeloupe and Martinique with a strong spatial variability. This report of *Gambierdiscus* species in Guadeloupe and Martinique questions the local belief regarding the origin of ciguateric fish in those islands. Indeed, the great majority of inhabitants in both French islands believe that contaminated seafood products came from fishing activities in the northern Caribbean islands, where the incidence rates of CFP are much higher. For instance, the CFP incidence rates in Guadeloupe and Martinique are 0.3 and 0.2 per 10,000 inhabitants per year, respectively, while these rates reached an average of 34.4 and 58.6 per 10,000 inhabitants per year in Antigua/ Barbuda and Montserrat islands, respectively; these islands are just north of Guadeloupe (Tester et al. 2010). Results obtained in this study suggest that ciguateric fishes could be, at least partially, locally contaminated by *Gambierdiscus* toxins in Guadeloupe and Martinique.

This study also gives indications of potential sites for survey studies related to *Gambierdiscus* and ciguatera outbreaks. In Guadeloupe, among the four most important sites registered with ciguatoxic fishes between 2004 and 2008 (Anse Bertrand, Abymes, Désirade, and Pointe Noire; Rosine et al. 2008), only one location coincided with a sampling site hosting *Gambierdiscus* (Chapelle in Anse Bertrand City). It should be highlighted that, in the present study, Pompierre (Saintes islands, South West of Guadeloupe) hosted the highest abundance of *Gambierdiscus* and notably that this area was also an intensive fishing zone. Therefore, Guadeloupe should be recommended as part of a potential monitoring program for CFP. In Martinique Island, Anse Tonnoir (Sainte Anne City), Tartane (Trinité City) and Pointe Faula (Vauclin City) sampling sites could be potentially important sites since these are cities where sampling sites have already been reported by the Health Agency of Martinique to involve verified cases of CFP (Daudens et al. 2013, Daudens-Vaysse et al. 2015). Also, Anse Tonnoir in Sainte Anne City showed the highest abundances of *Gambierdiscus*. Those dinoflagellates were also found at Fond Boucher, but no recent CFP cases have been reported to the Health Agency at this location.

In conclusion, this study determined, for the first time, the abundance of several potentially toxic benthic dinoflagellates in the French West Indies, namely Martinique and Guadeloupe. Dinoflagellates were identified to the genus level using light microscopy in order to avoid species misidentification and to perform a very large number of cell counts. During the 2014 dry season, benthic dinoflagellates were one order of magnitude more abundant in Guadeloupe than in Martinique. *Ostreopsis* was the most abundant genus, followed by *Prorocentrum*, *Coolia*, *Amphidinium*, and *Gambierdiscus*. Macrophyte species, their morphologies, and their phylogenetic groups could influence dinoflagellate abundances growing on them. In Guadeloupe, a toxic and persistent organochlorine pesticide, chlordecone, had a strong negative effect on benthic dinoflagellate abundances.

The presence of *Gambierdiscus* in both islands supports the possible idea that local fish might be contaminated locally and could, at least in part, explain CFP in Guadeloupe and Martinique. This is especially relevant when ciguatoxic fish are known not to be large pelagic species similar to those found in more contaminated islands in the North. The present study, with associated local epidemiological data, can be used to determine several key sites which could be recommended to be studied in the framework of a CFP monitoring program.

This preliminary survey is of great importance since it allows scientists and health and marine management services in Guadeloupe and Martinique to select preferential sampling sites and to determine accurate biological substrates for sampling in order to study (1) the potential seasonal variation of potentially toxic benthic dinoflagellates, as well as (2) the morphogenetic profiles of the different identified species, using SEM and molecular tools.

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