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DEPTH DISTRIBUTION OF BENTHIC DINOFLAGELLATES
IN THE CARIBBEAN SEA

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Running Head: Depth distribution of benthic dinoflagellates

Abstract

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

Introduction

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

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

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

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

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

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

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

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

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

Material and method

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

Abundance of benthic toxic dinoflagellates

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

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

Density of benthic dinoflagellates and leaf surface of H. stipulacea

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

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

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

Measurement of ecological factors

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

Data analysis

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

Results

Ecological parameters at Gosier and Rivière Sens

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

Biomass and leaf surface of Halophila stipulacea

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

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

Abundances of benthic dinoflagellates and depth distribution

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

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

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

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

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

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

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

Temperature, salinity and benthic dinoflagellates

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

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

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

Interaction between ecological parameters and benthic dinoflagellates

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

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

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

Density of benthic dinoflagellates

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

At Rivière Sens the highest abundances of dinoflagellates per square meter were 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 (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 densities of benthic dinoflagellates were similar between the wet and the dry season. (p=0.053).

Discussion

Influence of ecological factors

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

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

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

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

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

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

A positive correlation existed between *Coolia* spp. abundances and depth in this study. In the Mediterranean Sea this genus was observed at depths higher than 3 m (Cohu and Lemée, 2012). *Coolia* spp. distribution is often neglected in studies conducted in the tropical areas. Furthermore, among ecological study carried out in tropical areas and focusing on the depth none study has showed distribution of *Coolia* spp.. Only information about low abundances of *Coolia* spp. ($< 1\ 000\ \text{cells.gFW}^{-1}$) have been found in these studies (Delgado et al., 2005; Xu et al., 2014). *Coolia* spp. present in the Caribbean area exhibited optimal growth with a salinity of 33 and a temperature of 29°C (Morton et al., 1992). The results of this ecological study corroborated partially results of this study because highest abundances of *Coolia* spp. were found during the warmest period (the wet season), when temperature was included between 29.4°C and 31°C and with a salinity of seawater of 30. *Coolia* spp. were first reported to synthesize toxins in the early work (Holmes et al., 1995) but none strains examined by Penna et al., (2005) were toxic and Rhodes et al., (2000) have found both toxic and nontoxic strains. The toxicity variation of *Coolia* spp. is difficult to interpreted and cannot be linked with a problem of identification because taxonomic problems have not been reported for this genus (Penna et al., 2005). Effects of *Coolia* spp. on human health are still unknown (Zingone et al., 2006).

~~In fact, despite some initial studies suggested that the species produced cooliatxin. (Holmes et al., 1995), further tests indicated that *Coolia* spp. is not toxic (Delia et al., 2015).~~

Higher abundances of *Amphidinium* spp. were found in this survey with different temperature and salinity promoting optimal growth of *Amphidinium* spp. Indeed, the ecological study conducted on Caribbean benthic dinoflagellates has found an optimal growth temperature between 26°C and 28°C and a salinity of 34. This genus was found in

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

Species interactions

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

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

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

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

Substratum and dinoflagellates

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

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

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

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

Conclusion

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

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Figure and table

Figure 1: A: Location of Guadeloupe archipelago in the Caribbean Sea, B: Location of Rivière Sens (deep site) and Gosier (shallow site) in Guadeloupe.

Figure 2. Profiles of temperature and salinity during the wet (black) and the dry (grey) seasons at Gosier (graphs on the left) and Rivière Sens (graphs on the right).

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

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

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

Figure 5: Abundances of benthic toxic dinoflagellates according to depth during the wet (left) and the dry (right) season at Rivière Sens. Significant differences between depths are indicated with letters (Kruskal Wallis test and Dunn test, $\alpha=0,05$).

Figure 6: Mean density of benthic dinoflagellates $\pm$ SD according to depth at Gosier (above) and at Rivière Sens (below) for the wet (black) and the dry season (grey). “*” indicates no cell found but depth sampled and “NA” data no available because no macrophyte was found. Significant differences between depths are indicated with letters (Kruskal Wallis test and Dunn test, $\alpha=0.05$).

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
Average abundance							

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