Article

Epibenthic Harmful Marine Dinoflagellates from Fuerteventura (Canary Islands), with Special Reference to the Ciguatoxin-Producing *Gambierdiscus*

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Abstract: The relationship between the ciguatoxin-producer benthic dinoflagellate *Gambierdiscus* and other epibenthic dinoflagellates in the Canary Islands was examined in macrophyte samples obtained from two locations of Fuerteventura Island in September 2016. The genera examined included *Coolia*, *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Scrippsiella*, *Sinophysis*, and *Vulcanodinium*. Distinct assemblages among these benthic dinoflagellates and preferential macroalgal communities were observed. *Vulcanodinium* showed the highest cell concentrations (81.6×10^3 cells gr^{-1} wet weight macrophyte), followed by *Ostreopsis* (25.2×10^3 cells gr^{-1} wet weight macrophyte). These two species were most represented at a station (Playitas) characterized by turfy Rhodophytes. In turn, *Gambierdiscus* (3.8×10^3 cells gr^{-1} wet weight macrophyte) and *Sinophysis* (2.6×10^3 cells gr^{-1} wet weight macrophyte) were mostly found in a second station (Cotillo) dominated by Rhodophytes and Phaeophytes. The influence of macrophyte's thallus architecture on the abundance of dinoflagellates was observed. Filamentous morphotypes followed by macroalgae arranged in entangled clumps presented more richness of epiphytic dinoflagellates. Morphometric analysis was applied to *Gambierdiscus* specimens. By large, *G. excentricus* was the most abundant species and *G. australes* occupied the second place. The toxigenic potential of some of the genera/species distributed in the benthic habitats of the Canary coasts, together with the already known presence of ciguatera in the region, merits future studies on possible transmission of their toxins in the marine food chain.

Keywords: benthic dinoflagellates; *Gambierdiscus*; ciguatera; Canary Islands; epiphytic dinoflagellates

1. Introduction

Ciguatera fish poisoning (CFP), the most important food-borne illness caused by fish consumption in the world, is produced by ciguatoxins (CTX) which are suggested to be transferred from epiphytic dinoflagellates of *Gambierdiscus* and *Fukuyoa* genera into the food web [1,2]. The incidence of CFP in tropical and subtropical areas has been extensively reported since antiquity [3] but a spreading into more temperate regions of both CFP cases and *Gambierdiscus* and *Fukuyoa* populations have been reported in the last decade. In Europe, where CTXs are considered an emerging threat, the incidence of ciguatera episodes was first recorded in 2004 in the Canary Islands and Madeira [4–7]. There is awareness that global warming can cause spreading of CTX-producing dinoflagellates into higher latitudes not currently affected by CFP [8,9]. This concern has prompted Intergovernmental Panel on Climate Change to alert about the effect of global warming on the increase of CFP occurrence [10]. In fact, recent studies report *Gambierdiscus* and *Fukuyoa* species in more temperate waters of Japan, Mediterranean Sea, Canary Islands, and along the eastern coasts of North and South America [11–16].

Some authors have described that ciguatera incidence and the prevalence of *Gambierdiscus* and *Fukuyoa* cells are not always positively well correlated [17–20]. Different toxicity among *Gambierdiscus* species and changes in their interannual relative abundances were suggested to cause those differences between CFP outbreaks and *Gambierdiscus* detection [18]. Subsequent studies demonstrated high variability in the toxic potential among species. Higher toxicity has been reported, for example for *G. polynesiensis* in the Pacific [21–23], and *G. excentricus* in Caribbean Sea and the Canary Islands, in comparison with other species in the same regions [23,24]. This emphasizes the need for implementing adequate methodologies for the unequivocal identification of species in these genera, as well as for their quantification.

The difficult morphological differentiation among species of *Gambierdiscus* and, consequently, the problem for species-specific cell counts by traditional microscopy-based methodologies has been abundantly mentioned in the literature [25,26]. Therefore, the unequivocal identification of *Gambierdiscus* cells relies in most occasions on molecular techniques, mainly on rDNA sequences of cultures. Furthermore, semi-quantitative techniques (qPCR) have been described for most species and ribotypes of *Gambierdiscus* [27–29]. However, such methods cannot always be implemented, whereas light microscopy, despite its limitations, can still provide useful information.

In the present study, *Gambierdiscus* cells were morphologically characterized to determine to what extent their differences in morphology and size are useful for their specific identification. The methodology used was based on the parameters described by Bravo et al. [26] for the five species found in the Canary Islands so far, excepting the very recently reported *G. belizeanus* by Tudó et al. [30]. These morphological traits include cell depth measurement and the shapes of the second apical (2′) and second antapical (2′′′′) plates, as well as the position of the Po plate.

For some time, while the responsible agent of ciguatera was unknown, other benthic dinoflagellates apart from *Gambierdiscus* were associated with this syndrome, like *Ostreopsis* and *Prorocentrum*. As it was later discovered, this was due to the potential production of palytoxin and palytoxin-like compounds in *Ostreopsis*, and okadaic acid, dinophysistoxins-1, 2, and 4, and prorocentrolide in several benthic species of *Prorocentrum* like *P. lima* (see references in [31]). *P. hoffmannianum* has been isolated from benthic communities in the Canary Islands and confirmed to produce okadaic acid and three analogs [32]. Furthermore, the single species of *Vulcanodinium* described so far, *V. rugosum*, has been described to synthesize potent bioactive compounds like pinnatoxins and portimine, though it was never associated with human poisonings [33,34]. In consequence, although *Gambierdiscus* and *Fukuyoa* are the vector species for CTX in fish and CFP outbreaks, other species of those dinoflagellates cannot be discarded to cause some kind of harmful episode.

Studies in ciguatera endemic areas have described the effects of structural complexity of coral reefs on benthic harmful dinoflagellate communities. Thus, the different environmental driving factors that govern each community influence the benthic dinoflagellate assemblages [35,36]. Moreover, macrophyte host preferences as well as epiphytic dinoflagellate associations have been described in some regions [37,38]. The results, however, are sometimes contradictory due to the difficulty of understanding such complex benthic habitats [38]. The spatial distribution patterns of macrophytes depending on factors such as temperature, lighting, and wave exposure have been extensively studied on the coasts of the Canary Islands [39–41] and the composition and spatial distribution of marine macrophytes on the littoral of Fuerteventura exhibit a higher proportion of warm water species than on the rest of Islands of this archipelago [42–44]; it is not known, however, whether epiphytic harmful dinoflagellates are preferentially distributed in some of them. The specific genera examined in the present study comprised *Gambierdiscus*, *Prorocentrum*, *Coolia*, *Sinophysis*, *Ostreopsis*, *Vulcanodinium*, and *Scrippsiella*. All of them were surveyed from macrophytes from two locations in Fuerteventura Island with different macrophyte communities. The objectives of this study were: (1) to know if there is any preferential associations of benthic harmful dinoflagellates; (2) advance on the knowledge of the relationships of benthic dinoflagellate assemblages with different macrophyte communities; and (3)

to know the most abundant species of *Gambierdiscus* in the benthic macrophyte communities examined, for which a morphological study was carried out.

2. Materials and Methods

2.1. Study Sites

Cotillo is located in the NW of Fuerteventura Island (Canary archipelago, Figure 1A,B). In this station (28°41'18.34" N/14°0'48.14" W), macrophytes were sampled in a "charco"—a type of coastal pool of medium size quite abundant in the Canary Islands—of approximately 4×10^3 m^2 located to the left of Marfolín beach, on an extensive rocky platform that extends just north of the town of El Cotillo. This pool–maximum depth of 2 m at low tide and 3 m at high tide—has a mostly rocky bottom alternating with small sand spaces. This type of pools constitutes a particular environment with great environmental variability in a limited space that displays a high biodiversity. In Cotillo the rocky platform extends in depth up to more than one kilometer offshore reaching bathymetric levels greater than 20 m. Samples were taken from the "charco" by snorkeling during low tide up to 1.5 m deep and by scuba diving until 6 m deep.

Playitas is located on the eastern side of Fuerteventura Island (Figure 1A,B). The station (28°13'39.80" N/13°59'1.69" W) was situated just to the left of the port in the town Las Playitas. The platform extends with gentle slope in the infralitoral where small tidal ponds were sampled on foot. Then, samples were taken by snorkeling until 2–3 m and by scuba diving until 6 m deep.
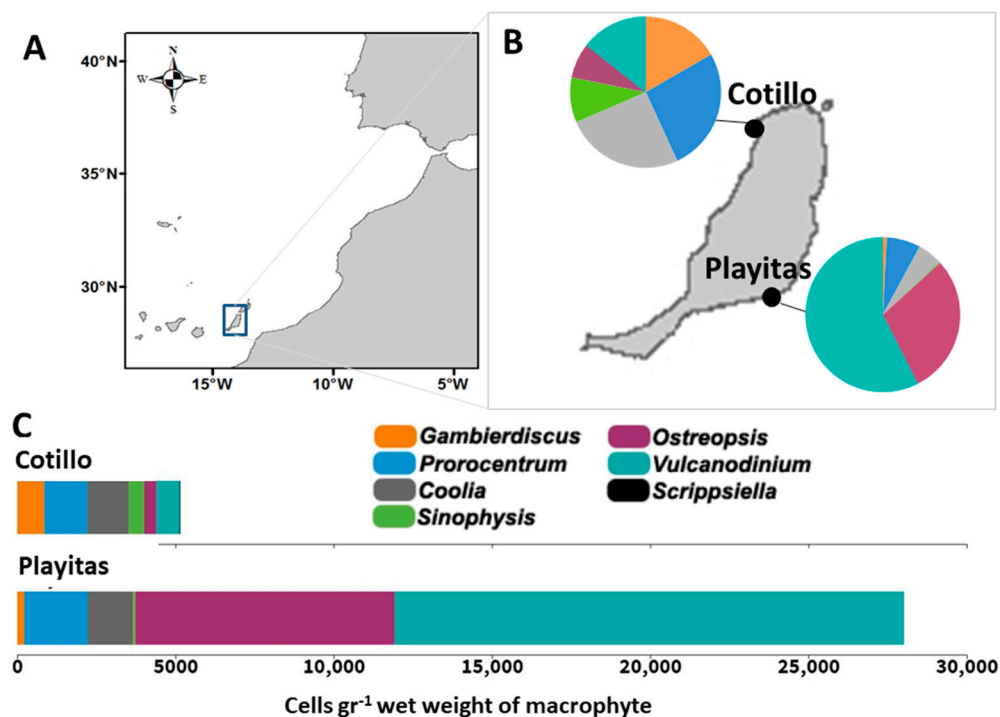
Figure 1. Location of Fuerteventura Island in the East Atlantic region (**A**). Relative (**B**) and total (**C**) mean abundances of epibenthic dinoflagellates in the two studied stations.

2.2. Field Sampling and Cell Enumeration

Sixty-seven samples of macrophytes were collected in the two stations in September 2016 (16th–17th in Playitas and 18th–19th in Cotillo) (Table 1, Figure 1). The macrophyte samples were carefully collected with surrounding water in plastic bags, placed in a plastic bottle and shaken to detach epiphytes. Afterwards, the gross materials were removed through a 300 μm opening nylon mesh and the remaining seawater was filtered again on a 20 mm nylon mesh to concentrate the cells.

Table 1. Number of samples and species of macrophytes collected in each station. The type of macrophyte according to the thallus structure (see Section 2.3) is indicated in parentheses. The code is used in the figures to indicate the macrophyte species.

Macrophytes	Code	Cotillo	Playitas
Amphiroa fragilissima (3), *Palisada perforata* (3), *Hypnea spinella* (3), *Blennothrix lyngbyacea* (4)	Turf		1
Amphiroa fragilissima (3), *Jania adhaerens* (3), *Centroceras gasparrinii* (4)	Turf		1
Asparagopsis taxiformis (4)	Asp	3	
Asparagopsis taxiformis (4), *Lophocladia trichoclados* (4)	Asp+Lo	1	
Blennothrix lyngbyacea (4)	Cya		3
Canistrocarpus cervicornis (2)	Can	1	1
Canistrocarpus cervicornis (2), *Blennothrix lyngbyacea* (4)	Can+Ci		1
Caulerpa cylindracea (3), *Blennothrix lyngbyacea* (4)	Cau+Ci		1
Caulerpa racemosa (3), *Centroceras gasparrinii* (4)	Cau+Ce		1
Cladostephus spongiosum (4)	Cla	1	
Cottoniella fusiformis (4)	Cot		2
Dictyota ciliolate (2)	Dic	2	
Dictyota dichotoma (2)	Dic	2	2
Dictyota humifusa (2)	Dic		1
Dictyota humifusa (2), *Jania capillacea* (3), *Lobophora schneideri* (2)	Dic++	1	
Dictyota implexa (2)	Dic	1	
Digenea simplex (3)	Dig	2	
Galaxaura rugosa (3)	Gal	3	
Galaxaura rugosa (3), *Lophocladia trichoclados* (4)	Gal+Lo		1
Halopteris filicina (4)	Hal	1	
Halopteris scoparia (4)	Hal	2	
Halopteris scoparia (4), *Jania virgate* (3)	Hal+Jan	1	
Hypnea spinella (3)	Hyp		2
Hypnea spinella (3), *Lophocladia trichoclados* (4), *Blennothrix lyngbyacea* (4)	Turf		1
Jania adhaerens (3), *Centroceras gasparrinii* (4)	Jan+Ce	1	
Jania adhaerens (3), *Hypnea spinella* (3)	Turf		1
Lobophora canariensis (1)	Lob	1	
Lobophora schneideri (1)	Lob	4	
Lophocladia trichoclados (4)	Lop	3	2
Lophocladia trichoclados (4), *Hypnea spinella* (3), *Blennothrix lyngbyacea* (4)	Lop++		1
Padina pavonica (1)	Pad	1	1
Spyridia filamentosa (4)	Spy		1
Spyridia hypnoides (4)	Spy		2
Spyridia hypnoides (4), *Blennothrix lyngbyacea* (4)	Spy+Bl		1
Spyridia hypnoides (4), *Hypnea spinella* (3), *Jania adhaerens* (3), *Centroceras gasparrinii* (4)	Turf		1
Stypopodium zonale (1)	Sty	5	1
Cyanophyceae (4)	Cya	2	

Aliquots from these samples were fixed in situ with formaldehyde for identification and enumeration in the laboratory.

Formaldehyde-fixed epiphyte samples were stained with Fluorescent Brightner 28 (Sigma, St Louis, MO, USA) [45] for dinoflagellates identification and counted under UV light using an Axiovert 125 epifluorescence inverted microscope (Carl Zeiss AG, Germany) at 400× magnification. Quantitative data were obtained for the following genera of benthic dinoflagellates: *Gambierdiscus*, *Prorocentrum*, *Coolia*, *Sinophysis*, *Ostreopsis*, *Vulcanodinium*, and *Scrippsiella*. Cell abundance was expressed as cells per gram wet weight of host macrophyte (abbreviated as cells g^{-1} in the results section). For this purpose, fresh macrophytes were weighted after being manually drained just after collection.

2.3. Macrophyte Sampling

The macrophyte community of the two sampling stations presented remarkable differences in species composition. During sampling, the most representative species (or groups of species) of each station were collected. Although macrophyte composition was not the target of the study, different macrophyte communities in Cotillo and Playitas were clearly evidenced (Table 1).

In the intertidal zone of Playitas, red algae (Rhodophyceae) belonging to the orders Ceramiales, Corallinales, and Gigartinales which formed a thick turf (named as turf in Table 1) was characteristic. There, the most representative species were: *Hypnea spinella*, *Jania adhaerens*, *Centroceras gasparrinii*, *Amphiroa fragilissima*, and *Palisada perforata*. Added to those and frequently as an epiphyte, the filamentous cyanobacteria *Blennothrix lyngbyacea* was also found. Among the brown algae (Phaeophyceae), much less abundant in the intertidal zone, erect foliose species of Dictyotales as *Padina pavonica* and *Stypopodium zonale* were collected (Table 1). Other species of Ceramiales, such as the erect, filamentous, and profusely branched *Lophocladia trichoclados* and *Cottoniella fusiformis*, as well as ribbon-like Dictyotales, such as *Canistrocarpus cervicornis*, *Dictyota dichotoma*, and *D. humifusa*, were sampled at depths of more than two meters.

Macrophyte community was mainly formed by a very diverse assemblage of erect brown and red algae in the "charco" sampled in Cotillo station. Dictyotales as *Dictyota* spp, *Stypopodium zonale*, *Padina pavonica*, and the also foliose *Lobophora schneideri* were dominant (Table 1). Species of Sphaceraliales forming erect arborescent tufts as *Halopteris scoparia* and *H. filicina* were also collected. Among the Rhodophyceae, species from Bonnemaisoniales (as the arborescent with duster-like appearance *Asparagopsis taxiformis*), Nemaliales (as the cylindrical dichotomously branched *Galaxaura rugosa*) and Ceramiales (as *Lophocladia trichoclados*) were the most common macrophytes in the "charco". At 2 m depth, *Asparagopsis taxiformis* was the dominant species and species of Dictyotales as *Lobophora schneideri* prevailed deeper.

Since epiphyte abundances are clearly related to differences in structure and wet weight to surface area ratios of macrophytes and an estimate of the surface/weight ratio has not yet been established, the macrophytes were categorized into four types based on external morphology classification, modified from definitions in Parsons and Preskitt [46]: (1) Type 1: Foliose (laminar thallus); (2) Type 2: Ribbon-like (several times forked ribbon-shaped thallus); (3) Type 3: Entangled clumps (thallus with cylindrical axes, 0.2–2.0 mm diameter, branched and entangled); (4) Type 4: Filamentous (thallus with thin cylindrical axes, ≤0.2 mm diameter, profusely branched and tree-like).

2.4. Epiphytic Dinoflagellate Assemblages

A principal component analysis (PCA) was performed to analyze the data describing the composition of epiphytic dinoflagellates. It was conducted using logarithmically transformed cell concentrations and the statistical software package SPSS. The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.66 and Bartlett's test of sphericity, which tests for the presence of correlations among variables, was significant at $p < 0.001$. In addition, non-parametric rank-based test (Kruskal–Wallis) was performed using the statistical software package SPSS version 14.0 (SPSS Inc., Chicago IL) to compare the distribution of the abundance values of dinoflagellate species from both stations.

2.5. Morphometric Analysis and Abundances of Gambierdiscus

Morphological analyses were performed on individual cells of *Gambierdiscus* isolated from epiphytic samples. Measurements of the epitheca and hypotheca of the same specimen were made by placing individual cells between two coverslips, which allowed them to be observed and photographed from their apical and antapical views. The morphologies of a total of 30–40 cells from each sample were studied. Cell morphology determinations were based on measurements of two thecal plates: the second apical (2′) plate, located on the epitheca, and the second antapical (2″″) plate on the hypotheca, following the methodology described by Bravo et al. [26]. Three morphometric parameters were used as follows: (1) R1 as an assessment of the rectangular vs. the hatchet shape of the 2′ plate; (2) R2 representing the position of Po in the lateral edge of the 2′ plate and, therefore, the degree of eccentricity of Po in the cell; and (3) R3 as an indicator of the elongation of the 2″″ plate. In addition, cell depth (D), corresponding to the dorso-ventral diameter was also used. These parameters were selected following the most relevant bibliography on *Gambierdiscus* morphology as mentioned by Bravo et al. [26]. These authors define the parameter values for each species based on a study

performed with culture cells. All measurements needed for those morphometric calculations were made on Calcofluor-stained cells using digital imaging software (ZEN lite, ZEISS Microscopy) and an epifluorescence microscope (Leica DMLA, Wetzlar, Germany) equipped with a UV light source and an AxioCam HRc (Carl Zeiss, Jena, Germany) digital camera. Concentrations of the five species of *Gambierdiscus* were estimated from the percentages of cells identified for each species and the total concentration value of the genus counted in each sample as explained above (section of field sampling and cell enumeration).

3. Results

3.1. Abundances of Epiphytic Dinoflagellates

Cells of *Gambierdiscus*, *Prorocentrum*, *Coolia*, *Sinophysis*, *Ostreopsis*, and *Vulcanodinium* genera were identified in the two sampled stations but appeared in different ratios. In Cotillo, *Prorocentrum*, *Coolia*, *Gambierdiscus*, and *Vulcanodinium* were present at percentages higher than 10% (26%, 25%, 17%, and 15%, respectively) whereas *Synophysis* and *Ostreopsis* represented 10% and 8%, respectively (Figure 1B). On other hand, *Vulcanodinium* and *Ostreopsis* prevailed in Playitas (58% and 29%, respectively), while *Prorocentrum*, *Coolia*, *Gambierdiscus* and *Sinophysis* were less abundant (7%, 5%, 1%, and 0.2%, respectively; Figure 1B). The genus *Scrippsiella* was only detected in three samples from Cotillo (reaching up to 486 cells gr^{-1}), was not included in the statistical analyses. Total dinoflagellate abundances were higher in Playitas station. The abundance mean values for all genera are plotted in Figure 1C. The differences between stations were highly significant both for genus composition and abundances. Statistical values (mean, standard deviations, maximum and minimum) of abundances of all species and stations are shown in Table 2. Significant differences ($p < 0.001$) were found between the distribution of the abundances of *Gambierdiscus*, *Sinophysis*, *Ostreopsis*, and *Vulcanodinium* from both stations; on the contrary, no significant differences were found for *Prorocentrum* and *Coolia* (Table 2).
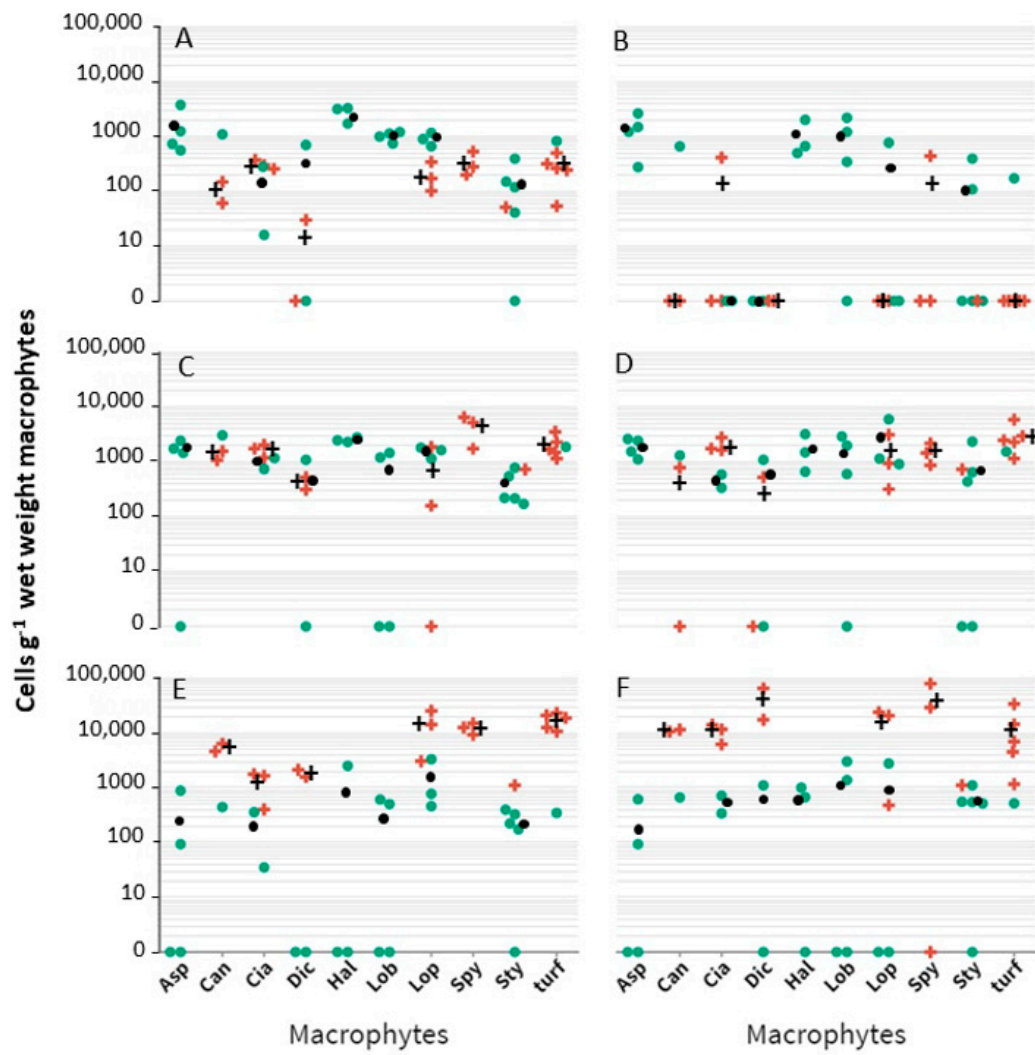
Table 2. Statistical values (mean, standard deviation, minimum, maximum, mean ranks and significance) of the abundances (cells gr^{-1} wet weight) of dinoflagellate genera in Fuerteventura.

	Station	Mean ± SD	Minimum	Maximum	Mean Ranks	Significance
Gambierdiscus	Cotillo	857 ± 899	0	3778	41.26	0.000
	Playitas	223 ± 188	0	800	22.96	
Prorocentrum	Cotillo	1356 ± 959	0	3393	31.22	0.262
	Playitas	1999 ± 1782	0	6616	36.59	
Coolia	Cotillo	1303 ± 1291	0	6065	32.59	0.654
	Playitas	1436 ± 1291	0	5954	34.73	
Sinophysis	Cotillo	495 ± 231	0	2646	40.11	0.000
	Playitas	70 ± 231	0	1103	24.54	
Ostreopsis	Cotillo	386 ± 670	0	3308	20.47	0.000
	Playitas	8195 ± 7548	401	25,204	51.18	
Vulcanodinium	Cotillo	736 ± 982	0	3970	23.46	0.000
	Playitas	16,098 ± 22,937	0	81,598	47.13	

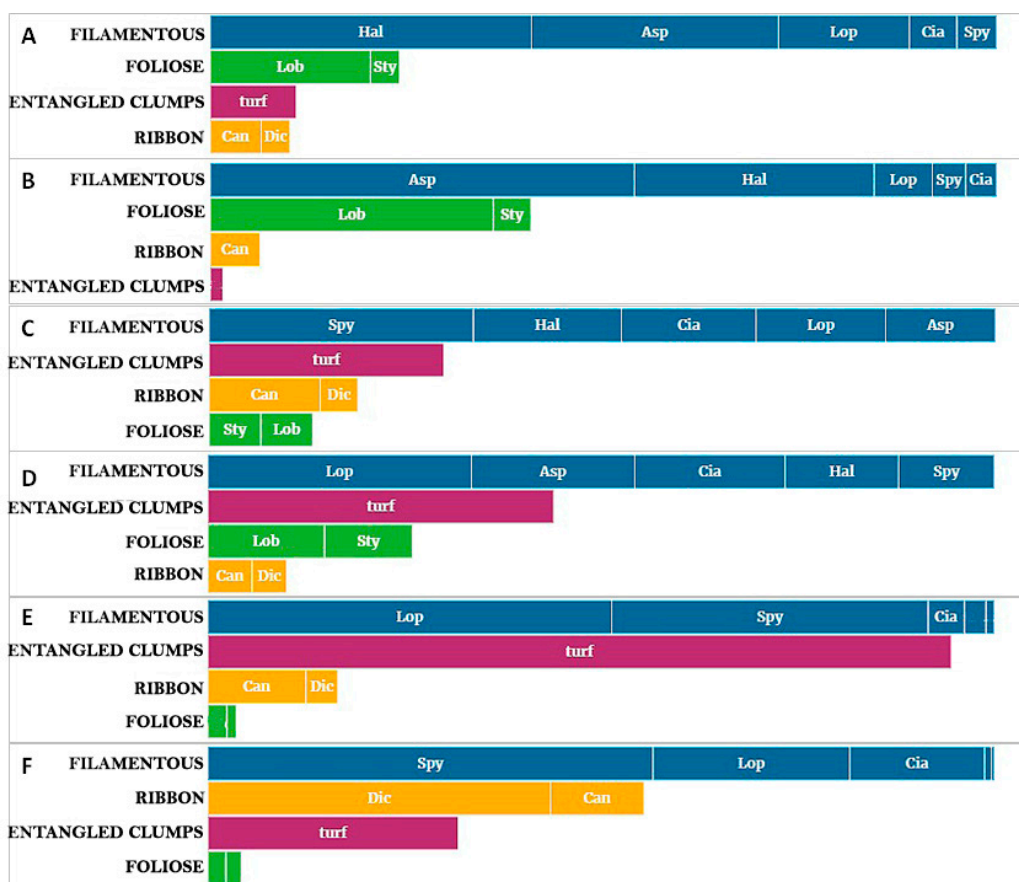### 3.2. Epiphytic Dinoflagellate Assemblages and Macrophyte Associations

Different dinoflagellate assemblages among the six dinoflagellate genera were revealed through Principal Component Analysis (PCA) of their abundances. Component 1 (PC1) grouped four species: *Ostreopsis*, *Prorocentrum*, *Coolia*, and *Vulcanodinium*, whereas component 2 (PC2) was more associated to *Gambierdiscus* and *Sinophysis* (Figure 2A). On PC2, these last two species were negatively correlated with *Ostreopsis*. The two components explained 62% of the variance (31.6% for component 1 and 30.6% for component 2). Factor loadings of the genera projected on the PCA plot show a clear separation of *Ostreopsis* and *Vulcanodinium* from *Gambierdiscus* and *Sinophysis* (Figure 2A), whereas the relationship of *Prorocentrum* and *Coolia* was not so evident. The components were differently associated to the two

stations. As shown in Figure 2A, while *Ostreopsis* and *Vulcanodinium* were more associated to Playitas, *Gambierdiscus* and *Sinophysis* were more to Cotillo station. The different macroalgae composition in the two stations and its differential association to the different genera of dinoflagellates is showed in Figure 2B.

Abundances of the different dinoflagellate genera in the main macrophytes of the two stations are plotted in Figure 3. The means of the abundances of *Gambierdiscus* and *Sinophysis* presented the highest values in the macrophytes of Cotillo compared to those of *Ostreopsis* and *Vulcanodinium*, which are higher in the macrophytes of Playitas.

The study of the abundances of the dinoflagellate genera in the different types of macrophytes showed that those with a filamentous structure clearly presented the highest abundances in all the genera studied (Figure 4). However, within that type of macrophytes remarkable differences were found depending on the dinoflagellates. Thus, *Gambierdiscus* and *Sinophysis* presented the highest abundance values in the filamentous *Halopteris* and *Asparagopsis*, whereas *Spyridia* and *Lophocladia* showed the highest abundances of *Ostreopsis* and *Vulcanodinium* (Figures 3 and 4). Macrophytes with an entangled clump structure as turf species characteristic of Playitas station ranked second for *Prorocentrum*, *Coolia*, and *Ostreopsis* (Figure 4). On the contrary, the foliose macrophyte *Lobophora* occupied second position for *Gambierdiscus* and *Sinophysis* and the ribbon-like *Dyctiota* and *Canistrocarpus* in the case of *Vulcanodinium*. Concentrations of *Prorocentrum* and *Coolia* presented a more homogenous distribution among all macrophyte species (Figure 3).

(A)   (B)

Figure 2. Plot of the scores derived from PCA of the concentrations of epibenthic dinoflagellate genera from the two stations, Cotillo (circles) and Playitas (diamonds) (**A**); and the macrophytes corresponding to each sample (**B**) (see macrophyte codes in Table 1). Crosses represent the factor loadings of the different dinoflagellate genera.

Figure 3. Abundances of the different dinoflagellate genera in different macrophytes from the two stations, Cotillo (circles, each of the sample value in green and mean values in black) and Playitas (crosses, each of the sample value in red and mean values in black). *Gambierdiscus* (**A**), *Sinophysis* (**B**), *Prorocentrum* (**C**), *Coolia* (**D**), *Ostreopsis* (**E**), *Vulcanodinium* (**F**). See macrophyte codes in Table 1.

Figure 4. Relative abundances of dinoflagellate genera: *Gambierdiscus* (**A**), *Sinophysis* (**B**), *Prorocentrum* (**C**), *Coolia* (**D**), *Ostreopsis* (**E**), *Vulcanodinium* (**F**) in different types of macrophytes according the thallus structure (see Section 2.3). See macrophyte codes in Table 1.

3.3. Morphological Characterization of Gambierdiscus Species

Based on cell sizes (cell depth denoted as D) and the parameters R1, R2, and R3 (related to the plate's morphology, see Material and Methods) 91% of the specimens were classified within the five *Gambierdiscus* species detected previously in the Canary Islands: *G. australes*, *G. caribaeus*, *G. carolinianus*, *G. excentricus*, and *G. silvae*. The values of the parameters (D, R1, R2, and R3) and the corresponding classification are scattered in Figure 5. *G. excentricus* was separated from all other species by the excentricity of Po (represented by parameter R2) (Figure 5A) excepting the overlap of some specimens with *G. australes*. In those cases, R1 and R3 relationship was useful for identification (Figure 5B). Figure 5A shows as *G. excentricus* and *G. silvae* were the most easily discriminated species basing in size and R2. In addition, the scattered plotting of R1 (denoting the shape of 2′ plate) and R3 (shape of 2‴ plate) efficiently separated *G. silvae* and *G. caribaeus* from the rest of the species (Figure 5B). For classification of those species, size was also useful following the description by the same authors previously mentioned. Low overlap percentages were observed between the groups of *G. australes* and *G. excentricus* (1.9% of the total cells) regarding excentricity of Po (represented by parameter R2), the most differentiating trait between those species (Figure 5A). Notwithstanding, the general appearance of the cell as well as the general shape of 2′ and 2‴ plates helped to classify them. *G. australes* and *G. caribaeus* were the most similar species. Both coincide in the three following traits: rectangular shape of 2′ plate (R1), cell size (D) and position of Po (R2) (Figure 5). The shape of 2‴, more elongated in *G. australes* than in *G. caribaeus*, was the most useful trait to discriminate them (Figure 5B). However, the overlapping in that parameter was also remarkable. Due to this, it was not possible to separate 9% of the total cells which were comprised in the *G. australes/caribaeus* group.
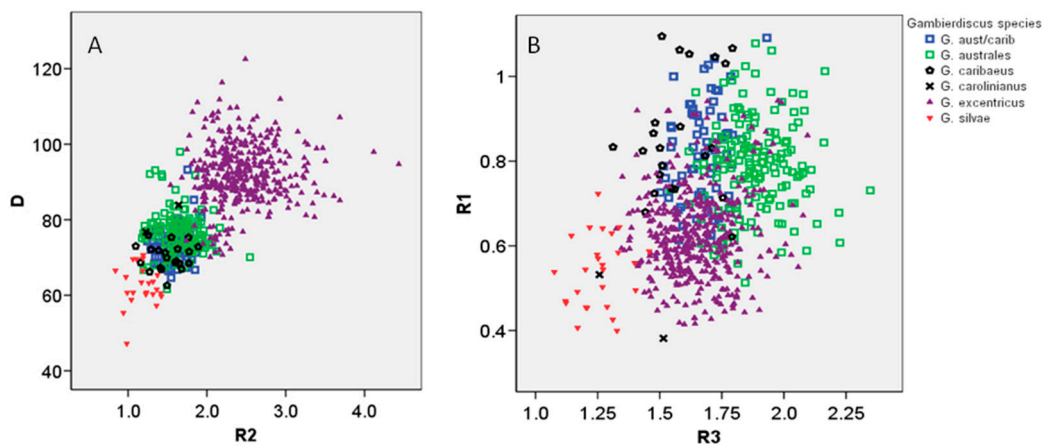
Figure 5. Scatterplots of morphological parameters observed in *Gambierdiscus* spp. cells from Cotillo and Playitas stations from Fuerteventura Island. (**A**) D against R2 and (**B**) R1 against R3.

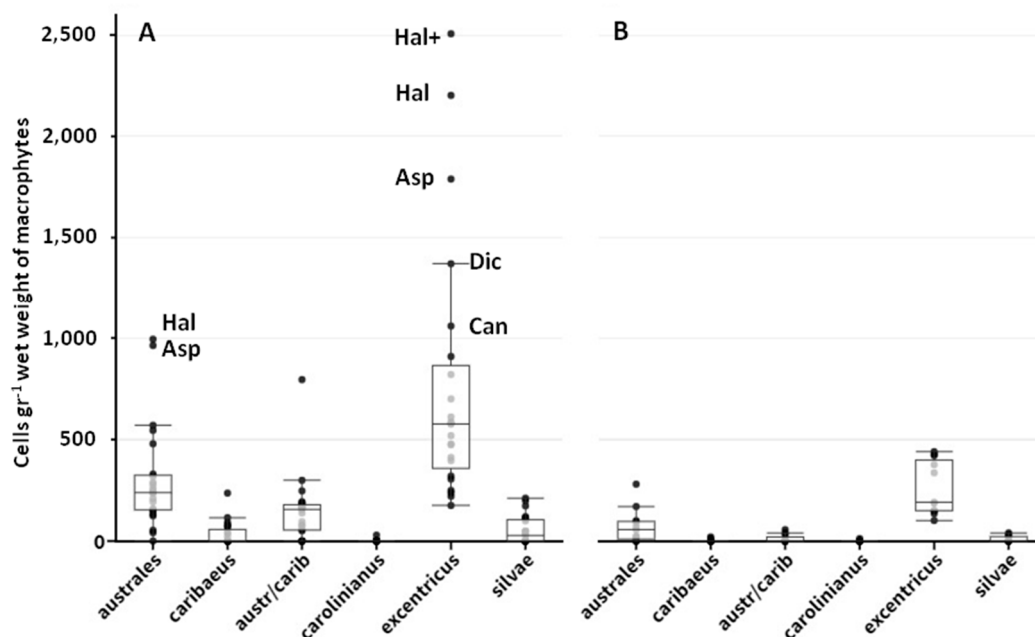### 3.4. Diversity and abundances of Gambierdiscus species

Total abundances of genus *Gambierdiscus* reached up to 3.8×10^3 cells gr^{-1} in Cotillo station and 8×10^2 cells gr^{-1} in Playitas. Means and standard deviations are showed in Table 2. The abundance distributions between the two stations showed significant differences ($p < 0.001$) (Table 2). Individual morphometric analyses as mentioned in the previous section revealed, at least five species of *Gambierdiscus* in the two stations: *G. australes*, *G. caribaeus*, *G. carolinianus*, *G. excentricus*, and *G. silvae*. Figure 6 shows the percent cell concentrations of *Gambierdiscus* species. Significant differences were only detected in the distribution of percentages of *G. australes* and *G. excentricus* between the two stations ($p < 0.01$). *G. excentricus* was the most abundant of the five species, representing as average 56% and 75% in Cotillo and Playitas, respectively, followed by *G. australes* (mean of 24% and 18% in each station, respectively). The number of specimens which could not be identified unequivocally, denominated as *G. australes/caribaeus*, was quite abundant in Cotillo but rare in Playitas station (mean of 12% and 3%, respectively). *G. caribaeus* and *G. silvae* presented mean abundances of 4% and 5% in Cotillo and 1% and 3% in Playitas, respectively. Finally, *G. carolinianus* was even less represented in the two stations (Figure 6).
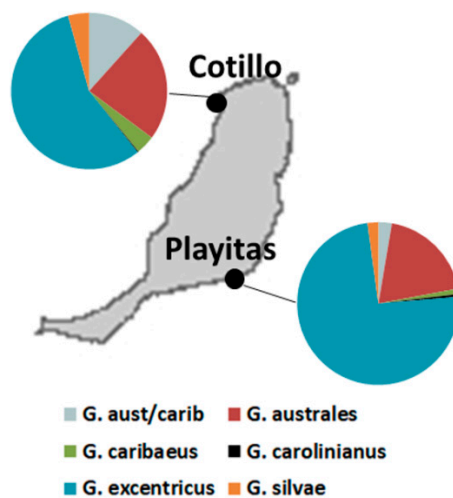
Figure 6. Relative mean abundances of *Gambierdiscus* species in Cotillo and Playitas stations on Fuerteventura Island.

Abundances per gram of macrophytes of the five *Gambierdiscus* species were estimated from the percentages of the morphologically identified cells and the quantification of cells

of the genus *Gambierdiscus*. Only *G. australes* and *G. excentricus* exceeded 500 cells gr^{-1}. Moreover, *G. excentricus* surpassed concentrations of 10^3 cells gr^{-1} in five samples from Cotillo station (Figure 7). The host macrophytes corresponding to these samples were: (1) *Halopteris scoparia* (Phaeophyceae) + *Jania virgata* (Rhodophyceae) (2500 cells gr^{-1}) (2) *Halopteris scoparia* (Phaeophyceae) (2200 cells gr^{-1}) (3) *Asparagopsis taxiformis* (Rhodophyceae) (1790 cells gr^{-1}) (4) *Dictyota implexa* (Phaeophyceae) (1370 cells gr^{-1}) and (5) *Canistrocarpus cervicornis* (Phaeophyceae) (1060 cells gr^{-1}) (Figure 7). Regarding depth in the water column, *Canistrocarpus cervicornis* and *Dictyota implexa* were the macrophytes collected at greater depths (3.5 m. and 6 m. respectively) where concentrations of *G. excentricus* were estimated to be higher than 10^3 cells gr^{-1}. That species accounted for 96% and 76% of *Gambierdiscus* spp. in those samples, respectively.

Figure 7. Box plot of cell abundances of *Gambierdiscus* species in Cotillo (**A**) and Playitas (**B**) stations in Fuerteventura Island. The macrophyte species that presented concentrations of *Gambierdiscus* close to 10^3 cells gr^{-1} are indicated (see macrophyte codes in Table 1).

4. Discussion

A great deal of research and communication efforts have been carried out during the last decade on the study of tropical and subtropical benthic HABs mainly those associated with ciguatera outbreaks and *Gambierdiscus*. However, with the exception of a few areas and dinoflagellate genera, the knowledge on benthic harmful microalgae abundance and distribution is still very scarce [47]. That knowledge has become even more essential considering the current expansion of some harmful benthic dinoflagellate species to temperate regions. Ciguatera is an emerging human poisoning in Europe since the first outbreak occurred in the Canary Islands archipelago and in Madeira in 2004 [3,48]. Since then, populations of the CTXs-producer dinoflagellates, *Gambierdiscus* and *Fukuyoa*, have been documented both in those regions of Macaronesia and in Mediterranean Sea, though no CFP episodes have been confirmed in the latter region [3]. Yet, there are few data in the literature on harmful benthic dinoflagellates in the Canary Islands other than *Gambierdiscus*. Studies on this topic are increasing since the emergence of ciguatera on the Islands. It is remarkable that two species of *Gambierdiscus*, *G. excentricus* and *G. silvae*, and two of *Coolia*, *C. canariensis* and *C. guanchica*, have been described in the last decade from samples from the Canary archipelago [49–52]. In addition, genera as *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Coolia*, and *Vulcanodinium* had already been reported in the same region [26,53].

4.1. Diversity and Abundance of Harmful Benthic Dinoflagellates

For the six epibenthic genera herein studied, both the mean and the maximum cell concentrations showed the following descending order: *Vulcanodinium*, *Ostreopsis*, *Prorocentrum*, *Coolia*, *Gambierdiscus*, and *Sinophysis* (Table 2). These genera are comparable to those reported in other studies in the Canary Islands [26,53], though Fernandez-Zabala et al. [53] limited the study to *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, and *Coolia*. As far as we know, there are very few reports on benthic dinoflagellates other than *Gambierdiscus* or *Fukuyoa* for other Islands from Macaronesia region. The genera *Ostreopsis*, *Prorocentrum*, and *Coolia* have been also reported for Cabo Verde Islands [53]. Moreover, a list of phytoplankton taxa including *Ostreopsis (O*. cf. *ovata)*, *Prorocentrum (P. lima* and *P. hoffmaniannum)*, *Coolia* sp., and *Gambierdiscus excentricus* are reported in Madeira [54,55]. Cell abundance comparisons from literature are controversial due to the methodological differences among studies. The main methodological problem is related to the differences on macrophyte surfaces and morphologies which make difficult the standardizations. Methodologies based on quantifying benthic dinoflagellates on artificial substrates have been developed in the last decade in order to normalize cell abundance to a standardized surface [56]. This methodology has been tested in the Canary Islands by Fernandez-Zabala et al. [53] showing that, in most cases, cell abundances of epiphytic dinoflagellates showed lower variability on artificial substrates than on macroalgae. However, a well-defined methodology to quantify epiphytic cells in macrophytes is still needed. In order to make the pertinent comparisons between macrophytes and artificial substrates, there should be a consensus on the methodologies of both procedures. This issue is particularly relevant to quantitate the potential associations between epiphytic dinoflagellates and certain macrophyte taxa.

Maximum concentrations of *Gambierdiscus* of 4.9×10^3 cells gr^{-1} blot dry weight of host macrophyte (n = 128, from samples collected from five Canary Islands) were already reported in Fuerteventura by Rodriguez et al. [15]. No mention of macrophyte species was given by those authors. Blot dry procedure consists in draining algae overnight over soft laboratory paper. A loss of 62% of weight on average has been reported when dry-blot macrophyte weight is used compared with the manually drained wet weight of the macrophyte used in the present paper; obviously with the corresponding increase in the concentrations of cells when blot-dried weight expression is used [26]. Taking this into consideration, estimated maximum values for *Gambierdiscus* from those authors and our results (3.1×10^3 and 3.8×10^3 cells gr^{-1} wet weight respectively) are of the same order of magnitude. On other hand, blooms of *Gambierdiscus* with concentrations higher than 10^4 cells gr^{-1} wet weight were reported in the port of La Restinga [53,57]. Further investigations carried out with standardized methodologies should be addressed to link dinoflagellate populations and their associated environmental conditions with CFP risk areas in the Canary Islands. Furthermore, the high heterogeneity in *Gambierdiscus* cell numbers in the region makes essential to investigate the relationships between some habitats and detected hotspot areas.

The maximum abundances of *Ostreopsis* found in the present study were lower than previously reported values in the region since concentrations up to 2.2×10^5 cells gr^{-1} wet weight algae had been documented [53]. Even if these numbers are lower than those for *Ostreopsis* blooms reported in NW Mediterranean Sea and New Zealand where they have been associated with human health problems by coastal aerosols [58], the risk of *Ostreopsis* proliferations in Canary Islands should be investigated. The genus *Prorocentrum* includes benthic species, such as *P. lima* and *P. hoffmannianum*, that produce okadaic acid and dinophysistoxins or derivatives which have been associated to Diarrhetic Shellfish Poisoning [32,59–61]. Although in the present study no taxonomic studies were carried out that allowed identification at species level, the different morphologies observed in cell size and shape reveals a high specific diversity which includes both *P. lima*-like cells and *P. hoffmannianum*-like specimens. Hence the great interest to carry out taxonomic studies from this potentially toxic genus in the region. Regarding the genus *Sinophysis* (often observed in Cotillo station), to our knowledge it has not been associated with toxin production. The only species reported so far in the Canary Islands, *S. canaliculata*, harbors cyanobionts of uncertain taxonomic position [62,63].

Vulcanodinium is not a genus usually included in studies of benthic dinoflagellates, although it has been documented in benthic communities of the Canary Islands [15,26]. Its high abundance in the present study is a remarkable new interesting finding given the high concentrations observed in Playitas station. *Vulcanodinium rugosum*, the only one species described so far from the genus, was described in 2011 from a French Mediterranean Lagoon and is responsible for producing neurotoxic pinnatoxins (PnTXs) which have been recurrently detected in the shellfish from that region [64,65]. The morphology of *Vulcanodinium* cells in the samples coincide with those described by Rhodes et al. and Zeng et al. [64,66] as motile cells, however their benthic/planktonic character should be studied. In the life strategy of this species, the phase in which vegetative division occurs is the benthic non-mobile spherical cells which are considered as cysts [66]. This type of cysts has been called division cysts which have been described in species considered planktonic but with an intense relationship with the benthos [67,68]. No human poisonings by PnTXs are known, however because of their high toxic potential, European Food Safety Authority (EFSA) have pointed out the need for more information on the oral toxicity of these compounds for risk assessment as seafood contaminant [65]. Therefore, future taxonomic, life cycle, and toxin studies are required from the organism found in the Canary Islands.

4.2. Associations of Benthic Harmful Dinoflagellates and Macrophyte Communities

Our data showed preferential associations of benthic dinoflagellates in benthic communities of the Canary archipelago. The population distributions of *Gambierdiscus* and *Synophysis* were significantly opposite to that of *Ostreopsis* and *Vulcanodinium*. Moreover, the two principal components from PCA were preferentially associated with two different algae communities, those of Cotillo and Playitas, respectively. Our results agree with the distinct distributions of *Gambierdiscus* and *Ostreopsis* reported by other studies (as for example [69]). These authors reported *Ostreopsis* spp. in greater concentration in reef areas with high wave energy, coinciding with that mentioned in the Mediterranean by Vila et al. [70]. This is also supported by the results of Grzebyk et al. [71] which reported highest abundances of *Ostreopsis* in turbulent coral reef habitats. However, blooms of this genus have also been registered in protected areas [31,72]. To better understand these patterns, proper identification of *Ostreopsis* assemblages in each case, and more information about their ecology and the environmental factors associated with their proliferations are needed. On other hand, distribution of *Gambierdiscus* has been more associated with sheltered zones protected from the wind and adversely affected by terrestrial inputs [71]. These authors also cite *Ostreopsis* and *Prorocentrum* to be more tolerant to terrestrial loads and exploiting different ecological niches than *Gambierdiscus*. These opposite niches can be determined by the spatial distribution of environmental factors, such as hydrodynamics and terrestrial contributions.

Macrophytes as important elements of benthic niches are interrelated with environmental factors. Among them, wave exposure integrates a wide variety of environmental factors being critical for the biodiversity of coastal ecosystems. It is known that hydrodynamic conditions influence the distribution of intertidal and subtidal organisms [73,74]. In this way, direct and indirect effects of waves have been reported an important driver of the distribution and biodiversity of marine macrophytes in coastal ecosystems [75]. Comparing the characteristics of the habitats studied here, Playitas station is more exposed to wave impact and with macrophyte communities mainly composed by mix red turf algae. This is very different to Cotillo, a more protected habitat with a very different macrophyte composition. Our data suggests that the "charco" in Cotillo station would provide a better niche for the development of *Gambierdiscus* and *Sinophysis*. Instead, the rocky platform exposed to waves in Playitas would configure a habitat more suitable for *Ostreopsis* and *Vulcanodinium*. Our results suggest that dinoflagellate-macrophyte associations are determined by the characteristics of the studied habitats. The environmental conditions and the microhabitats found in each location would determine the dominant organisms. On the other hand, their populations and the resulting associations could change over time. It must be taken into account that this study represents a fixed image at a certain time of

the year. In that sense, further studies integrating spatial and temporal scales are needed, as these dimensions are highly relevant for management purposes and sampling strategy [76].

The fact is that macrophytes serve as habitat and function as complex ecological systems depending on their size, structure and longevity. They exhibit a great variety of epiphytic algae, as well as other microorganisms and associated mobile animals (including meiofauna, macrofauna, and fish). Therefore, given such complexity, discerning the relationship between macrophytes and epiphytic dinoflagellates still remains a difficult task. Notwithstanding, some trends appear in the literature about substrate preferences of the main benthic dinoflagellate genera (*Ostreopsis*, *Prorocentrum*, *Coolia and Gambierdiscus*), linked with macrophyte morphology and taxonomy (see Boisnoir et al. [38] and references therein). Regarding *Gambierdiscus*, this genus seems to be associated with a wide diversity of macrophyte taxa, although the epiphytic behaviour (growth and attachment) varies by species and host algae [77]. Recent authors have emphasized the importance of microhabitats in benthic communities of ciguatera endemic areas and the complexity of habitats as a determinant factor for the heterogeneity in *Gambierdiscus* and other epiphytic dinoflagellate distributions [36]. Environmental factors such as light and wave impact have a heterogeneous distribution and, therefore, generate a great deal of heterogeneity in the macrophyte communities and the associated dinoflagellates.

Since the beginning of studies on communities where ciguatera-producing organisms thrive, many authors have remarked that the type of substrate plays an important role in their distributions. Yet, the role of some macrophytes as potentially preferential substrates is controversial. Some of the first ciguatera studies mentioned that rhodophytes were most prone to harbor epiphytic harmful dinoflagellates [78,79]. However, other authors described opportunistic patterns regarding substrate interactions with occurrences on rhodophytes, phaeophytes, chlorophytes, and vascular plants [20,80]. The examination of substrate preferences is controversial due to the difficulty of standardizing cell abundances calculated per weight of the host macrophyte. As far as we know, no estimates of surface/weight ratio have been established which prevent accurate comparisons among the different species of macrophytes. Trying to avoid this handicap we conducted comparisons between types of macrophytes depending on their thallus architecture. The different thallus architecture determines the total surface available for epiphytic dinoflagellates and defines a range of microhabitats which offer shelter and facilitate survival. The available surface and the microhabitats number increase progressively from the two-dimensional foliose to the three-dimensional, flexible filamentous thallus with a high surface:volume ratio (types 1–4 respectively, see material and methods). Our results revealed filamentous macrophytes as preferred substrates for all dinoflagellate genera, suggesting that it shapes a very heterogeneous habitat which increases the diversity and richness of the epiphytic communities. Macrophytes that formed entangled groups also showed high concentrations of dinoflagellates, especially of the genus *Ostreopsis*. Nevertheless, this classification aimed to define general trends of host preferences has some limitations. For example, the delimitation between the two macrophyte types mentioned is not strict. In our study, the "entangled clumps" type coincided mainly with turf algae that occasionally included some specimens of filamentous algae. Despite these considerations, differences in macrophyte preferences between dinoflagellate genera were observed (i.e., the association between the "entangled clumps" type formed by turfs of rhodophytes and *Ostreopsis* vs. the preference of ribbon-like macrophytes by *Vulcanodinium*).

4.3. Gambierdiscus Results

In the Canary archipelago, ciguatera outbreaks could be related with local *Gambierdiscus* spp. including those identified until date: *G. australes*, *G. caribaeus*, *G. carolinianus*, *G. excentricus G. silvae* and *G. belizeanum* [15,30]. The morphometric study of these first five species performed by Bravo et al. [26] was applied in the present study with the aim to identify them in samples of Fuerteventura –take into account that the publication of the detection of *G. belizeanum* in the region was almost coincident with that of the present manuscript. Despite of their morphological similarity, 91% of the specimens were successfully classified at species level. *G. excentricus* and *G. australes* were the most abundant species in

that order representing 83% (61% and 22%, respectively) of total *Gambierdiscus* spp. Taking into account that 9% of analyzed specimens were classified within the group *G. australes*/*G. caribaeus*, *G. australes* is almost certainly underrated. The dominance of *G. excentricus* and *G. australes* matches previous molecular results based on LSUrDNA and SSUrDNA sequences of cultures and single cells isolated from Eastern Canary Islands [15,26]. Quantification based on morphology is very time consuming and not totally effective, but species-specific quantitative PCR assays have not been yet undertaken in that region as it has been the case in other areas such as the Gulf of Mexico and the Pacific Ocean [28,81,82].

The species of the genus *Gambierdiscus* produce ciguatoxins (CTXs) and maitotoxins (MTXs) but only the transfer of CTXs up the food chain results in their metabolism and accumulation in fish tissues, thus potentially causing CFP in humans. Although highly toxic, MTXs do not induce CFP because of their low oral potency and inability to accumulate in the muscle tissue of fish [83,84]. It has very recently been reported that different species of *Gambierdiscus* contain different proportions of the two types of toxins and therefore a very different toxic potential [23,24,85]. For this reason, in order to assess the potential risk of CFP occurrence it is necessary to know the specific diversity and distribution of *Gambierdiscus* in the region as well as the CTXs and MTXs contained by each one. *G. excentricus* displays the highest content of CTXs so far [23,24,50] and its CTX-like toxicity has been comparable to that of *G. polynesiensis*, the predominant CTX producer in the South Pacific, a ciguatera endemic region. In contrast to the consistent toxicity characteristics of *G. excentricus*, analyses of *G. australes* have yielded variable results depending on the strains and their origins [24,30,85,86]. The toxicity of the rest of *Gambierdiscus* species from the Canary Islands has been very scarcely studied; neuroblastoma cell-based assay (neuro-2a CBA) revealed lower CTX-like toxicity than the former ones (or even none for *G. caribaeus*), although high intraspecific variability has been also reported [24,30].

References

1. Parsons, M.L.; Aligizaki, K.; Dechraoui, M.Y.; Fraga, S.; Morton, S.; Penna, A.; Rhodes, L. *Gambierdiscus* and *Ostreopsis*: Reassessment of the state of knowledge of their taxonomy, geography, ecophysiology, and toxicology. *Harmful Algae* **2012**, *14*, 107–129. [CrossRef]

2. FAO; WHO. Report of the Expert Meeting on Ciguatera Poisoning. In Proceedings of the Food Safety and Quality No. 9, Rome, Italy, 19–23 November 2018.

3. Soliño, L.; Costa, P.R. Global impact of ciguatoxins and ciguatera fish poisoning on fish, fisheries and consumers. *Environ. Res.* **2020**, *182*, 109111. [CrossRef] [PubMed]

4. Pérez-Arellano, J.L.; Luzardo, O.P.; Brito, A.P.; Hernández Cabrera, M.; Zumbado, M.; Carranza, C.; Angel Moreno, A.; Boada, L.D. Ciguatera fish poisoning, Canary Islands. *Emerg. Infect. Dis.* **2005**, *11*, 1981–1982. [CrossRef] [PubMed]

5. Gouveia, N.; Delgado, J.; Vale, P. Primeiro registo da ocorrência de episódios do tipo ciguatérico no arquipélago da Madeira. In *X Reuniao Ibérica de Fitoplâncton Tóxico e Biotoxinas—Livro de Resumos*; IPIMAR: Lisboa, Portugal, 2009; p. 41.

6. Bravo, J.; Suárez, F.; Ramírez, A.; Acosta, F. Ciguatera, an emerging human poisoning in Europe. *J. Aquac. Mar. Biol.* **2015**, *3*, 53.

7. Otero, P.; Pérez, S.; Alfonso, A.; Vale, C.; Rodríguez, P.; Gouveia, N.N.; Gouveia, N.; Delgado, J.; Vale, P.; Hirama, M.; et al. First toxin profile of Ciguateric fish in Madeira Arquipelago (Europe). *Anal. Chem.* **2010**, *82*, 6032–6039. [CrossRef]

8. Tester, P.A.; Feldman, R.L.; Nau, A.W.; Kibler, S.R.; Wayne Litaker, R. Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. *Toxicon* **2010**, *56*, 698–710. [CrossRef]

9. Kibler, S.; Tester, P.; Kunkel, K.; Moore, S.; Litaker, R. Effects of ocean warming on growth and distribution of dinoflagellates associated with ciguatera fish poisoning in the Caribbean. *Ecol. Model.* **2015**, *316*, 194–210. [CrossRef]

10. IPCC. *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate 2019*; IPCC: Geneva, Switzerland, 2019.

11. Litaker, R.W.; Vandersea, M.W.; Faust, M.A.; Kibler, S.R.; Nau, A.W.; Holland, W.C.; Chinain, M.; Holmes, M.J.; Tester, P.A. Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. *Toxicon* **2010**, *56*, 711–730. [CrossRef]

12. Nishimura, T.; Sato, S.; Tawong, W.; Sakanari, H.; Yamaguchi, H.; Adachi, M. Morphology of *Gambierdiscus scabrosus* sp. nov. (Gonyaulacales): A new epiphytic toxic dinoflagellate from coastal areas of Japan. *J. Phycol.* **2014**, *50*, 506–514. [CrossRef]

13. Villareal, T.A.; Hanson, S.; Qualia, S.; Jester, E.L.E.; Granade, H.R.; Dickey, R.W. Petroleum production platforms as sites for the expansion of ciguatera in the northwestern Gulf of Mexico. *Harmful Algae* **2007**, *6*, 253–259. [CrossRef]

14. Laza-Martinez, A.; David, H.; Riobo, P.; Miguel, I.; Orive, E. Characterization of a Strain of *Fukuyoa paulensis* (Dinophyceae) from the Western Mediterranean Sea. *J. Eukaryot. Microbiol.* **2016**, *63*, 481–497. [CrossRef] [PubMed]

15. Rodriguez, F.; Fraga, S.; Ramilo, I.; Rial, P.; Figueroa, R.; Riobo, P.; Bravo, I. Canary Islands (NE Atlantic) as a biodiversity 'hotspot' of *Gambierdiscus*: Implications for future trends of ciguatera in the area. *Harmful Algae* **2017**, *67*, 131–143. [CrossRef] [PubMed]

16. Aligizaki, K.; Nikolaidis, G. Morphological identification of two tropical dinoflagellates of the genera *Gambierdiscus* and *Sinophysis* in the Mediterranean Sea. *J. Biol. Res. Thessalon.* **2008**, *9*, 75–82.

17. Bagnis, R.; Bennett, J.; Prieur, C.; Legrand, A.M. The dynamics of three toxic benthic dinoflagellates and the toxicity of ciguateric surgenfish in French Polynesia. In *Toxic Dinoflagellates*; Anderson, D.M., White, A.W., Baden, D.G., Eds.; Elsevier: New York, NY, USA, 1985; pp. 177–182.

18. Chinain, M.; Germain, M.; Deparis, X.; Pauillac, S.; Legrand, A.M. Seasonal abundance and toxicity of the dinoflagellate *Gambierdiscus* spp (Dinophyceae), the causative agent of ciguatera in Tahiti, French Polynesia. *Mar. Biol.* **1999**, *135*, 259–267. [CrossRef]

19. Turquet, J.; Quod, J.-P.; Ten-Hage, L.; Dahalani, Y.; Wendling, B. Example of a *Gambierdiscus toxicus* flare-up following the 1998 coral bleaching event in Mayotte island (Comoros, South-West Indian Ocean). In *Harmful Algal Blooms 2000*; Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J., Eds.; Intergovernmental Oceanographic Commission of UNESCO: Paris, France, 2001; pp. 50–51.

20. Gillespie, W.C.; Holmes, M.J.; Burke, J.B.; Doley, J. Distribution and periodicity of *Gambierdiscus toxicus* in Queensland, Australia. In *Toxic Dinoflagellates*; Anderson, D.M., White, A.W., Baden, D.G., Eds.; Elsevier: New York, NY, USA, 1985; pp. 183–188.

21. Chinain, M.; Darius, H.T.; Ung, A.; Cruchet, P.; Wang, Z.; Ponton, D.; Laurent, D.; Pauillac, S. Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. *Toxicon* **2010**, *56*, 739–750. [CrossRef]

22. Rhodes, L.; Harwood, T.; Smith, K.; Argyle, P.; Munday, R. Production of ciguatoxin and maitotoxin by strains of *Gambierdiscus australes*, *G. pacificus* and *G. polynesiensis* (Dinophyceae) isolated from Rarotonga, Cook Islands. *Harmful Algae* **2014**, *39*, 185–190. [CrossRef]

23. Litaker, R.; Holland, W.; Hardison, D.; Pisapia, F.; Hess, P.; Kibler, S.; Tester, P. Ciguatoxicity of *Gambierdiscus* and *Fukuyoa* species from the Caribbean and Gulf of Mexico. *PLoS ONE* **2017**, *12*, e0185776. [CrossRef]

24. Rossignoli, A.E.; Tudo, A.; Bravo, I.; Diaz, P.A.; Diogene, J.; Riobo, P. Toxicity Characterisation of *Gambierdiscus* Species from the Canary Islands. *Toxins* **2020**, *12*, 134. [CrossRef]

25. Chinain, M.; Faust, M.A.; Pauillac, S. Morphology and molecular analyses of three toxic species of *Gambierdiscus* (Dinophyceae): *G. pacificus*, sp. nov., *G. australes*, sp. nov., and *G. polynesiensis*, sp. nov. *J. Phycol.* **1999**, *35*, 1282–1296. [CrossRef]

26. Bravo, I.; Rodriguez, F.; Ramilo, I.; Rial, P.; Fraga, S. Ciguatera-Causing Dinoflagellate *Gambierdiscus* spp. (Dinophyceae) in a Subtropical Region of North Atlantic Ocean (Canary Islands): Morphological Characterization and Biogeography. *Toxins* **2019**, *11*, 423. [CrossRef]

27. Vandersea, M.; Kibler, S.; Holland, W.; Tester, P.; Schultz, T.; Faust, M.; Holmes, M.; Chinain, M.; Litaker, R. Development of semi-quantitative PCR assays for the detection and enumeration of *Gambierdiscus* species (Gonyaulacales, Dinophyceae). *J. Phycol.* **2012**, *48*, 902–915. [CrossRef] [PubMed]

28. Smith, K.; Biessy, L.; Argyle, P.; Trnski, T.; Halafihi, T.; Rhodes, L. Molecular Identification of *Gambierdiscus* and *Fukuyoa* (Dinophyceae) from Environmental Samples. *Mar. Drugs* **2017**, *15*, 243. [CrossRef] [PubMed]

29. Kretzschmar, A.L.; Verma, A.; Kohli, G.; Murray, S. Development of a quantitative PCR assay for the detection and enumeration of a potentially ciguatoxin-producing dinoflagellate, *Gambierdiscus lapillus* (Gonyaulacales, Dinophyceae). *PLoS ONE* **2019**, *14*, e0224664. [CrossRef] [PubMed]

30. Tudo, A.; Gaiani, G.; Varela, M.R.; Tsumuraya, T.; Andree, K.B.; Fernandez-Tejedor, M.; Campas, M.; Diogene, J. Further advance of *Gambierdiscus* Species in the Canary Islands, with the First Report of *Gambierdiscus belizeanus*. *Toxins* **2020**, *12*, 692. [CrossRef] [PubMed]

31. Ben-Gharbia, H.; Yahia, O.K.-D.; Amzil, Z.; Chomerat, N.; Abadie, E.; Masseret, E.; Sibat, M.; Triki, H.Z.; Nouri, H.; Laabir, M. Toxicity and Growth Assessments of Three Thermophilic Benthic Dinoflagellates (*Ostreopsis* cf. *ovata*, *Prorocentrum lima* and *Coolia monotis*) Developing in the Southern Mediterranean Basin. *Toxins* **2016**, *8*, 297. [CrossRef]

32. Rodriguez, F.; Riobo, P.; Crespin, G.D.; Daranas, A.H.; de Vera, C.R.; Norte, M.; Javier Fernandez, J.; Fraga, S. The toxic benthic dinoflagellate *Prorocentrum maculosum* Faust is a synonym of *Prorocentrum hoffmannianum* Faust. *Harmful Algae* **2018**, *78*, 1–8. [CrossRef]

33. Rhodes, L.; Smith, K.; Selwood, A.; Munday, R.; Suda, S.; Hallegraeff, G.; McNabb, P. A dinoflagellate producer of pinnatoxins. *Harmful Algae News* **2011**, *43*, 20.

34. Selwood, A.I.; Wilkins, A.L.; Munday, R.; Shi, F.; Rhodes, L.L.; Holland, P.T. Portimine: A bioactive metabolite from the benthic dinoflagellate *Vulcanodinium rugosum*. *Tetrahedron Lett.* **2013**, *54*, 4705–4707. [CrossRef]

35. Tindall, D.R.; Morton, S.L. Community Dynamics and Physiology of Epiphytic/Benthic Dinoflagellates Associated with Ciaguatera. In *Physiological Ecology of Harmful Algal Blooms*; Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., Eds.; Springer: Berlin/Heidelberg, Germany, 1998; Volume G41, pp. 293–313.

36. Yong, H.L.; Mustapa, N.I.; Lee, L.K.; Lim, Z.F.; Tan, T.H.; Usup, G.; Gu, H.; Litaker, R.W.; Tester, P.A.; Lim, P.T.; et al. Habitat complexity affects benthic harmful dinoflagellate assemblages in the fringing reef of Rawa Island, Malaysia. *Harmful Algae* **2018**, *78*, 56–68. [CrossRef]

37. Nakahara, H.; Sakami, T.; Chinain, M.; Ishida, Y. The role of macroalgae in epiphytism of the toxic dinoflagellate *Gambierdiscus toxicus* (Dinophyceae). *Phycol. Res.* **1996**, *44*, 113–117. [CrossRef]

38. Boisnoir, A.; Pascal, P.-Y.; Cordonnier, S.; Lemee, R. Spatio-temporal dynamics and biotic substrate preferences of benthic dinoflagellates in the Lesser Antilles, Caribbean sea. *Harmful Algae* **2019**, *81*, 18–29. [CrossRef] [PubMed]

39. Sansón, M.; Reyes, J.; Afonso-Carrillo, J. Flora marina. In *Naturaleza de las Islas Canarias: Ecología y Conservación*; Fernández Palacios, J., Martín Esquivel, J., Eds.; Editorial Turquesa: Santa Cruz de Tenerife, Spain, 2001; pp. 193–198.

40. Sangil, C.; Sanson, M.; Afonso-Carrillo, J. Spatial variation patterns of subtidal seaweed assemblages along a subtropical oceanic archipelago: Thermal gradient *vs* herbivore pressure. *Estuar. Coast. Shelf Sci.* **2011**, *94*, 322–333. [CrossRef]

41. Afonso-Carrillo, J.; Sansón, M. Algas, hongos y fanerógamas marinas de las Islas Canarias. In *Clave Analítica. Materiales Didácticos Universitarios, Serie Biología 2*; Servicio de Publicaciones de la Universidad de La Laguna: Santa Cruz de Tenerife, Spain, 1999; p. 254.

42. González-Ruiz, S.; Reyes, J.; Sansón, M.; Afonso-Carrillo, J. Flora marina de Cotillo, Noroeste de Fuerteventura (Islas Canarias). *Vieraea* **1995**, *24*, 13–38.

43. González-Ruiz, S.; Sansón, M.; Reyes, J. Distribución vertical de las algas en el litoral de Cotillo (Noroeste de Fuerteventura, Islas Canarias). *Vieraea* **1995**, *24*, 39–50.

44. Reyes, J.; Sansón, M.; Afonso-Carrillo, J. Flora y vegetación marina. Algas y sebas. In *Patrimonio Natural de la isla de Fuerteventura*; Rodríguez Delgado, O., Ed.; Cabildo de Fuerteventura, Gobierno de Canarias y Centro de la Cultura Popular Canaria: Tenerife, Spain, 2005; pp. 117–140.

45. Silva, E.S. Contribution a L'étude du microplancton de Dakar et des régions maritimes voisines. *Bull. Inst. Fr. Afr. Noire.* **1956**, *2*, 335–371.

46. Parsons, M.L.; Preskitt, L.B. A survey of epiphytic dinoflagellates from the coastal waters of the island of Hawaii. *Harmful Algae* **2007**, *6*, 658–669. [CrossRef]

47. Zingone, A.; Berdalet, E.; Bienfang, P.; Enevoldsen, H.; Evans, J.; Kudela, R.; Tester, P. Harmful algae in benthic systems: A GEOHAB core research program. *Cryptogam. Algol.* **2012**, *33*, 225–230. [CrossRef]

48. Boada, L.D.; Zumbado, M.; Luzardo, O.P.; Almeida-González, M.; Plakas, S.M.; Granade, H.R.; Abraham, A.; Jester, E.L.E.; Dickey, R.W. Ciguatera fish poisoning on the West Africa Coast: An emerging risk in the Canary Islands (Spain). *Toxicon* **2010**, *56*, 1516–1519. [CrossRef]

49. Fraga, S.; Penna, A.; Bianconi, I.; Paz, B.; Zapata, M. *Coolia canariensis* sp. nov. (Dinophyceae), a new nontoxic epiphytic bentic dinoflagellate from the Canary Islands. *J. Phycol.* **2008**, *44*, 1060–1070. [CrossRef]

50. Fraga, S.; Rodriguez, F.; Caillaud, A.; Diogene, J.; Raho, N.; Zapata, M. *Gambierdiscus excentricus* sp. nov. (Dinophyceae), a benthic toxic dinoflagellate from the Canary Islands (NE Atlantic Ocean). *Harmful Algae* **2011**, *11*, 10–22. [CrossRef]

51. Fraga, S.; Rodriguez, F. Genus Gambierdiscus in the Canary Islands (NE Atlantic Ocean) with Description of *Gambierdiscus silvae* sp. nov., a New Potentially Toxic Epiphytic Benthic Dinoflagellate. *Protist* **2014**, *165*, 839–853. [CrossRef] [PubMed]

52. David, H.; Laza-Martinez, A.; Rodriguez, F.; Fraga, S.; Orive, E. *Coolia guanchica* sp. nov. (Dinophyceae) a new epibenthic dinoflagellate from the Canary Islands (NE Atlantic Ocean). *Eur. J. Phycol.* **2019**, *55*, 1–13. [CrossRef]

53. Fernandez-Zabala, J.; Tuya, F.; Amorim, A.; Soler-Onis, E. Benthic dinoflagellates: Testing the reliability of the artificial substrate method in the Macaronesian region. *Harmful Algae* **2019**, *87*. [CrossRef] [PubMed]

54. Kaufmann, M. *Gambierdiscus* and related benthic dinoflagellates from Madeira archipelago (NE Atlantic). *Harmful Algae News* **2013**, *47*, 18–19.

55. Kaufmann, M.J.; Santos, F.; Maranhao, M. Checklist of nanno- and microphytoplankton off Madeira Island (Northeast Atlantic) with some historical notes. *Nova Hedwig.* **2015**, *101*, 205–232. [CrossRef]

56. Tester, P.A.; Kibler, S.R.; Holland, W.C.; Usup, G.; Vandersea, M.W.; Leaw, C.P.; Teen, L.P.; Larsen, J.; Mohammad-Noor, N.; Faust, M.A.; et al. Sampling harmful benthic dinoflagellates: Comparison of artificial and natural substrate methods. *Harmful Algae* **2014**, *39*, 8–25. [CrossRef]

57. Soler-Onís, E.; Fernandez-Zabala, J.; Ojeda-Rodriguez, A.; Amorin, A. Bloom of *Gambierdicus caribaeus* in the temperate-subtropical waters of El Hierro, Canary Islands (North East Atlantic). *Harmful Algae News* **2016**, *55*, 14–17.

58. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Tartaglione, L.; Grillo, C.; Melchiorre, N. Putative palytoxin and its new analogue, ovatoxin-a, in *Ostreopsis ovata* collected along the Ligurian coasts during the 2006 toxic outbreak. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 111–120. [CrossRef]

59. Lee, J.-S.; Igarashi, T.; Fraga, S.; Dahl, E.; Hovgaard, P.; Yasumotol, T. Determination of diarrhetic shellfish toxins in various dinoflagellate species. *J. Appl. Phycol.* **1989**, *1*, 147–152. [CrossRef]

60. Fattorusso, E.; Ciminiello, P.; Costantino, V.; Magno, S.; Mangoni, A.; Milandri, A.; Poletti, R.; Pompei, M.; Viviani, R. Okadaic acid in mussels of adriatic sea. *Mar. Pollut. Bull.* **1992**, *24*, 234–237. [CrossRef]

61. Bravo, I.; Fernández, M.L.; Ramilo, I.; Martínez, A. Toxin composition of the toxic dinoflagellate *Prorocentrum lima* isolated from different locations along the Galician coast (NW Spain). *Toxicon* **2001**, *39*, 1537–1545. [CrossRef]

62. Escalera, L.; Reguera, B.; Takishita, K.; Yoshimatsu, S.; Koike, K.; Koike, K. Cyanobacterial endosymbionts in the benthic dinoflagellate *Sinophysis canaliculata* (Dinophysiales, Dinophyceae). *Protist* **2011**, *162*, 304–314. [CrossRef] [PubMed]

63. Garcia-Portela, M.; Riobo, P.; Rodriguez, F. Morphological and molecular study of the cyanobiont-bearing dinoflagellate *Sinophysis canaliculata* from the canary islands (Eastern central atlantic). *J. Phycol.* **2017**, *53*, 446–450. [CrossRef]

64. Rhodes, L.; Smith, K.; Selwood, A.; McNabb, P.; Munday, R.; Suda, S.; Molenaar, S.; Hallegraeff, G. Dinoflagellate *Vulcanodinium rugosum* identified as the causative organism of pinnatoxins in Australia, New Zealand and Japan. *Phycologia* **2011**, *50*, 624–628. [CrossRef]

65. Sosa, S.; Pelin, M.; Cavion, F.; Herve, F.; Hess, P.; Tubaro, A. Acute Oral Toxicity of Pinnatoxin G in Mice. *Toxins* **2020**, *12*, 87. [CrossRef] [PubMed]

66. Zeng, N.; Gu, H.; Smith, K.F.; Rhodes, L.L.; Selwood, A.I.; Yang, W. The first report of *Vulcanodinium rugosum* (Dinophyceae) from the South China Sea with a focus on the life cycle. *N. Z. J. Mar. Freshw. Res.* **2012**, *46*, 511–521. [CrossRef]

67. Salgado, P.; Fraga, S.; Rodriguez, F.; Bravo, I. Benthic flattened cells of the phylogenetically related marine dinoflagellates *Protoceratium reticulatum* and *Ceratocorys mariaovidiorum* (Gonyaulacales): A new type of cyst? *J. Phycol.* **2018**, *54*, 138–149. [CrossRef]

68. Bravo, I.; Figueroa, R.I. Towards an ecological understanding of dinoflagellate cyst functions. *Microorganisms* **2014**, *2*, 11–32. [CrossRef]

69. Richlen, M.L.; Lobel, P.S. Effects of depth, habitat, and water motion on the abundance and distribution of ciguatera dinoflagellates at Johnston Atoll, Pacific Ocean. *Mar. Ecol. Prog. Ser.* **2011**, *421*, 51–66. [CrossRef]

70. Vila, M.; Garcés, E.; Masó, M. Potentially toxic epiphytic dinoflagellate assemblages on macroalgae in the NW Mediterranean. *Aquat. Microb. Ecol.* **2001**, *26*, 51–60. [CrossRef]

71. Grzebyk, D.; Berland, B.; Thomassin, B.A.; Bosi, C.; Arnoux, A. Ecology of ciguateric dinofagellates in the coral reef complex of Mayotte island (S.W. Indian Ocean). *J. Exp. Mar. Biol. Ecol.* **1994**, *178*, 51–66. [CrossRef]

72. Nascimento, S.; Franca, J.; Goncalves, J.; Ferreira, C. *Ostreopsis* cf. *ovata* (Dinophyta) bloom in an equatorial island of the Atlantic Ocean. *Mar. Pollut. Bull.* **2012**, *64*, 1074–1078. [CrossRef] [PubMed]

73. Fowler-Walker, M.J.; Wernberg, T.; Connell, S.D. Differences in kelp morphology between wave sheltered and exposed localities: Morphologically plastic or fixed traits? *Mar. Biol.* **2006**, *148*, 755–767. [CrossRef]

74. Jonsson, P.R.; Granhag, L.; Moschella, P.S.; Aberg, P.; Hawkins, S.J.; Thompson, R.C. Interactions between wave action and grazing control the distribution of intertidal macroalgae. *Ecology* **2006**, *87*, 1169–1178. [CrossRef]

75. Nishihara, G.N.; Terada, R. Species richness of marine macrophytes is correlated to a wave exposure gradient. *Phycol. Res.* **2010**, *58*, 280–292. [CrossRef]

76. Giussani, V.; Asnaghi, V.; Pedroncini, A.; Chiantore, M. Management of harmful benthic dinoflagellates requires targeted sampling methods and alarm thresholds. *Harmful Algae* **2017**, *68*, 97–104. [CrossRef]

77. Rains, L.K.; Parsons, M.L. *Gambierdiscus* species exhibit different epiphytic behaviors toward a variety of macroalgal hosts. *Harmful Algae* **2015**, *49*, 29–39. [CrossRef]

78. Yasumoto, T.; Oshima, Y.; Murakami, R.; Nakajima, I.; Bagnis, R.; Fukuyo, Y. Toxicity of benthic dinoflagellates in a coral reef. *Bull. Jpn. Soc. Sci. Fish.* **1980**, *46*, 327–331. [CrossRef]

79. Bomber, J.W.; Rubio, M.G.; Norris, D.R. Epiphytism of dinoflagellates associated with the disease ciguatera—Substrate-specificity and nutrition. *Phycologia* **1989**, *28*, 360–368. [CrossRef]

80. Bomber, J.W.; Norris, D.R.; Mitchell, L.E. Benthic dinoflagellates associated with ciguatera from the Florida Keys. II. Temporal, spatial and substrate heterogeneity of *Prorocentrum lima*. In *Toxic Dinoflagellates*; Anderson, D.M., White, A.W., Baden, D.G., Eds.; Elsevier: New York, NY, USA, 1985; pp. 45–50.

81. Nishimura, T.; Hariganeya, N.; Tawong, W.; Sakanari, H.; Yamaguchi, H.; Adachi, M. Quantitative PCR assay for detection and enumeration of ciguatera-causing dinoflagellate Gambierdiscus spp. (Gonyaulacales) in coastal areas of Japan. *Harmful Algae* **2016**, *52*, 11–22. [CrossRef]

82. Tester, P.A.; Vandersea, M.W.; Buckel, C.A.; Kibler, S.R.; Holland, W.C.; Davenport, E.D.; Clark, R.D.; Edwards, K.F.; Taylor, J.C.; Vander Pluym, J.L.; et al. *Gambierdiscus* (Dinophyceae) species diversity in the Flower Garden Banks National Marine Sanctuary, Northern Gulf of Mexico, USA. *Harmful Algae* **2013**, *29*, 1–9. [CrossRef]

83. Yokoyama, A.; Murata, M.; Oshima, Y.; Iwashita, T.; Yasumoto, T. Some Chemical Properties of Maitotoxin, a Putative Calcium Channel Agonist Isolated from a MarineDinoflagellate. *J. Biochem.* **1988**, *104*, 184–187. [CrossRef] [PubMed]

84. Munday, R. *Toxicology of Seafood Toxins: A Critical Review*; CRC Press: Boca Raton, FL, USA, 2014.

85. Pisapia, F.; Holland, W.; Hardison, D.; Litaker, R.; Fraga, S.; Nishimura, T.; Adachi, M.; Nguyen-Ngoc, L.; Sechet, V.; Amzil, Z.; et al. Toxicity screening of 13 *Gambierdiscus* strains using neuro-2a and erythrocyte lysis bioassays. *Harmful Algae* **2017**, *63*, 173–183. [CrossRef] [PubMed]

86. Estevez, P.; Sibat, M.; Leao-Martins, J.M.; Reis Costa, P.; Gago-Martinez, A.; Hess, P. Liquid Chromatography Coupled to High-Resolution Mass Spectrometry for the Confirmation of Caribbean Ciguatoxin-1 as the Main Toxin Responsible for Ciguatera Poisoning Caused by Fish from European Atlantic Coasts. *Toxins* **2020**, *12*, 267. [CrossRef] [PubMed]

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