

Original Article

Playing hide and seek: Distribution with depth of potentially harmful epibenthic dinoflagellates of Southern EL HIERRO Island, Canary Islands (Ne Atlantic)

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ABSTRACT

The study of epibenthic assemblages of harmful dinoflagellates (BHABs) is commonly conducted in shallow infralittoral zones (0 – 5 m) and are seldom investigated at deeper waters. In this study, the distribution with depth of five BHAB genera (*Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Coolia* and *Amphidinium*) was investigated in the south of El Hierro island (Canary Islands, Spain). Sampling involved the use of a standardized artificial substrate deployed at three depth levels (5, 10 and 20 m) that were visited at three different times throughout one year. The influence of three depth-correlated abiotic parameters, i.e. light, water motion and water temperature, on the vertical and seasonal distribution of the BHAB assemblage was also assessed. Two vertical distribution patterns were observed consistently through time: cell abundances of *Ostreopsis* and *Coolia* decreased from 5 to 20 m while those of *Gambierdiscus*, *Prorocentrum* and *Amphidinium* showed the reverse pattern, although significant differences were only observed between 5 and 10 - 20 m depth. In April, two members of the latter group, *Gambierdiscus* and *Amphidinium*, were even absent at 5 m depth. The recorded environmental parameters explained a high percentage of the observed distribution. In particular, model selection statistical approaches indicated that water motion was the most significant parameter. An analysis of *Gambierdiscus* at species level revealed the co-occurrence of four species in the study area: *G. australes*, *G. belizeanus*, *G. caribaeus* and *G. excentricus*. The species *G. excentricus*, reported here for the first time in El Hierro, showed a more restricted vertical and seasonal distribution than the other species, which may explain not being detected in previous studies in the area. The results obtained in this study highlight the importance of considering a wider depth range and different seasons of the year when investigating the ecology of BHABs and assessing their risk and impacts on human health and the environment. Only then, efficient monitoring programs will be implemented in the Canary Islands and globally in areas affected by these events.

1. Introduction

In the last decades, studies on toxic benthic dinoflagellates (Benthic Harmful Algal Blooms – BHABs) have experienced a notable increase. This has been mainly prompted by the apparent expansion in the distributional range of these organisms from tropical to more temperate

areas and the associated toxic incidents caused by their toxins, that affect human health and the environment (Berdalet et al., 2017; Hallegraeff et al., 2021; Chinain et al., 2021). Unlike their planktonic counterparts, which live freely in the water column, BHABs occur in structurally complex benthic environments, characterized by the presence of diverse substrates to which they are associated (GEOHAB, 2012).

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Therefore, BHABs are commonly sampled by underwater manual collection of their most common hosting substrates, namely particular species of macrophytes (Lobel et al., 1988). Habitats located in shallow infralittoral zones (0–5 m) have the advantage of facilitating sampling tasks, since in general different macrophyte species are available, and their selection and collection is simple without the necessity of special equipment. Consequently, most of what is known on the ecology and monitoring of BHABs is based on studies that have been carried out in shallow zones and thus, the relevance of deeper ones has been overlooked. However, depth affects a range of different biotic and abiotic environmental parameters, namely light, water temperature, water motion, nutrients concentration, salinity or macroalgae distribution, which are known to influence the cell abundance and distribution of BHAB dinoflagellates (Parsons et al., 2012; Tester et al., 2020). Typically, in the same area, shallow zones are characterized by high variability and exposure to the action of certain physical stressors, e.g. water motion, while deeper zones usually present calmer, more stable conditions (e.g. Tuya et al., 2007). Due to the existence of species-specific habitat requirements, some species could find more appropriate growth conditions in deeper zones, which would act as climatic refuge areas (Tester et al., 2020). In the last few years, ecological studies in deeper zones (>5 m) have experienced a gradual increase, although they are still scarce. Some of them have investigated the presence of BHABs at depth (e.g. Villareal et al., 2007; Tester et al., 2013), while others have investigated the existence of vertical distribution patterns (e.g. Cohu and Lemée, 2012; Boisnoir et al., 2018). Understanding these patterns may undoubtedly have a great impact on monitoring programs, as it would improve the detection and cell abundance estimation of potentially toxic species. However, the limited number of studies carried out so far, together with some contradictory results obtained between them, has prevented reaching a consensus on the vertical distribution of BHAB (Tester et al., 2020).

Recently, a considerable number of studies on the diversity and

distribution of BHABs have been carried out in the Canary Islands (Fraga et al. 2008, 2011; Fraga and Rodríguez, 2014 Soler-Onís et al., 2016; Rodríguez et al., 2017, 2018; Fernández-Zabala et al., 2019; Bravo et al., 2019, 2020; David et al., 2020; Tudó et al., 2020). Most efforts have been made on the genus *Gambierdiscus* Adachi & Fukuyo due to its already evidenced implication in the ciguatera poisoning (CP) outbreaks documented in the region since 2004 (Pérez-Arellano et al., 2005; Boada et al., 2010; Núñez et al., 2012). The studies have been systematically based on shallow coastal areas and, therefore, the knowledge on the biodiversity, cell abundance and distribution of this genus in the archipelago has been limited exclusively to these zones. However, in the Canary Islands, it is possible to find deep zones close to the coast, especially in the geologically younger islands, in which the insular platform is generally narrow (Tenerife, La Palma and El Hierro). Although less accessible for study than shallow zones, deep zones potentially include suitable habitats for BHAB dinoflagellates, such as *Gambierdiscus* spp., and, for this reason, these should not be disregarded when assessing BHAB diversity and public health risk in the region.

Therefore, in the present study, the vertical distribution and temporal dynamics of BHABs in relation to important depth-correlated physical parameters were investigated in the south of El Hierro, the youngest (ca. 0.9 Ma), most isolated island of the Canary Islands. The conclusions derived from this study contribute to improve the detection and cell estimation of BHAB dinoflagellates for a better assessment of associated risks in the region, while helping to clarify ecological patterns of these organisms across vertical (depth) gradients on a global scale.

2. Material and Methods

2.1. Study site and sampling design

This study was conducted in the southwestern part of El Hierro Island, the westernmost, warmest island of the Canary Islands (Fig. 1A and

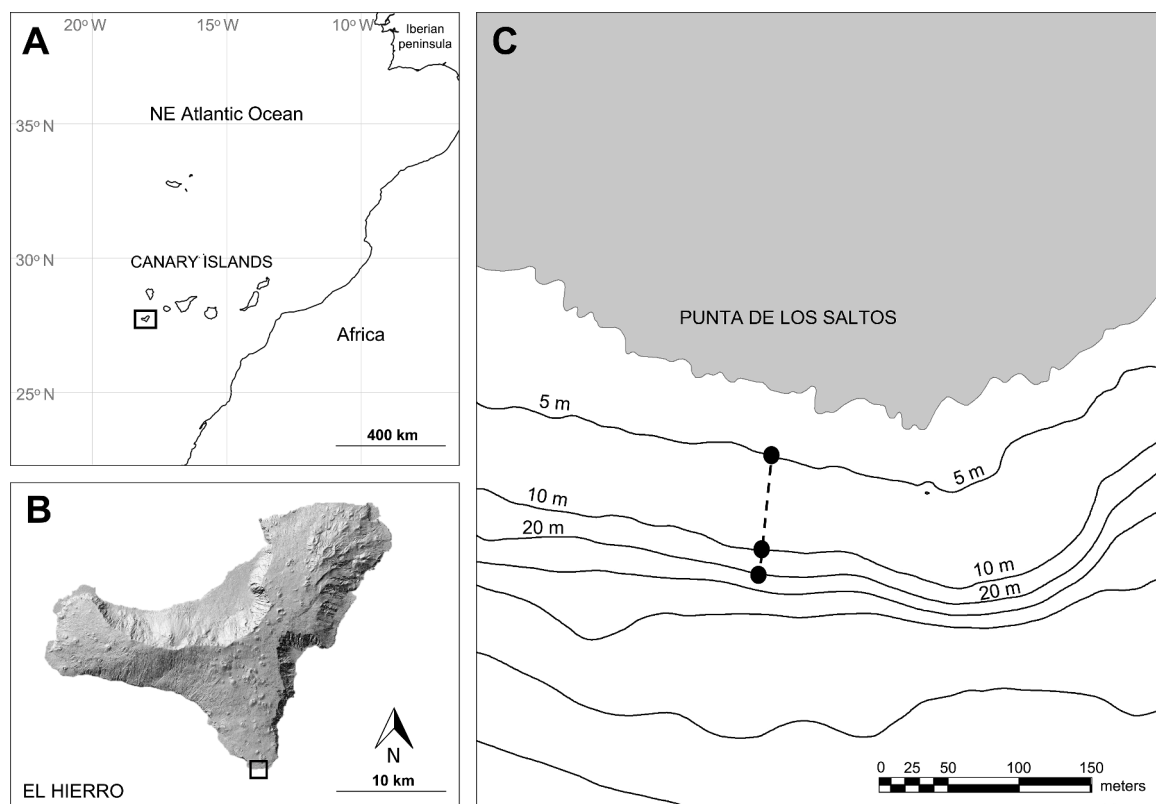


Fig. 1. Location of the study site including: (A) the position of the Canary Islands in the Northeast Mid-Atlantic Ocean, (B) the location of the sampling area “Punta de Los Saltos” in the south of El Hierro island and (C) the vertical transect (---) at which three depth levels (•; 5, 10 and 20 m) were established.

B). Sampling took place at Punta de Los Saltos (27°38'15"N, 17°59'18"W), a site located approximately 100 m from the coast within the marine reserve area of "Punta de La Restinga - Mar de Las Calmas" (Fig. 1C). The area is characterized by oligotrophic temperate-warm waters that range between ca. 19 °C in March - April and 26 °C in September - October. The geographical location of this area protects it from the dominant NE trade winds and currents present in the archipelago during most of the year except in winter, when Atlantic squalls approach the islands producing NW swells (Braun et Molina, 1988). The area is also characterized by scarce rains due to the low influence of trade winds on the south of the island in contrast with the northern side, where these have a greater influence and produce horizontal rains. This makes runoff infrequent in southern coastal waters and therefore fresh water influence in the study site was negligible. The bottom is mostly basaltic steep ledges down to about 20 - 25 m depth, from which large black sand flats occur with dispersed rocky outcrops.

Rocky areas are covered by a continuous, diverse and well preserved macroalgae assemblage. The shallow infralittoral section (0 - 10 m) is dominated by *Lobophora schneideri* Vieira (Dictyotales, Dictyotaceae). This species presents an average coverage of ca. 70%, which can sometimes exceed 90%. Other species associated with *L. schneideri* are *Styopodium zonale* (Lamouroux) Papenfuss, *Dictyota* spp., *Canistrocarpus cervicornis* (Kützinger) De Paula & De Clerck, *Ellisolandia elongata* (Ellis & Solander) Hind & Saunders, *Asparagopsis taxiformis* (Delile) Trevisan and *Pseudochlorodesmis furcellata* (Zanardini) Borgesen. These species are often patchy (Fig. 2A and B). In the deepest section (10 - 20 m), the algal assemblage is dominated by *L. schneideri*, *Lophocladia trichoclados* Schmitz and *Dasya baillouviana* (S.G. Gmelin) Montagne. This assemblage is common in areas with low wave exposure. Other common species in this section are *Padina pavonica* (Linnaeus) Thivy, *Dictyota* spp., *C. cervicornis*, *Sebdenia canariensis* Soler-Onís, Haroun, Viera-Rodríguez & Prud'homme ex Gabriel & Fredericq or *Sebdenia dichotoma* Berthold (Fig. 2C). The algal assemblage in this area remains stable in terms of abundance and diversity throughout the year (Betancor et al., 2014). The almost homogeneous macroalgal assemblage was one of the criteria used in site selection to reduce the possible confounding effect of different macroalgal species on the tested hypothesis.

Sampling occurred in April, August, and November 2017. These months were selected to include contrasting seasons in the study area. Underwater, the same experimental procedure was always followed. Three depth levels, at 5, 10 and 20 m, were established following a vertical transect (ca. 100 m in length, Fig. 1C) that was visited repeatedly. At each depth level, 8 replicated plastic-framed fiberglass screens (7 for quantitative study and 1 for biodiversity assessment) were employed for sampling BHAB dinoflagellates. Briefly, screens were attached to a plastic bar that was placed parallel to the coast at each specific depth. After 24 h, screens were collected carefully and placed individually in plastic zip bags with surrounding water (see Fernández-Zabala et al., 2019 for further details). At the same time, a sample of macroalgae near the artificial substrates was collected at each sampling as a reference for evaluation of the successful colonization of fiberglass screens by BHAB genera. Samples obtained for the quantitative study were immediately fixed with 4% formaldehyde in seawater, while the 8th replicate obtained for biodiversity assessment was kept live.

In all the cases, HOBO Pendant® Temperature/Light and G data loggers were set up next to the artificial substrates to record, respectively, seawater temperature (°C) and light (lux) every 15 minutes, and water motion (m s^{-2}) every minute during 24 h. Temperature/light data loggers were attached to the sea bottom while G data loggers were placed on the top of fishing floats of 50 g weight that were suspended in the water column and attached to the plastic bar by means of a rotatory fishing line (see Fig. 2B from Fernández-Zabala et al., 2019).

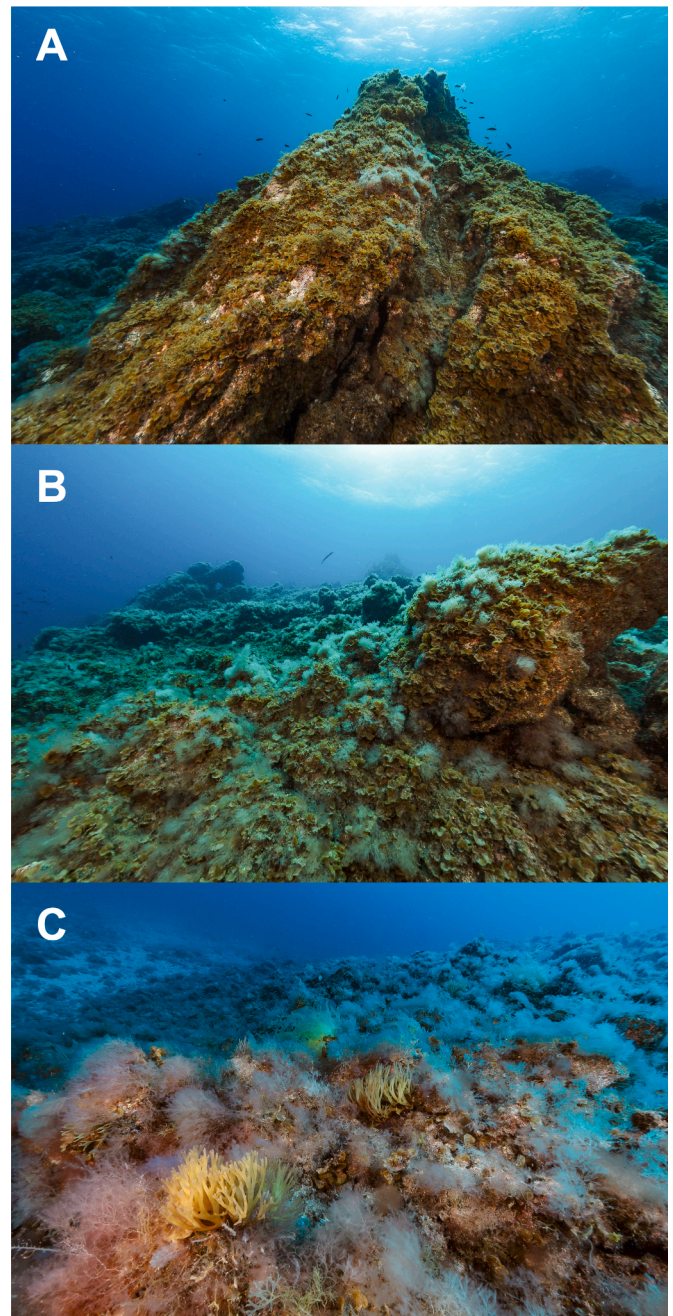


Fig. 2. Macroalgal assemblage at (A) 5 m, (B) 10 m and (C) 20 m depth at the study site.

2.2. Sample processing, dinoflagellate species identification and cell counting

Back to the laboratory, live samples were immediately processed for the establishment of dinoflagellate clonal cultures. Although specimens from all genera were isolated, only data regarding *Gambierdiscus* species will be discussed in the present work. Briefly, cells were isolated under a LEICA DMI3000 B (LEICA, Germany) inverted light microscope, transferred individually into a well of 24-well culture plate filled with f/10 culture medium (coastal filtered seawater-based) and incubated at 24 °C, 10 - 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 8:16 h day/night photoperiod. To avoid any teratological forms, as soon as a sufficient number of cells was reached, cells were harvested for morphological examination.

Cultured cells were examined under a JEOL JSM-6380 LV (JEOL, Japan) scanning electron microscope (SEM). To that end, cells were

preserved with formaldehyde (4%), collected on isopore polycarbonate membrane filters (diameter: 13 mm, pore size: 5 µm, Millipore, Ireland) by gentle filtration, rinsed in distilled water and dehydrated with increasing ethanol concentrations (10, 30, 197 60, 80 and 100%) for 10 min each. The filters were then mounted on a stub (Sigma-Aldrich, Germany), dried overnight at 50 °C and coated with gold-palladium using a Sputtering Polaron E5000 (Polaron PLC, UK).

For further investigations of *Gambierdiscus* species diversity, molecular analyses were also conducted from clonal cultures. Cells were harvested by centrifugation (13000 rpm) using a MiniSpin® centrifuge (Eppendorf, USA). Genomic DNA was extracted using a NucleoSpin® Plant II kit (Macherey-Nagel, Germany) following the manufacturer's instructions. The D1 - D3 regions (LSU rDNA) were then amplified in a BioRad® T100 thermocycler (BioRad, France) using a PCR Mastermix (Takara Bio, Shiga, Japan) and the primers D1R - LSUB (Scholin et al., 1994; Litaker et al., 2003) for a final reaction volume of 25 µl. The PCR protocol consisted in 40 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 1 min; and a final elongation step of 72 °C for 10 s. The PCR products were purified with a QIAquick® PCR purification kit (Quiagen, Germany). Purified products were sent for sequencing (Macrogen, Madrid, Spain). The final sequences were edited using MEGA 7 (Kumar et al., 2016).

Samples collected for quantitative study were processed as described in Fernández-Zabala et al. (2019). Cell counting was carried out at the genus level under the inverted light microscope at 20X magnification using Sedgewick-Rafter chambers (Edler and Elbrächter, 2010). In most cases, more than one Sedgewick-Rafter chamber was necessary to count a sufficient number of cells per replicate. For each replicate, at least 400 cells (1-2 chambers) of the dominant genus (always *Ostreopsis*) and ca. 100 cells of the remaining genera, in up to 3 chambers, were counted. When cells were at very low concentrations, 10 ml Utermöhl chambers were used (Edler and Elbrächter, 2010). Final cell abundances were expressed as cells 100 cm⁻² of fiberglass screen.

Once total BHAB dinoflagellate cell abundances were quantified, the sets of 7 replicates were used to estimate the relative cell abundances of *Gambierdiscus* species at each depth and sampling time. To that end, each replicate was settled and a random aliquot of the concentrated material was placed on a slide. The first 2 - 3 cells of *Gambierdiscus* in each aliquot were isolated under the inverted light microscope. This process was carried out at least once from each replicate until 20 cells were reached for each depth. This number was conditioned by the low *Gambierdiscus* cell abundances present in the samples. Cells were then stained using calcofluor-white and studied under a LEICA DM6000 B light microscope (LEICA, Germany) equipped with epifluorescence. *Gambierdiscus* species were identified mainly based on the differences between plate suture ratios defined by Bravo et al. (2019): i) R1 or ratio between the 2/1' and 2/3' (indicator of plate 2' morphology), ii) R2 or ratio between 2/4' and 2/3' (indicator of Po eccentricity) and the measures of the cell depth (dorso-ventral diameter) and width (transverse diameter). In addition, other morphological features as the cell surface pattern were employed. The plate numbers used in this study followed the modified Kofoidian tabulation system proposed by Besada et al., 1982. The relative abundance of each species in the 20 cells sub-sample was used to estimate the abundance of each species, in terms of cells 100 cm⁻², for each depth and sampling time.

For biodiversity assessment of BHAB genera other than *Gambierdiscus*, the remaining field material was prepared for SEM following the procedures described above.

2.3. Data Analysis

2.3.1. Variation of BHAB dinoflagellate abundance and distribution with depth through sampling times

Differences in the abundances of dinoflagellates throughout depth and time were analysed using GLMMs (Generalized Linear Mixed Models) with depth (3 levels: 5, 10 and 20 m) as a fixed factor, and times

(3 levels) as a random factor. A negative binomial distribution of errors, with an 'identity' link function, was selected to reach the assumptions of linearity and normality of errors, which were checked by a visual inspection of residuals and Q-Q plots. All GLMMs were performed using the 'lme4' library (Kuznetsova et al., 2017), which were implemented in the R statistical software (R Core Team, 2016).

2.3.2. Effect of depth-related environmental parameters on BHAB dinoflagellates

To visualize multivariate similarities in dinoflagellate assemblage structure across the range of environmental conditions, a Redundancy Analysis (RDA), implemented through the 'vegan' R package (Oksanen et al. 2019), was carried out as a constrained ordination technique on log (x + 1) transformed data. A bi-plot was then constructed, and further customized via the 'ggord' R package (Beck, 2017), displaying each BHAB dinoflagellate genus and depth-related parameters (light, water temperature and water motion), as vectors of varying length and direction. The significance of these multivariate configurations was tested by the 'anova.cca' function, which also assessed the significance of each axis (component) to explain a significant amount of variation of the multivariate dataset. Collinearity among physical parameters was detected using VIFs (Variable Inflation Factors). VIFs were always < 5, indicating low collinearity among predictor variables for the RDA configuration in the bidimensional space.

To determine which of the three depth-related environmental parameters explained most of the variation in the abundance and distribution of each dinoflagellate genera, a model selection strategy was adopted through the 'MuMIn' R library (Barton, 2019). Briefly, this package performs a multimodel averaging by incorporating model selection uncertainty. All candidate models were firstly ranked by the Akaike Information Criterion (AIC) corrected for small samples (AICc). Then, the relative importance of each predictor was calculated, as the sum of Akaike weights over all possible models. In all cases, low collinearity among predictors (VIF < 5) was corroborated. All fitted models were lineal (i.e. 'Gaussian' error distribution with an 'identity' link function). Visual inspection of model assumptions, via residuals and quantile-quantile (QQ) plots (Harrison et al., 2018), was performed on selected models to validate the lack of departures from linearity.

3. Results

3.1. BHAB genera diversity, abundance and vertical distribution patterns across sampling times

Fifteen species within 5 genera belonging to 3 different orders were identified: *Coolia* Meunier, *Gambierdiscus*, *Ostreopsis* Schmidt (Gonyaulacales), *Prorocentrum* Ehrenberg (Prorocentrales) and *Amphidinium* Claperède & Lachmann (Amphidinales) (Table 1). At all depths and times studied, the same BHAB dinoflagellate genera were present on

Table 1
List of BHAB species identified in this study.

<i>Ostreopsis</i>	<i>O. fattorussoi</i> Accoroni, Romagnoli & Totti, <i>O. cf. ovata</i> Fukuyo, <i>O. siamensis</i> Schmidt
<i>Coolia</i>	<i>C. malayensis</i> Leav, Lim & Usup <i>C. tropicalis</i> Faust <i>Coolia</i> sp.
<i>Gambierdiscus</i>	<i>G. australes</i> Chinain & Faust <i>G. belizeanus</i> Faust <i>G. caribaeus</i> Vandersea, Litaker, Faust, Kibler, Holland & Tester <i>G. excentricus</i> Fraga <i>P. hoffmanianum</i> Faust
<i>Prorocentrum</i>	<i>P. lima</i> (Ehrenberg) Stein <i>P. panamense</i> Grzebyk, Sako & Berland <i>P. rhathymum</i> Loeblich III, Sherley & Schmidt
<i>Amphidinium</i>	<i>A. carterae</i> Hulburt

both macroalgae and fiberglass screens indicating that, after 24 h, the colonization process of the artificial substrates by BHAB dinoflagellate genera reflected donor assemblages on adjacent natural substrates.

In most cases, dinoflagellate cell abundance estimates showed coefficients of variation (CV) <50%, which reflects the low sample variability obtained from the use of 7 fiberglass screen replicates (Table S1). Only in those cases where mean cell abundances were low (<90 cells 100 cm^{-2}) or cells were absent from one of the replicates, *Gambierdiscus*, *Prorocentrum* and *Amphidinium* showed CVs >50% (Table S1).

The five genera were present at all depths and sampling times except in April at 5 m depth, when neither *Gambierdiscus* nor *Amphidinium* cells were detected. According to the cell concentrations, *Ostreopsis* was the dominant genus at all depths and sampling times, with cell abundances ranging between 1480 ± 332 and $62,069 \pm 7300$ cells 100 cm^{-2} . In most cases, these values were 1–3 orders of magnitude higher than those recorded for the other genera (Table S1). In all cases, *Ostreopsis*

mean cell abundance decreased with depth (Fig. 3; Table S1), as it was significantly higher at 5 m than at 10 m and at this depth than at 20 m ($p < 0.01$ in both cases, Table 2). This pattern was consistent over sampling times, although mean cell abundances registered in April were higher than those registered in August and November (Fig. 3). In most cases, *Coolia* was the second most abundant genus of this study at 5 and 10 m depth with cell abundances ranging between 395 ± 151 and 879 ± 286 cells 100 cm^{-2} . However, at 20 m depth *Prorocentrum* was the second most abundant genus in all the cases with cell abundances that ranged between 307 ± 111 and 1304 ± 217 cells 100 cm^{-2} , while *Coolia* cell abundances ranged between 255 ± 109 and 549 ± 128 cells 100 cm^{-2} (Table S1). Similarly to the pattern observed for *Ostreopsis*, *Coolia* mean cell abundance decreased with depth (Fig. 3), as it was significantly higher at 5 m than at 10 m and at 10 m than at 20 m ($p = 0.025$ and $p < 0.01$ respectively, Table 2). However, differently from *Ostreopsis*, mean cell abundances of *Coolia* were higher in August and November

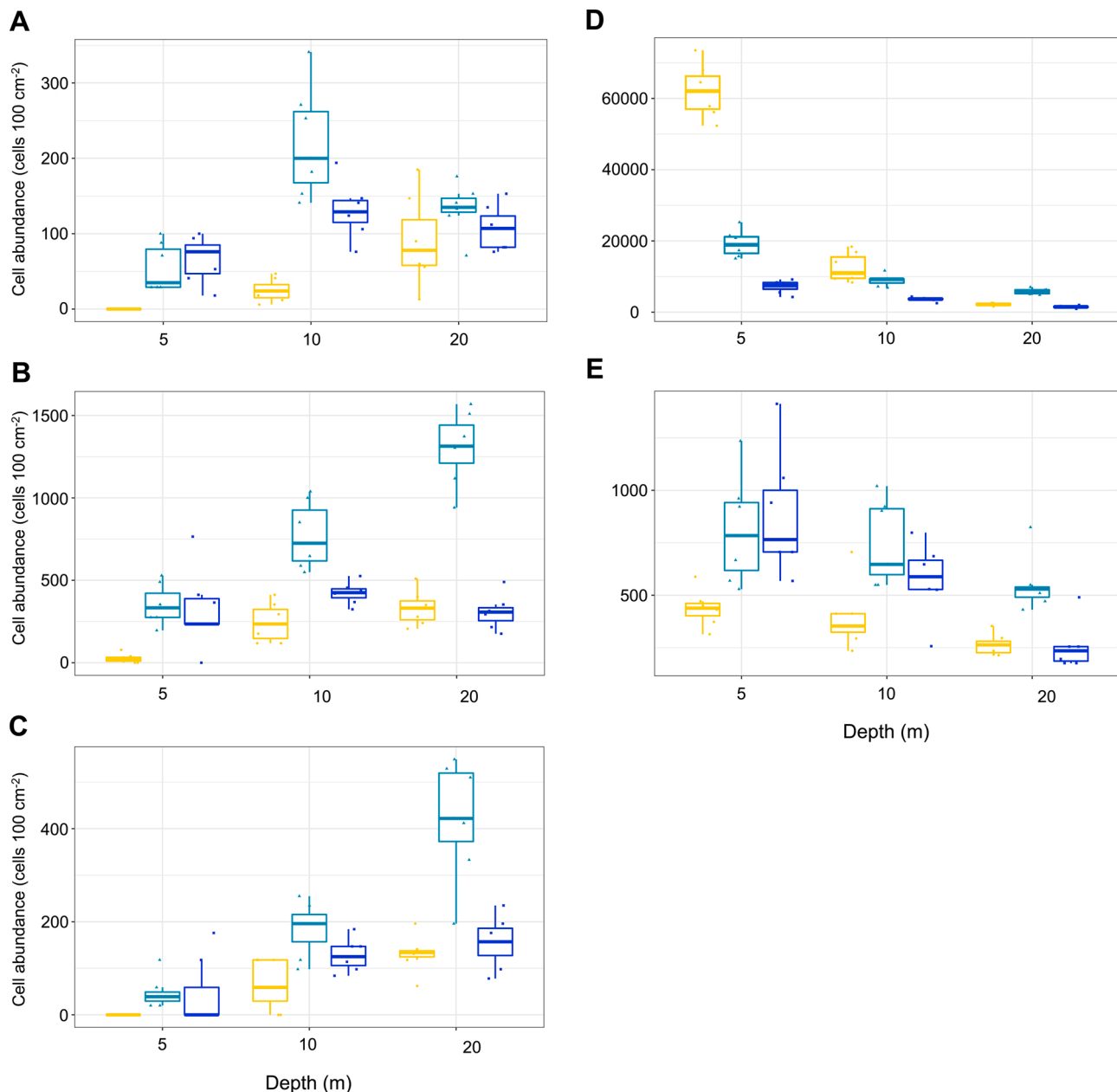


Fig. 3. Box plots of the cell abundances (cells 100 cm^{-2}) of the BHAB genera found at 3 depth levels (5, 10 and 20 m) through times: April (yellow/circles), August (green/triangles) and November (blue/squares). Left column plots correspond to those genera which cell abundances increased with depth: (A) *Gambierdiscus*; (B) *Prorocentrum* and (C) *Amphidinium*. Right column plots correspond to those genera which cell abundances decreased with depth: (D) *Ostreopsis* and (E) *Coolia*.

Table 2

Results of GLMs testing for differences in the abundance of BHAB dinoflagellate genera among the three depth levels (5, 10 and 20 m). Significant P-values ($P < 0.05$) are highlighted in bold. For each pair of comparisons reference levels are '10 m' and '5 m' respectively.

Coefficients	<i>Ostreopsis</i> Estimate	Z	P	<i>Coolia</i> Estimate	Z	P	<i>Gambierdiscus</i> Estimate	Z	P	<i>Prorocentrum</i> Estimate	Z	P	<i>Amphidinium</i> Estimate	Z	P
Intercept	8.91	25.205	<0.01	6.31	37.76	<0.01	4.52	9.52	<0.01	6.07	15.57	<0.01	4.77	10.75	<0.01
20 m	-0.907	-6.76	<0.01	-0.48	-5.029	<0.01	0.35	1.31	0.18	0.24	1.039	0.29	0.603	1.55	0.12
5 m	1.103	8.22	<0.01	0.21	2.23	0.025	-1.307	-5.02	<0.01	-0.905	-3.73	<0.01	-1.73	-4.103	<0.01
Intercept	10.01	28.27	<0.01	6.53	39.04	<0.01	3.21	6.67	<0.01	5.16	13.13	<0.01	3.04	6.55	<0.01
10 m	-1.103	-8.22	<0.01	-0.21	-2.23	0.025	1.307	5.02	<0.01	0.905	3.73	<0.01	1.73	4.103	<0.01
20 m	-2.01	-14.303	<0.01	-0.705	-7.21	<0.01	1.66	5.56	<0.01	1.14	4.62	<0.01	2.33	5.45	<0.01

than in April (Fig. 3).

Gambierdiscus, *Prorocentrum* and *Amphidinium* typically showed lower cell abundances than the other genera, ranging between 0 and 220 ± 72 , 26 ± 27 and 1304 ± 217 and 0 and 422 ± 125 cells 100 cm^{-2} respectively (Table S1). In contrast with the pattern observed for *Ostreopsis* and *Coolia*, these three genera were found to increase their mean cell abundance with depth (Fig. 3). Mean cell abundances of *Gambierdiscus*, *Prorocentrum* and *Amphidinium* significantly increased from 5 to 10 and 20 m ($p < 0.01$ in all cases, Table 2). However, no significant differences were found between 10 and 20 m (Table 2). In general, mean cell abundance of these genera were, as observed for *Coolia*, higher in August and November than in April (Fig. 3).

3.2. Influence of environmental parameters on the abundance and distribution of BHAB genera

Light, water motion and water temperature showed a clear depth-related pattern, decreasing as depth increased (Table S2). Water temperature recorded in the study site ranged between 19.5 and 24.9 °C. The maximum water temperature difference recorded in 24 h between 5 and 20 m depth was registered in April (1.8 °C), followed by November (1 °C) and August (0.8 °C). Water temperature average was higher in August (ca. 24 °C), followed by November (ca. 23.3 °C) and April (ca. 20.4 °C). Maximum light was recorded at 5 m depth in August (39,955 lux). At all sampling times, light was attenuated ca. 70% between 5 and 20 m depth.

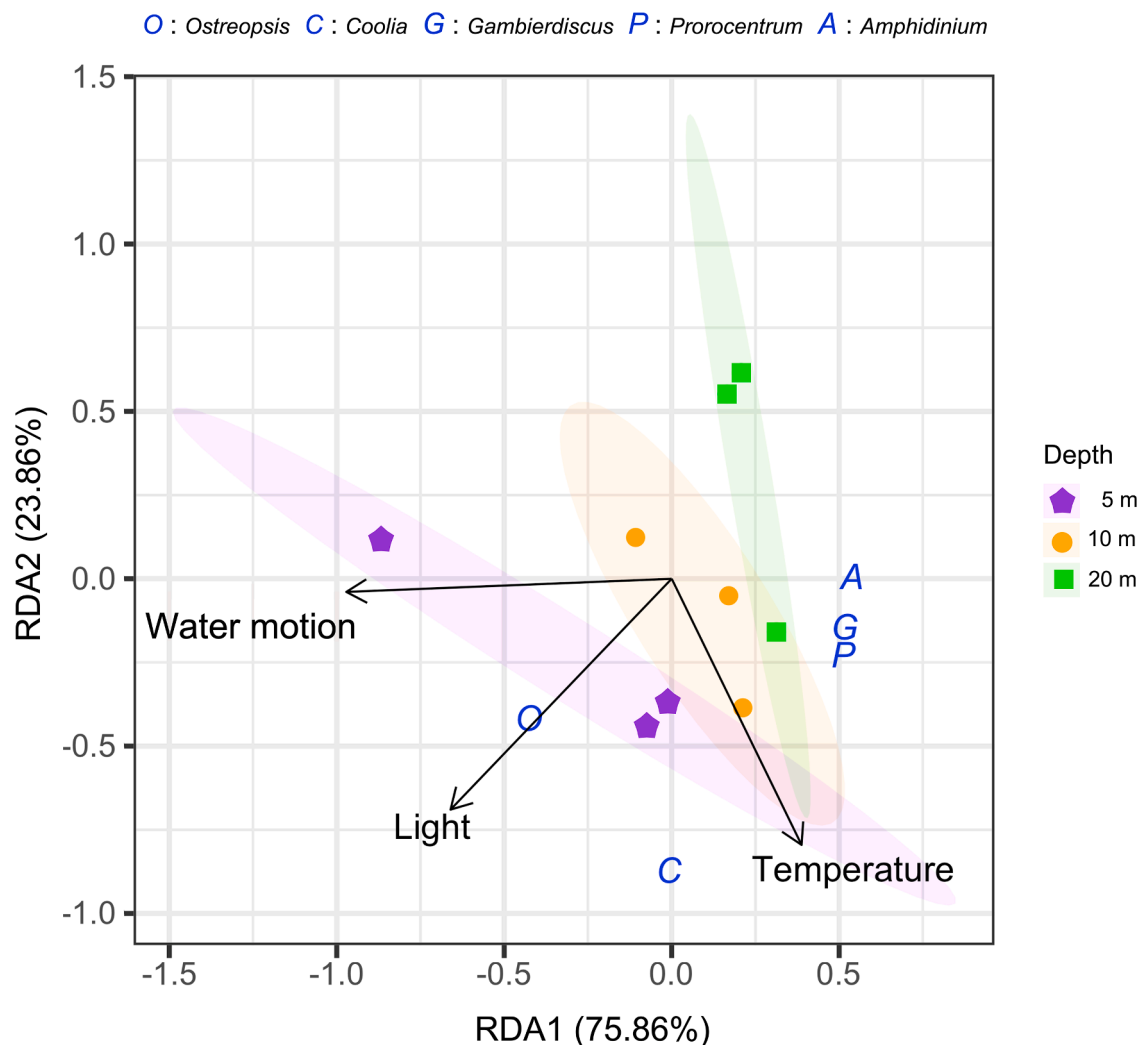


Fig. 4. Redundancy Analysis (RDA) ordination plot denoting relationships between BHAB dinoflagellate genera (*Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum* and *Amphidinium*) and environmental parameters (water temperature, water motion and light). Ellipsoids show confidence limits (95%) areas encompassing BHAB assemblages at each depth (5, 10 and 20 m).

Maximum water motion was registered at 5 m depth in April (18.1 m s^{-2}). The highest water motion variation between 5 and 20 m depth during 24 h (visualized through the standard deviation (SD) of this parameter) was registered in April (from 0.8 to 0.1 m s^{-2}). In contrast, this variation was lower in August and November (from 0.3 to 0.1 m s^{-2}) (Table S2).

Environmental parameters explained 84% (adjusted $R^2 = 0.84$ from the RDA) in the variance of the abundance and distribution of the BHAB dinoflagellate assemblage. Water motion was the parameter that most contributed to explain the observed variation, followed by light and water temperature (Fig. 4). Considering the different genera, *Gambierdiscus*, *Prorocentrum* and *Amphidinium* were mostly influenced by water motion (Fig. 4; $p < 0.01$ in all the cases, Table 3; Table S3) followed by water temperature (Fig. 4; $p < 0.05$, $p = 0.01$ and $p < 0.05$ respectively, Table 3; Table S3). *Coolia* was mainly influenced by water temperature (Fig. 4; $p = 0.01$, Table 3; Table S3) and light (Fig. 4; $p = 0.04$, Table 3; Table S3). Finally, the distribution of *Ostreopsis* was mostly influenced by light (Fig. 4; $p < 0.01$, Table 3; Table S3) followed by water motion (Fig. 4; $p = 0.07$, Table 3; Table S3). The way environmental parameters influenced the BHAB genera was different. Water motion was negatively correlated with *Gambierdiscus*, *Prorocentrum*, and *Amphidinium* and positively (but $p = 0.07$) correlated with *Ostreopsis*. Light was positively correlated with *Ostreopsis* and *Coolia*. Finally, temperature was positively correlated with *Coolia*, *Gambierdiscus*, *Prorocentrum* and *Amphidinium* (Fig. 4; Table 3).

3.3. Abundance and vertical distribution of *Gambierdiscus* species through sampling times

During the quantitative analysis of *Gambierdiscus* species, a total of 180 cells were identified to species level. In this analysis, three species were identified: *Gambierdiscus australes* Chinain & Faust, *Gambierdiscus caribaeus* Vandersea, Litaker, Faust, Kibler, Holland & Tester and *Gambierdiscus excentricus* (Fig. 5). In general, relative cell abundances of *Gambierdiscus* species remained similar between depths and sampling times (Fig. 6). Whenever *Gambierdiscus* was detected (not detected at 5 m in April), *G. caribaeus* was the most abundant species, accounting for 60 – 85% of total *Gambierdiscus* cell abundance, which corresponded to 20 – 154 cells 100 cm^{-2} . *G. australes* was usually the second most abundant species, accounting for 10 – 35%, which corresponded to 2 – 55 cells 100 cm^{-2} . Finally, *G. excentricus* was the least abundant species, reaching a maximum of 15% of the total abundance, which

Table 3

Relative importance of environmental parameters influencing BHAB dinoflagellate genera abundance and distribution throughout depths and sampling times from multimodel averaging of GLMs (full method: sum of Akaike Weights over all possible models). Significant variables are highlighted in bold.

Genus	Parameter	Estimate	Adjusted SE	z statistic	p-value
<i>Ostreopsis</i>	Light	0.00004	0.00001	2.97	<0.01
	Water motion	1.4	0.08	1.76	0.07
	Temperature	- 0.02	0.01	0.19	0.85
<i>Coolia</i>	Light	0.000009	0.000005	2.003	0.04
	Water motion	- 0.01	0.47	0.02	0.1
	Temperature	0.09	0.03	2.48	0.01
<i>Gambierdiscus</i>	Light	0.0000003	0.00002	0.02	0.98
	Water motion	- 3.02	0.06	5.38	<0.01
	Temperature	0.13	0.06	2.02	<0.05
<i>Prorocentrum</i>	Light	0.000002	0.00001	0.22	0.82
	Water motion	-1.868	0.3	6.29	<0.01
	Temperature	0.1075	0.03	3.02	0.01
<i>Amphidinium</i>	Light	0.0000003	0.00002	0.1	0.99
	Water motion	- 3.02	0.56	5.39	<0.01
	Temperature	0.13	0.06	2.02	<0.05

corresponded to 0 – 20 cells 100 cm^{-2} (Fig. 6).

In April, cell abundances of *G. caribaeus* and *G. australes* increased from 10 to 20 m. In August and November, their cell abundances increased from 5 to 10 m, but decreased from 10 to 20 m (Fig. 6). In contrast to these species, *G. excentricus* showed a more restricted distribution, being always absent at 5 m depth, and absent in November at all depths. In April and August, the cell abundance of this species increased with depth from 10 to 20 m (Fig. 6).

In addition to the three species already mentioned, the qualitative analysis of samples revealed the presence of *Gambierdiscus belizeanus* Faust in the study area (Fig. 5; Table 1).

4. Discussion

4.1. Vertical distribution patterns of BHAB genera and influence of environmental parameters in El Hierro

This study investigated the presence and distribution with depth of BHAB dinoflagellates in the south of El Hierro Island at three different times (April, August, November). Five genera were recorded from 5 to 20 m depth, but the structure of the assemblage changed with depth and sampling time. Results indicated that BHAB genera followed two vertical distribution patterns throughout the studied depth range that were temporally consistent: *Ostreopsis* and *Coolia* always presented the highest cell abundances at the surface and consistently decreased with depth, while *Gambierdiscus*, *Prorocentrum* and *Amphidinium* always presented the highest cell abundances below 5 m. The consistent record of maximum cell abundances of some BHAB genera at 10 – 20 m demonstrated that, in the south of El Hierro Island, depth zones may offer a set of environmental conditions that result more favorable for particular genera, while hindering others.

4.1.1. Water motion

The results of this study indicated that water motion was the parameter that mostly contributed to explain the BHAB assemblage structure and distribution. This parameter has been commonly disregarded in many field and laboratory studies, in which water temperature has been considered the main, sometimes unique, factor influencing the BHAB dynamics. However, already in early studies on the ecology of benthic dinoflagellates, the hydrodynamic characteristics of the studied systems was considered a key environmental parameter shaping benthic dinoflagellate assemblages (Tindall and Morton, 1998). Water motion may affect BHAB dinoflagellates at different levels. Dinoflagellates are known to be very sensitive to water turbulence at the cellular level possibly related to several mechanisms such as mechanical damage, swimming interference and influence on nutrient availability and cell physiology, among others (Estrada and Berdalet, 1998). At the population and community level, water turbulence caused by waves and currents also influence cell abundances of epibenthic dinoflagellates as these are only loosely attached to their substrates, being readily removed by waves and currents and dispersed into the water column (Tindall and Morton, 1998, Accoroni and Totti, 2016).

In this study, water motion showed a negative significant effect on *Gambierdiscus*, *Prorocentrum* and *Amphidinium*, which explained their maximum cell abundances away from the sea surface, at 10 or 20 m, where water motion values rapidly decreased. The negative influence of water motion on *Gambierdiscus* and *Amphidinium* was most probably responsible for the absence of these two genera at 5 m in April, when maximum water motion values were recorded. Richlen and Lobel (2011) also found that water motion influenced negatively cell abundances of these genera, and reported a similar a distribution pattern with depth at the Johnston Atoll in the Pacific Ocean. Various studies have assessed the effect of water motion on the abundance and distribution of *Gambierdiscus*, while the number of studies on *Prorocentrum* and *Amphidinium* are comparatively much more limited. In agreement to this study, the large majority of the studies document higher cell abundances of

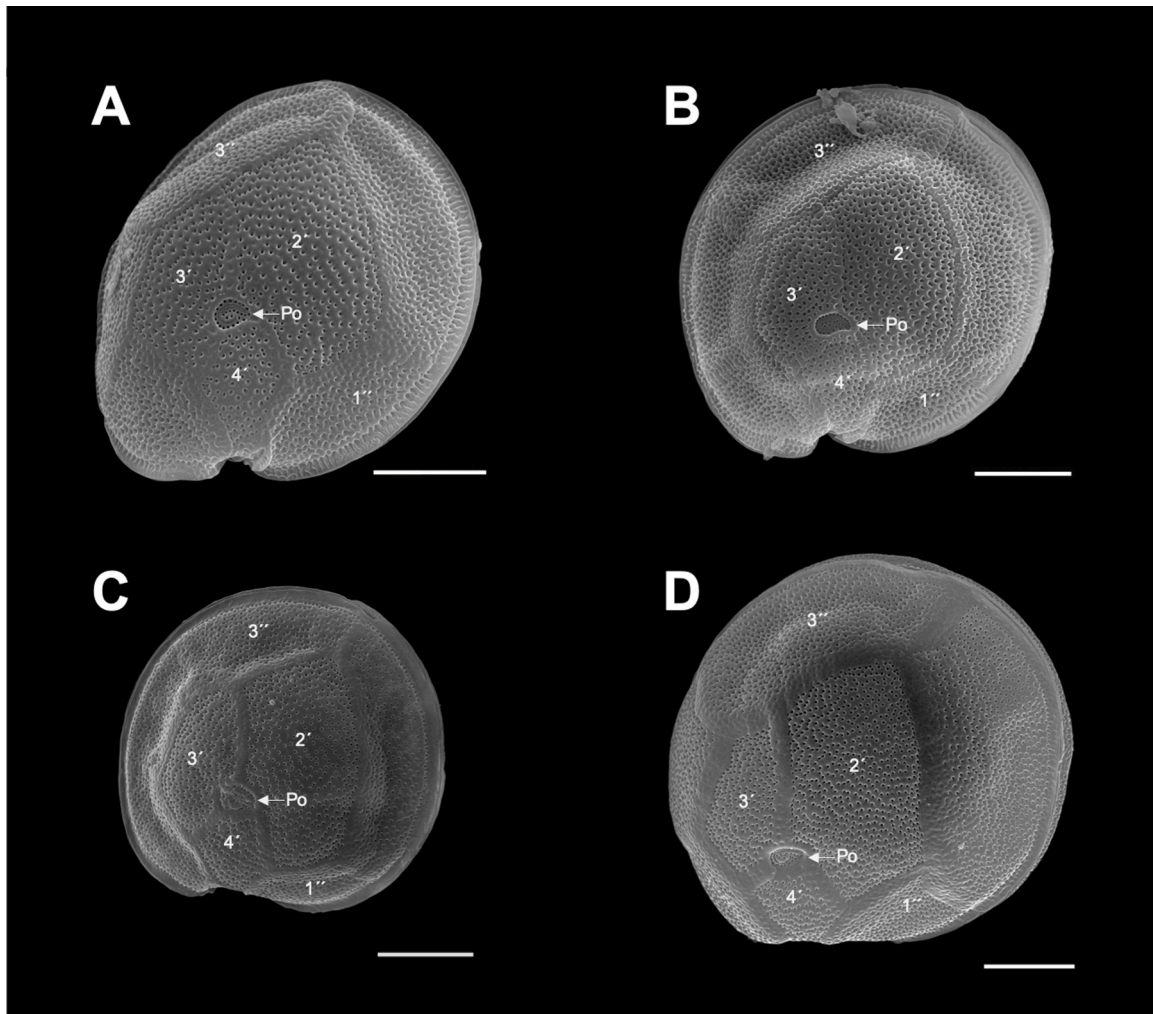


Fig. 5. Scanning electron micrograph images of the epithecas from *Gambierdiscus* species found at the study site: (A) *G. australes*, (B) *G. belizeanus* (C) *G. caribaeus* (D) *G. excentricus*. Scale bar: 20 μm .

Gambierdiscus from sheltered environments, which would correspond to the deeper zones sampled at the present study, than from exposed ones (reviewed in Parsons et al., 2012 and Tester et al., 2020).

In contrast with *Gambierdiscus*, *Prorocentrum* and *Amphidinium*, water motion, at the recorded levels, was found to have a positive influence on the growth of *Ostreopsis*. Such positive influence was not only evident on the vertical gradient, with maximum cell abundances at the shallowest level, but also between sampling times: *Ostreopsis* cell abundances were 3 to 6 times higher at 5 m depth in April, when maximum water motion values were recorded, than in later sampling times. Water motion is one of the environmental factors frequently evoked when discussing dynamics of *Ostreopsis* blooms, but a consensus has not yet been reached. However, many studies have observed that moderate hydrodynamic conditions seem to positively affect *Ostreopsis* growth (Vila et al., 2001, Totti et al., 2010, Selina et al., 2014, Santos et al., 2019), while strong hydrodynamics, often recorded at shallow depths, may prevent growth (Santos et al., 2019) or remove cells from substrates (Mabrouk et al., 2011).

Regarding the influence of water motion on the distribution of *Coolia*, the already referred vertical pattern, characterized by higher cell densities at the surface and marked decline with depth, and the results of the RDA, suggest it is less sensitive to water motion than *Gambierdiscus*, *Prorocentrum* and *Amphidinium*. However, compared with *Ostreopsis*, *Coolia* appeared to be favoured by lower water motion levels, as evidenced by the higher cell abundances at 5 m depth in August and

November when water motion values significantly decreased. In agreement with the results of this study, Vila et al. (2001) observed in the Mediterranean that *Ostreopsis* and *Coolia* tolerated certain turbulent conditions (“shaken”, “slightly shaken”), but *Ostreopsis* had a higher tolerance.

The measure of water motion to assess its influence on the abundance and distribution of BHAB has been carried out in different ways. Some studies have assessed it visually, establishing categories such as calm, moderate or strong (Vila et al., 2001, Totti et al., 2010). However, the use of subjective measures makes its relation with BHAB imprecise. The use of quantitative methods is more appropriate to establish precise relations. Some studies employed indirect quantitative methods such as “clod-cards” or the wave height (Richlen and Lobel, 2011, Santos et al., 2019) while, in this study, the water motion was directly recorded (m s^{-2}) by means of digital devices (accelerometers). The use of different approaches to estimate the water motion precludes a realistic comparison of results between studies. Therefore, until the incorporation of precise, standard quantitative estimates of water motion in ecological studies, the influence of this parameter on BHAB dynamics will remain uncertain. In any case, results obtained at the present study support previous observations indicating different water motion tolerance levels for the studied BHAB genera: *Ostreopsis* (less susceptible) < *Coolia* < *Gambierdiscus*, *Prorocentrum* and *Amphidinium* (more susceptible). These distinct levels of susceptibility to water motion seem to be a key ecological trait influencing the vertical distribution of BHAB genera.

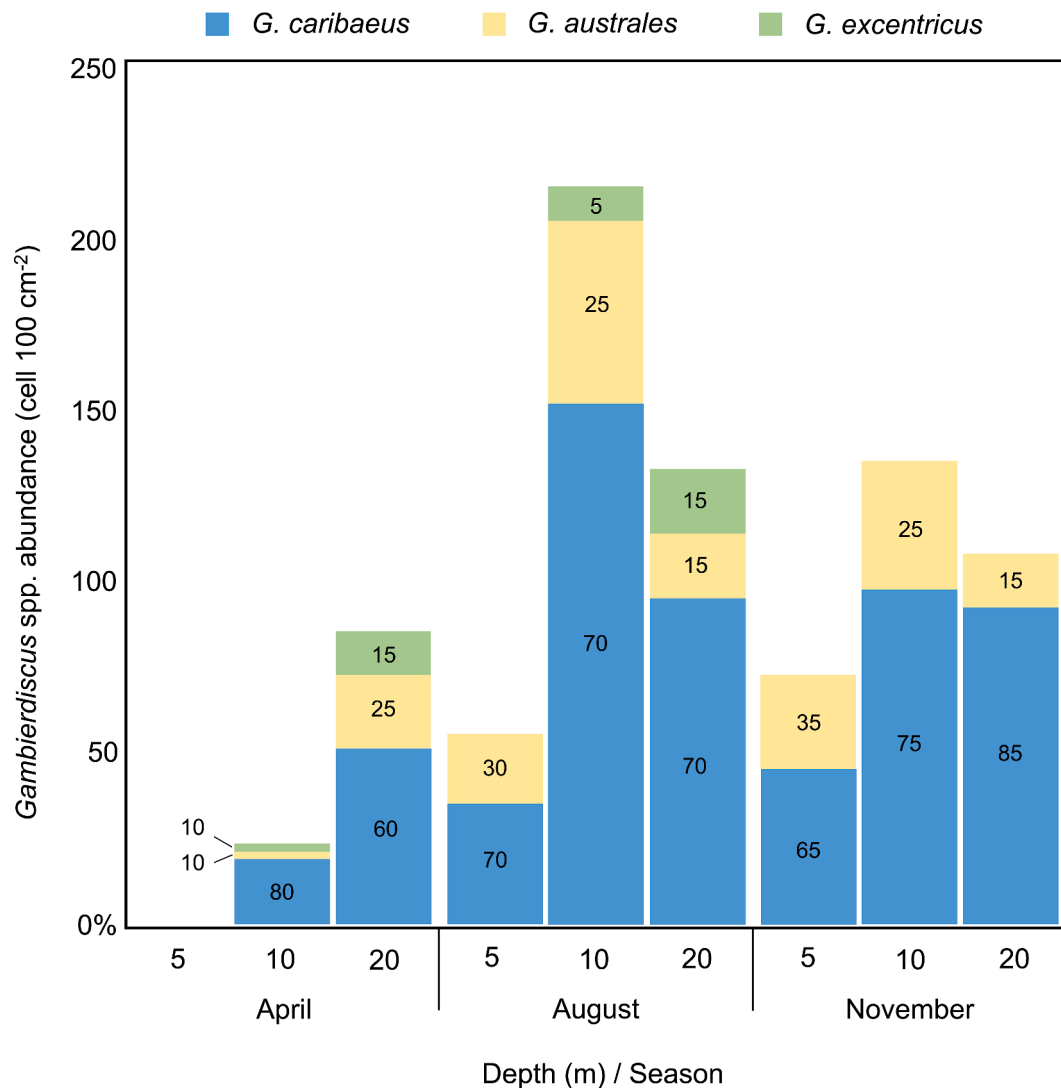


Fig. 6. Total and relative cell abundance of *Gambierdiscus* species throughout depths and sampling times. Relative abundance values (%) are denoted inside bars.

4.1.2. Light

Light was the second parameter that most influenced the BHAB assemblage distribution. As observed for water motion, variations in light occurred mostly between depth levels and, therefore, its influence was more evident on the vertical distribution of BHAB assemblage. However, daylength cannot be discarded as an important light limiting factor of growth between sampling times, particularly for *Gambierdiscus*, *Prorocentrum* and *Amphidinium*, which showed significant reduction in cell abundances in November at 10 and 20 m, when daylength is significantly shorter.

In the vertical gradient, light showed a significant positive influence on the growth of *Ostreopsis* and *Coolia*. This was especially true for *Ostreopsis*, for which light was the most determining parameter. In agreement with this result, most previous studies have found maximum *Ostreopsis* cell abundances at shallow, well-illuminated zones (e.g. Totti et al., 2010 or Richlen and Lobel, 2011), which have led them to suggest a dependence, or a higher tolerance, to high light compared to other genera (Accoroni and Totti, 2016). However, the presence of populations of *Ostreopsis* at 20 m depth with significant cell densities indicates that, even at that depth, the lower light limit was not reached, suggesting their realized light niche is quite wide. Laboratory studies that have investigated the role of light influencing the growth of this genus are limited (reviewed in Tester et al., 2020) but, in agreement with the results of this study, these studies indicate that *Ostreopsis* is

capable of growing at a wide range of light intensities (Monti and Cecchin, 2012).

Likewise, *Coolia* cell abundances were always found to be higher at the shallower level, suggesting similar light requirements to *Ostreopsis*. Both genera are frequently present in the water column (Mangialagio et al., 2011, Jauzein et al., 2018, Santos et al., 2019) and seem less dependent on a substrate than other epibenthic dinoflagellates (tychoplanktonic). The higher tolerance for being exposed to high light intensities could be an adaptative advantage for the tychoplanktonic ecological strategy that characterizes these two genera.

However, under the same light intensities, *Ostreopsis* cell abundances were always higher than those of *Coolia*, as it was also observed in other field studies (Cohu and Lemée, 2012, Fernández-Zabala et al., 2019, Bravo et al., 2020). The reason behind the consistent dominance of the BHAB assemblage by *Ostreopsis* at the surface may reflect a better adaptation of the genus to the surface light conditions. In accordance with this observation, results obtained by Heil et al. (1993) suggested that high light intensities could induce *Ostreopsis* cells to aggregate producing mucus layers to protect themselves, which could represent a competitive strategy against other epibenthic dinoflagellates in shallow areas.

Light did not influence the distribution with depth of the other studied genera significantly but, in any case, the consistent increase in cell abundances below 5 m, with maxima occurring at 10 m depth

(*Gambierdiscus*) and at 20 m (*Prorocentrum* and *Amphidinium*) suggests that these genera are well adapted to low-light conditions and contrast with the high-light adapted genera *Ostreopsis* and *Coolia*. However, *Gambierdiscus* seems to have a different behaviour than *Prorocentrum* and *Amphidinium*. The fact that *Gambierdiscus* had its highest recorded densities at 10 m depth in the warmest sampling time (August), suggests its optimum light window is found close to the light environment found at that depth, and values above or below it, are already towards the upper and lower light tolerance limit, respectively. On the other hand, *Prorocentrum* and *Amphidinium*, had their highest recorded cell abundances in the warmest sampling time at 20 m depth, suggesting the optimum light window could extend below the light environment recorded at that depth.

Our results indicate that the studied genera have different ecological light preferences that were partly responsible of their vertical distribution. Thus, the five genera may be ordered according to decaying light tolerance: *Ostreopsis* < *Coolia* < *Gambierdiscus* < *Prorocentrum* and *Amphidinium*.

4.1.3. Water temperature

Temperature was the parameter that less influenced the abundance and distribution of the BHAB assemblage. This contrasts with other studies, in which water temperature has been traditionally considered a major influence on the growth of BHAB dinoflagellates. Even so, this study showed that water temperature had a positive significant influence on the growth of *Coolia*, *Gambierdiscus*, *Prorocentrum* and *Amphidinium*, but it was much more related with time dependent gradients (sampling times) than to vertical gradients. This was evidenced by the consistent record of higher cell abundances at all depths in August the warmest month, and lower cell abundances in April, the coolest month. The distribution with depth observed for *Gambierdiscus*, *Prorocentrum* and *Amphidinium* cannot be assigned to the influence of water temperature, as the cell abundances of these genera were, in most cases, higher at 10–20 m depth where slightly lower temperatures were registered. In the case of *Coolia*, water temperature might in addition have contributed, at least partly, to the observed vertical distribution, as cell abundances always decreased with depth concurrently with recorded subtle water temperature drops.

According to the results of this study, water temperature did not influence the cell abundance and distribution observed for *Ostreopsis*. This fact might have masked the overall influence of this parameter over the BHAB assemblage, as *Ostreopsis* was always the most abundant genus throughout depth and time. However, it is important to highlight that the growth of *Ostreopsis* seemed to be favoured, as observed from its temporal pattern, by a low-water temperature: maximum cell densities were recorded in April, when the lowest mean water temperature of this study was recorded. This contrasts with what has been generally described from more temperate areas, such as the Mediterranean, where proliferations of *Ostreopsis* frequently coincide with relatively higher water temperatures. However, there are also studies conducted in that region which have observed that the highest abundances of this genus are not necessarily associated with the highest water temperatures (Accoroni and Totti, 2016). In this sense, Accoroni et al. (2014) suggested that, although a relatively high temperature threshold is needed for the onset of *Ostreopsis* proliferation (probably in relation to cyst germination), high cell abundance maintenance may occur at lower temperatures. In accordance with these observations, results from laboratory experiments by Scalco et al. (2012) with different strains of *O. cf. ovata* from the Mediterranean, suggested that photosaturation at high temperature and irradiance conditions could be also responsible for the occurrence of blooms outside the warmest period of the year. Results obtained in the present study, and observations in other areas of the archipelago (Fernández-Zabala et al., 2019), document high cell abundances of *Ostreopsis* during most part of the year, with maximum cell abundances not necessarily associated with the highest water temperatures.

Results obtained in the present study indicate that within the warm-temperate regime of El Hierro, the studied BHAB genera show different temperature affinities: *Ostreopsis* (low-water temperature affinity) < *Coolia*, *Gambierdiscus*, *Prorocentrum* and *Amphidinium* (high-water temperature affinity).

4.2. Abundance and vertical distribution of *Gambierdiscus* species through sampling times

In the Canary Islands, the first outbreaks of ciguatera poisoning (CP), a food-borne disease caused by the neurotoxins produced by species of the genus *Gambierdiscus* (Chinain et al., 2021), were registered at the beginning of this century and nowadays represent the major algae-related risk for human health in the region (Pérez-Arellano et al., 2005; Boada et al., 2010; Núñez et al., 2012). Given its implications for human health in the Canary Islands, the genus *Gambierdiscus* was also investigated at species level in the south of El Hierro Island to clarify its ecological patterns across vertical and temporal gradients.

Four out of the six species of *Gambierdiscus* described in the archipelago were identified in the study area, i.e. *G. australes*, *G. belizeanus*, *G. caribaeus* and *G. excentricus*. *G. excentricus* and *G. belizeanus* were easily discriminated among the four species based on the eccentricity Po (R2) and the heavily areolate cell surface pattern respectively, which are unique features of these species. In the case of *G. australes* and *G. caribaeus*, plate suture ratios (R1 and R2) were informative enough to discriminate between them. In addition, molecular analysis carried out from the cultures of these species facilitated their later morphological identification.

From these species, *G. belizeanus* was never detected during the quantitative analysis. Therefore, its contribution to the vertical distribution pattern observed for the genus seems negligible.

The distribution of *Gambierdiscus* in the south of El Hierro was characterized by a marked dominance of *G. caribaeus* at all depths and across sampling times (60–85% of the total cell abundance) and, therefore, the vertical distribution pattern already described for the genus was mainly reflecting the vertical pattern of this species. This result, together with a high-density bloom of this species reported by Soler-Onís et al. (2016) at a nearby sampling point, suggests that the environmental conditions present in the south of El Hierro favour the dominance of *G. caribaeus* over the rest of *Gambierdiscus* species. The distribution with depth of *G. australes*, the second most abundant species (10–35% of the total cell abundance), was similar to *G. caribaeus* in all the sampling times, indicating very similar ecological requirements (Fig. 6). Both species showed a vertical and seasonal distribution suggesting high-water temperature affinity, low-light adaptation and low tolerance to water motion, as previously observed at the genus level. The highest densities of these species were recorded at 10 m depth in the warmer months (August and November), at temperatures within their optimal range as suggested by laboratory experiments (Ramilo et al., 2021), while their lowest densities were recorded at 10 m in the coldest month (April). This distribution also suggests that, when their thermal requirements are fulfilled, they seem to prefer the intermediate light intensities found at 10 m depth. This observation is in accordance with experimental results for *G. caribaeus* from Xu et al. (2016), which showed that, at the temperature yielding its maximum growth rates, this species grew better at the higher irradiance level tested.

G. excentricus was the least abundant species (0–15% of the total cell abundance) and showed the most restricted distribution in this study; it was only present at 10 and 20 m in April and August. According to its latitudinal distribution range, *G. excentricus* has been regarded as a cool-water adapted species when compared with other species of this genus (Tester et al., 2020). In agreement with this observation, laboratory experiments by Ramilo et al. (2021) on the temperature influence on the growth of *Gambierdiscus* species from the Canary Islands have shown that *G. excentricus* presents a lower optimal thermal range than *G. caribaeus* and *G. australes* and, therefore, yields its maximum growth

rate ($0.08 \text{ divisions day}^{-1}$) at temperatures $2.5 - 3.5^\circ\text{C}$ less than the other two species (25°C). These experiments have also demonstrated that the growth rate of *G. excentricus* is the lowest of the three species (0.08 vs $0.18 \text{ divisions day}^{-1}$) at the temperatures yielding their maximum growth rates. These results may explain why *G. excentricus* showed, in all the cases, the lowest cell abundances.

The environmental data recorded in this study did not allow us to explain why *G. excentricus* was not detected in November. However, the most plausible explanation is that the environmental conditions between August and November could have become unfavorable for this species. In this sense, daily maximum sea surface temperatures (SSTs) between August and November ($>25^\circ\text{C}$, Fig. 7), could have exceeded the upper thermal tolerance limit of *G. excentricus*. This could suggest that the upper thermal tolerance limit of this species in the field is lower than that recorded in laboratory-controlled conditions (29°C , Ramilo et al., 2021). Very little is still known on the overwintering strategies of *Gambierdiscus* species. A possible explanation for the disappearance of *G. excentricus* might be related to the formation of resting stages under unfavourable environmental conditions (Anderson et al., 2003). However, these stages have so far not been reported from field samples. For the moment, there is only evidence from laboratory observations made in cultures of *G. balechii* (Fraga et al., 2016). In any case, our observations indicate that *Gambierdiscus* species have a different phenology, but further studies are required to better understand the temporal variability of *G. excentricus* in field.

As discussed in section 4.1, water temperature had a positive influence on *Gambierdiscus* at the genus level, and therefore its distribution with depth could not be directly assigned to this parameter. However, due to its cooler-water affinity, the influence of water temperature in the distribution with depth of *G. excentricus* cannot be discarded, as it might have given place to more suitable growth conditions for this species below 5 m depth. Although water temperature differences recorded between depth levels at the present study were slight, these could be enough to favour its growth. Results by Kibler et al. (2012) under experimental conditions, where small differences of only 1°C of water temperature greatly affected growth potentials of *Gambierdiscus* species, support this hypothesis. The vertical distribution of *G. excentricus* was, in addition, most probably influenced by the already discussed low tolerance of *Gambierdiscus* to high light intensities, which could be even lower for *G. excentricus* than for the other two species, leading to its

disappearance at 5 m in August. Unfortunately, the influence of light on this species has not yet been assessed experimentally.

Prior to this study, *G. excentricus* had been identified in all the islands of the Canary Islands except from El Hierro (Rodríguez et al., 2017; Bravo et al., 2019, 2020; Tudó et al., 2020). Tudó et al. (2020) suggested that the waters of El Hierro, which are the warmest in the archipelago, could be unsuitable for the growth of this species due to its cool-water affinity compared to other *Gambierdiscus* species. However, in those studies sampling was carried out in relatively shallow coastal sites between September and November, when our study has shown a lower probability of finding *G. excentricus*.

Based on our observations, the spatial distribution of *Gambierdiscus* proposed for the Canary Islands could be biased by the time of sampling and the restricted depth ranges considered. Most studies conducted in the region have suggested that the species of this genus are distributed in relation to the SST gradient that exists between warm western and cold eastern islands (Rodríguez et al., 2017; Bravo et al., 2019, 2020; Tudó et al., 2020; Ramilo et al., 2021). Tudó et al. (2020) already noticed the difficulty of explaining the distribution of the genus based exclusively on SSTs, and recognized that the information on the distribution of *Gambierdiscus* spp. could still be incomplete or environmental factors other than the temperature gradient could be influencing it.

While more studies are undeniably needed to fully understand the diversity, abundance and distribution of this genus in the archipelago, the results obtained in the present study showed that the set of different environmental conditions created by depth gradients at a local scale should be considered together with larger-scale processes to avoid “playing hide and seek” with the species of this genus. Results from the present study showed that this is especially relevant in two situations: i) in highly exposed sites and when sampling during high-water motion periods, which can prevent the presence of *Gambierdiscus* spp. at shallow zones (e.g. 0 - 5 m) and ii) in the warm-water western islands such as El Hierro, where cooler-water adapted species, i.e. *G. excentricus*, show restricted distributions. This last consideration is particularly important in the present climate change scenario. As water temperature increases, these species are expected to seek thermal refuge in deeper zones during warmest periods of the year (Tester et al., 2020). If true, in coming years certain species such as *G. excentricus* may disappear from the upper infralittoral zones (0 - 20 m) in El Hierro and start disappearing from shallow zones in other islands, following the west (warm) to east (cold)

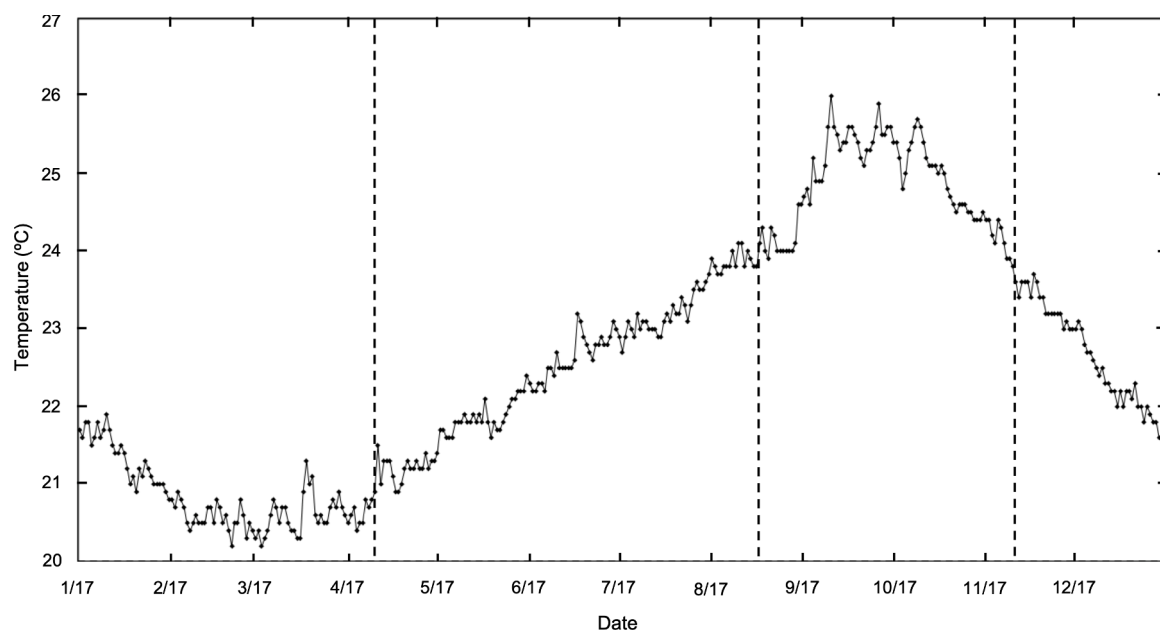


Fig 7. Daily maximum SSTs at the study site covering the year 2017. Dotted lines denote times in which samplings took place: 25 - 26 April, 17 - 18 August and 6 - 7 November. Data provided by the Copernicus Marine Environment Monitoring Service (CMEMS, <http://marine.copernicus.eu/>)

thermal gradient present in the region.

5. Conclusions

This study has demonstrated the existence of two vertical distribution patterns in the BHAB assemblage of southern El Hierro: cell abundances of *Ostreopsis* and *Coolia* decreased from 5 to 20 m depth while cell abundances of *Gambierdiscus*, *Prorocentrum* and *Amphidinium* showed the reverse pattern, mostly evident from 5 to 10 m. Although these patterns appeared to be consistent through time, further spatio-temporal studies using a reliable, standardized sampling approaches (e.g. artificial substrates) are needed to verify if they stand in the rest of the region, or even other geographical areas. Water motion and light determined vertical and temporal distribution of the BHAB assemblage in a large percentage. Therefore, they are valuable predictors of BHAB vertical patterns in the study region: *Ostreopsis* and *Coolia* are high light adapted genera (shallow genera) and *Gambierdiscus*, *Prorocentrum* and *Amphidinium* are low water motion adapted genera (depth genera).

An analysis of the vertical and seasonal distribution of *Gambierdiscus* at species level revealed the existence of interspecific differences, highlighting the need for conducting ecological studies at species rather than at genus level. As a consequence, the cell abundance and distribution of *Gambierdiscus* species in the Canary Islands might have been underestimated by considering only limited sampling periods and depth ranges, allowing the species such as *G. excrucians* to "play hide and seek". Therefore, it seems urgent to reevaluate sampling protocols to improve the detection, quantification and risk assessment of this and other BHAB genera in the Canary Islands.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

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Supplementary materials

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