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Highlights:
- Glorieuses islands can be considered as pristine ecosystems, not subject to human pressure
- Planktonic microbial communities were studied in terms of ecological descriptors for tracking changes in the way coral-reef systems function
- A category 5 tropical cyclone could explain the post-event environmental situation
- A low active microbial food web was observed after the cyclone.
- Three variables among the autotrophic and heterotrophic compartments can be considered for assessing environmental changes in Glorieuses marine waters.

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Conceptualization: MB, CD, PC, LB
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Abstract

Ecological baselines for the structure and functioning of ecosystems in the absence of human activity can provide essential information on their health status. The Glorieuses islands are located in the Western Indian Ocean (WIO) and can be considered as "pristine" ecosystems that have not been subjected to anthropogenic pressure. Their nutrient context and the microbial assemblages were assessed by determining the abundance of heterotrophic prokaryotes (archaea and bacteria), picocyanobacteria, picoeukaryotes, microphytoplankton and protozooplankton communities in five stations, during two contrasted periods (November 2015 and May 2016). Chlorophyll-a concentrations were always under 1 µg/L and associated to very low levels in orthophosphates, nitrate and dissolved organic carbon, revealing an ultra-oligotrophic status for the Glorieuses waters. Picocyanobacteria confirmed the ultra-oligotrophic status with a predominance of *Synechococcus*. Zeaxanthin associated with the presence of picocyanobacteria represented the major pigment in both surveys. Three indices of diversity (species richness, Shannon and Pielou indexes) from microscopy observations highlighted the difference of diversity in microphytoplankton between the surveys. A focus on a 16S metabarcoding approach showed a high dominance of picocyanobacteria, Alpha- and Gammaproteobacteria, regardless of station or period. Multivariate analyses (co-inertia analyses) revealed a strong variability of ecological conditions between the two periods, with (i) high nutrient concentrations and heterotrophic nanoflagellate abundance with an active microbial food web in November 2015, and (ii) high heterotrophic prokaryote and picoeukaryote abundance in May 2016. The impact of a category 5 tropical cyclone (Fantala) on the regional zone in April 2016 is also advanced to explain these contrasted situations. Relative importance of top-down factors between bacterial and heterotrophic nanoflagellates was observed in November 2015 with an active microbial food web. All the results indicate that three microbial indexes potentially can be considered to assess the ecological change in Glorieuses marine waters.

1.Introduction

Coral reef environments are generally recognized among the most threatened and vulnerable marine ecosystems, and are highly susceptible to stress and disturbance, especially from anthropogenic pressure (Costanza et al., 1997; Mellin et al., 2008) and climate change (Miller et al., 2009). Recently, Barbier (2017) alerted the scientific world as to coastal development and the loss or degradation of 30% of coral reefs worldwide over the three last decades. What is more, these latter constitute a natural shelter for the myriad organisms living there, such as reef fish and invertebrates (Barbier et al., 2011). Tropical pelagic habitats (such as mangrove wetlands, seagrass meadows and corals reefs)

are highly socio-economically and ecologically essential (Nagelkerken, 2009), providing goods and services to human welfare and economies, including greenhouse gas dynamics and pollution filtration processes such as carbon storage and cycling (e.g. in mangrove habitats, Fujimoto et al., 1999; Balmford, 2002).

Ecological indicators are seen as a way to reduce the complexity of ecosystems to a small number of key criteria that could retain the essential information needed for tracking changes in the state of the environment, as pointed out by Hayes et al. (2015). These authors cited several international studies on a global scale, including those mandated in 2008 by the European Marine Strategy Framework Directive (EC, 2008). Microbial planktonic communities encompass a great diversity of organisms such as bacteria, phytoplankton and protozoa (ciliates and nanoflagellates). The composition and structure of microbial communities are basic indicators of the state of the ecosystem, including the type of factors regulating the dynamics of these communities (Suttle, 2005; Estrada and Vaque, 2014). Phytoplankton, roughly defined as the autotrophic component of the planktonic food web, are considered as having an important ecological function as primary producers that directly and indirectly fuels the food webs (Domingues et al., 2008). Recently, Gittings et al. (2019) evaluated tropical phytoplankton phenology using satellite data. They proposed to categorize phytoplankton as an "ecosystem indicator" to assess "ecosystem health" in response to environmental and climatic disturbances.

In their review, Varkitzi et al. (2018) proposed a subset of indicators for each biodiversity component to be tested for use in the assessment of good environmental status of the water column in Mediterranean Sea, phytoplankton being the unique biological variables tested. While some indicators are available for phytoplankton such as diversity indices (based essentially on individuals of size greater than 10 µm, microalgae), indicators are much less numerous for other components, and almost non-existent for picophytoplankton (size smaller than 2 µm) and prokaryotes (Varkitzi et al., 2018). The differences can be attributed to the fact that phytoplankton is listed as a key biological element in Water Framework Directive (EC, 2008). Other studies highlighted the approach based on a composition index built with phytoplankton pigments (HPLC) gathered during interannual samplings in French coastal water masses, providing information on the seasonality of disturbances (Lampert and Hernandez-Farinas, 2018). Autotrophic picoplankton (including picoeukaryotes), with a cell size comprised between 0.2 and 2 µm, have been discovered as a major phytoplankton component, contributing to 60% of the total primary production in an open ocean ecosystem, located in the north tropical oligotrophic Indian Ocean (Platt et al., 1983). Picoplankton communities are dominated by two genera of *Chroococcales (Cyanophyceae)*: *Synechococcus*, cosmopolite organisms in coastal waters, and *Prochlorococcus* commonly found in the open ocean between 40°N and 40°S (Johnson et al., 2006). As a result, they can contribute up to 80 % of total daily or annual carbon

production, that will subsequently be transferred to higher trophic levels through grazing by microzooplankton (Dupuy et al., 2016; Armengol et al., 2019).

Interactions between anthropogenic and climatic stressors represent a serious challenge for managing and predicting the water quality and ecological status of interface ecosystems (estuaries, coastal lagoons) (Hemraj et al., 2017). However, the effects of climate change can lead to constantly changing ocean dynamics, and so an understanding of biological indices would be appreciable to follow the ecological status of fragile ecosystems such as coral-reef systems (Burke et al., 2001). Generally, it is extremely difficult to identify the effects of climate change among other forcing stressors, mainly because ecosystems that are isolated from human pressure are rare. Glorieuses islands (Grande Glorieuse, Île du Lys), belonging to the Iles Eparses and part of the TAAF (Terres Australes et Antartiques Françaises), located in the Western Indian Ocean (WIO), display an extraordinary and rich biodiversity in terms of marine species (Durville et al., 2003; Poupin et al., 2014; Conand et al., 2014; Chabanet et al., 2015; Bouvy et al., 2016; Dupuy et al., 2016; Quetel et al., 2016). These islands are associated with a tropical wet climate (Quod et al., 2007; Quetel et al., 2016) and are recognized as being uninhabited (other than a limited military presence) as well as geographically isolated (located 222 km north of Madagascar and 253 km north of Mayotte). As a result, Glorieuses islands can be considered as equivalent tropical pristine ecosystems, only being touched by natural disturbances such as climatic actions. Geographically, the Mozambique Channel and the Western Indian Ocean (WIO) are dominated by a series of southward drifting anti-cyclonic and cyclonic eddies, which cause the upwelling of cooler, nutrient-rich waters, resulting in an elevated phytoplankton biomass (Barlow et al., 2014; Lamont et al., 2014). In addition, the WIO is also subjected to periodical extreme weather events such as cyclones, with serious impact on shorelines and sediment transport, as described by Duvat et al. (2017) on Farquhar Atoll (Seychelles), after the passage of a category 5 tropical cyclone (Fantala) in April 2016. There is a limited understanding of the influence of mesoscale eddies and cyclones on pelagic biological compartments in the WIO and Mozambique Channel, essentially focusing on phytoplankton (Barlow et al., 2014; Ternon et al., 2014). Nevertheless, cyclones can cause a dramatic change in the ecological functioning of tropical coastal lagoons in Australia (McKinnon et al., 2003) and in Bengal Bay (Srichandan et al., 2015), with drastic effects on phytoplankton assemblages, caused by modifications in turbidity and nutrient regime.

In 2012, the Glorieuses lagoon was classified as a Marine Protected Area, protecting 10% of the fish-species richness of Iles Eparses in the WIO, using international measures such as fishing bans within a 12 nautical miles-limit, so as to minimize the human impact of fisheries on the archipelago (Quetel et al., 2016). Indeed, the biodiversity of these ecosystems has been particularly threatened by overfishing in local fisheries from both Mayotte and Madagascar over the past years (Chabanet et al.,

2015; Quetel et al., 2016). Therefore, the TAAF decided to set up monitoring and management tools to efficiently protect the biodiversity of the Glorieuses islands.

In this context, the "SIREME" research program (Suivi et inventaire des récifs coralliens de Mayotte et des Iles Eparses) aimed to assess the quality of coral reef ecosystems by studying reef fish, coral and plankton biodiversity, in the knowledge that a comprehensive evaluation of microbial communities may be important to provide a qualitative baseline for coral reef communities in pristine environments, compared to other reef ecosystems (Dinsdale et al., 2008).

Our main goal in the SIREME program was to assess ecological indicators (from both biological and chemical parameters) as a future reference point, in order to monitor the state of the coral reefs over time, by comparing results between stations and seasons (beginning and end of the wet period).

In this study, we address objectives focused on microbial communities in Glorieuses marine waters through three ecological questions: (i) What biological groups (bacterioplankton, phytoplankton, protozooplankton) could be used as potential variables to evaluate the ecological change of marine waters? (ii) Are there seasonal differences in microbial planktonic communities that could be explained by the variations in environmental conditions (trophic status)? (iii) Could the tropical cyclone have contributed, after its passage in April 2016 in the regional zone, to the ecological context in May 2016?

To answer these questions, the abundance of microbial planktonic communities (heteroprokaryotes, phytoplankton, protozooplankton) and the bacterio- and microphytoplankton diversity (metabarcoding and microscopy approaches) in five marine sites around Glorieuses islands were determined at two periods (November 2015 and May 2016). So as to gain insight into the ecological status of the ecosystem, pigment biomarkers by HPLC were determined to follow the dynamics of functional and taxonomic phytoplankton groups.

2. Material and Methods

2.1. Study site and samplings

The Iles Eparses are small coral reef islands, located in the Western Indian Ocean (WIO) close to Madagascar, and became the 5th district of the French Southern and Antarctic Lands (TAAF) in February 2007. The Glorieuses archipelago (11°33'S to 12°21'S and 46°26'E to 47°17'E) consists in two islands, Grande Glorieuse and Île du Lys, forming a coral reef and lagoon (Figure 1). Grande Glorieuses is roughly circular and measures approximately 3 km across. Five locations were sampled during two periods (November 19 to 21, 2015 and May 23 to June 2, 2016) (Table 1; Figure 1). The choice of the sampling sites followed the Global Coral Reef Network (GCRMN) guidelines (Conand et al., 1998): stations were representative of coral reef geomorphology and habitats (fore-reef, terraces, lagoon, pinnacles, etc.): GLO1, GLO2 and GLO6 are located on external slope, GLO5 on a

high horizontal underwater floor and GLO7 on the internal slope of the lagoon. Sampling sites were associated with the highest possible coral vitality according to the general state of the reef studied; they had low exposure to confounding factors such as tides and heavy swells (Chabanet et al., 2015). Maximal depths of stations varied between 3 and 14 m (Table 1). Water samples were taken at a depth of 2 m using a Niskin bottle, transferred immediately to acid-washed polyethylene bottles, and kept in the dark at *in situ* temperatures until being processed in the laboratory within 2 h.

2.2. Physical-chemical variables

At each sampling site, a CTD profiler (YSI 600 XLM) was deployed to record temperature, depth, pH, and dissolved oxygen concentrations. Dissolved organic carbon (DOC) analyses were performed on 30 ml sub-samples collected in pre-combusted (450 °C overnight) glass vials, preserved with 35 µl of 85% phosphoric acid. Samples were stored in the dark until analysis, using a Shimadzu TOC VCPH analyzer (Rochelle Newall et al., 2008). Samples for measuring dissolved nutrients (NH_4-N, NO_3-N, NO_2-N, PO_4-P) were filtered onto Whatman GF/F fiberglass filters, stored at - 20°C and analyzed as described by Strickland and Parsons (1968).

2.3. Biological variables

For archaeal and bacterial abundance (heterotrophic prokaryotes: HPROK), samples were fixed with prefiltered (0.2 µm) buffered formaldehyde (2% final concentration), stored in liquid nitrogen (-196°C) and analyzed upon return to Montpellier University. HPROK cells were enumerated by flow cytometry, according to the protocol described by Marie et al. (1997), slightly modified with the use of a higher fluochrome concentration (Bouvy et al., 2016). One milliliter of fixed sub-samples was incubated with SYBR Green I (Molecular Probes, Eugene, OR, USA) at a final concentration of 1/375, for 15 min at 4°C in the dark. For each subsample, three replicate counts were performed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an air-cooled argon laser (488 nm, 15 mW). Stained bacterial cells, excited at 488 nm, were enumerated according to their right angle light scatter (RALS), and the green fluorescence (FL1) was measured using a 530/30 nm filter. These cell parameters were recorded on a four-decade logarithmic scale mapped onto 1024 channels. Fluorescent beads (1-2 µm, Polysciences, Warrington, PA, USA) were systematically added to each sample. True count beads (Becton Dickinson, San Jose, CA, USA) were added to determine the volume analyzed. Standardized RALS and FL1 values (cell RALS and FL1 divided by 1 µm beads, RALS and FL1 respectively) were used to estimate the relative size and the nucleic acid content of bacterial cells respectively (Troussellier et al., 1999). List-mode files were analyzed using BD Cellquest Pro software (ver.5.2.1). HPROK cells with High Nucleic Acid (HNA) content were identified and considered as active cells in the HPROK community (Gasol et al., 1999).

Pico and nano-phytoplankton samples were fixed with prefiltered (0.2µm) formaldehyde (2% final concentration), and counted using a FACSAria Flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with a HeNe air-cooled laser (633 nm, 20 mW). Picocyanobacteria cells (CYAN) represented by *Prochlorococcus* sp. (PRO) and *Synechococcus* sp (SYN), as well as autotrophic picoeukaryote (PICO) cells, were excited at 633 nm and enumerated depending on their FALS and RALS properties, and by their orange fluorescence (576/26 nm) and red fluorescence (> 650 nm) from phycoerythrin and chlorophyll pigments, respectively. Fluorescent beads (1-2 µm for picoplankton cells and 2-6 to 10-20 µm diameter for nanoplankton cells) were systematically added to each sample. True count beads (Becton Dickinson, San Jose, CA, USA) were added to determine the volume analyzed. List-mode files were analyzed using BD FACSDiva software. In order to enumerate heterotrophic nanoflagellates (HNF; cell size comprised between 2 and 20 µm), water samples were fixed with paraformaldehyde (4% final concentration) and stored at 4 °C in the dark. Twenty-five milliliters of preserved water samples were then stained with DAPI (final concentration of 2.5 µg/mL) for 15 min, filtered onto a black Nuclepore filter (0.8 µm), stored at 4°C, and counted using an epifluorescence microscope (Olympus AX 70 microscope, magnification x 1000) with UV excitation (Bouvy et al., 2016).

For protozooplankton (ciliates) and microphytoplankton (i.e. cells or colonies larger than 20 µm) abundance, water samples (500 ml) were concentrated by gravity filtration onto a Nuclepore filter (5 µm pore-size), and fixed with alkaline lugol iodine (2% final concentration) (Bouvy et al., 2016). The remaining 30 mL were then stored at 4°C in the dark until analysis in the laboratory. Microorganisms were enumerated in a Utermöhl settling chamber (Hydro-Bios combined plate chamber) using a reverse microscope (Zeiss Axiovert, magnification 400) for ciliates, and an inverted microscope (Olympus IX70), equipped with a digital camera (Motic Moticam Pro) for microphytoplankton. The ciliates were identified with and without lorica (aloricates and loricates). Microphytoplankton species abundances were used to calculate three diversity indices: species richness (number of taxa per station), Shannon-Wiever diversity index (Shannon and Wiever, 1963), and Pielou's evenness index (Pielou, 1966).

2.4. Photosynthetic pigments

The identification and quantification of photosynthetic pigments included in phytoplankton (total and below 3 µm fraction) was performed through HPLC (High Performance Liquid Chromatography), using Waters D600 equipment and an ODS C-18 column only with UV-detection (Wright et al., 1991). Pigments were detected by a Waters 2996 photo-diode detector (optic resolution 1.2 nm) from 400 to 700 nm for chlorophylls and carotenoids, and by a 2475 Multi λ fluorescence detector (Leruste et al., 2016). Photosynthetic pigments composition in samples was determined by using Empower

Pro3 (Waters) software. For each sample, 200 to 300 ml, depending on phytoplankton biomass, were filtered onto Whatman GF/F filters (47 mm diameter) and stored in liquid nitrogen (-196°C) until analysis. A chromatogram was extracted at 440 nm, which is considered as being a threshold for obtaining the average of the pigment peak. Each peak was attributed to a pigment, checking the elution order and its retention in time, and comparing its associated spectrum between 400 and 700. nm with a library of pigment spectra. A background noise line, due to the age of the column used or to the HPLC device itself, could be detected on the chromatogram. Therefore, a readjustment was realized on the peak areas in order to minimize the impact of this noise on the results. Pigments were quantified using the slope of the regression line of their corresponding standards and their peak areas to obtain their concentrations (expressed in µg/L). The regression line for each standard was determined through the quantity of 4 aliquots and their peak areas.

Photosynthetic pigments that are dominant in phytoplanktonic cells can be seen as fingerprint markers of algal groups: chlorophyll-a (CHL-A) is a biomarker of the total phytoplankton biomass, peridin (PERI) for Dinophytes, fucoxanthin (FUCO) for fucoxanthin-rich diatoms. Diadinoxanthin (DIAD) is typical to marine planktonic diatoms (Lavaud et al., 2002). Alloxanthin (ALLO) is then used as a biomarker for Cryptophytes, whereas prasinoxanthin (PRAS) is typical to Prasinophytes (Bustillos-Guzman et al., 2004). Violaxanthin (VIOL), neoxanthin (NEO), and chlorophyll b (CHL-B) are found in Chlorophytes, also known as green algae (Roy et al., 2011). Zeaxanthin (ZEAX) is mainly found in cyanobacteria especially in coastal tropical water ecosystems, but can also be found in Chlorophytes and Prasinophytes (Vidussi et al., 2001). Finally, 19'But-fucoxanthin (BUTF) and 19'Hex-fucoxanthin (HEXF) were used to identify Haptophytes (Roy et al., 2011).

2.5. Sequence processing – Bacterial diversity

Water subsamples (100 ml) were filtered through 0.22 µm pore-size polycarbonate filters (Nuclepore, pressure < 10 kPa) and stored at -20°C until nucleic acid extraction. DNA extractions were conducted using the PowerWater DNA isolation Kit (Mo Bio Laboratories), as described by the manufacturer for maximum yield. Amplification of the V4-V5 region of the 16S rRNA genes was performed for bacteria using the 515F (5′-GTGYCAGCMGCCGCGGTA-3′) and the 928R (5′-CCCCGYCAATTCMTTTRAGT-3′) primers (Wang and Qian, 2009). Amplicon sequencing was carried out with Illumina MiSeq technology (2 x 250 pb), in collaboration with the GeT core facility (Toulouse, France). Bacterial 16S rRNA paired-end reads were merged with a maximum of 10% mismatches in the overlap region using FLASh (Magoč and Salzberg, 2011). Denoising procedures consisted in discarding reads with no expected length and the ones containing ambiguous bases (N). After de-replication, the clusterization tool was run with SWARM (Mahé et al., 2014), which uses a local clustering threshold. Chimeras were then removed using VSEARCH (Rognes et al., 2016) and

low abundance sequences were filtered at 0.005% (Bokulich et al., 2013), discarding singletons from the datasets. Taxonomic affiliation was performed with both RDP Classifier (Wang et al., 2007) and Blastn+ (Camacho et al., 2009) against the 128 SILVA database (Quast et al, 2013). Samples were randomly resampled to the lowest number of retrieved bacterial sequences per sample, that is, 9 909 sequences to allow comparisons between samples. This procedure was automated in the FROGS pipeline (Escudie et al., 2018). Raw data is available under Sequence Read Archive (SRA) format within the BioProject PRJNA720390. The variation of OTU microbial sequences was determined by Non-metric Multi-Dimensional Scaling (NMDS). An OTU sequence/station matrix was created for sequence data to estimate station similarity using the Bray Curtis metric. The similarity matrix was then ordinated by NMDS. A SIMPER (similarity percentage) analysis was performed to determine which sequence contributed most to the similarity or dissimilarity between stations.

2.6. Data processing - co-inertia analysis

The differences between sites for all variables were tested using the non-parametric Mann-Whitney U-test. Differences were considered as significant at $p < 0.05$ (Sigma Stat version 3.5). The relationships between environmental parameters and biological variables were studied using multivariate analyses, with data from the 5 sampling stations. All the data were log $(x + 1)$ transformed. Principal component analysis (centered PCA) was performed for each of the two following data sets: an Environmental System based on 10 parameters, and a Biological System based on 12 variables. The results of the two analyses were associated using a co-inertia analysis, which allows two tables with a different number of variables to be compared (Doledec and Chessel, 1994). Two sets of factor scores were obtained for the sampling points: scores of the rows "seen by the environmental parameters", and scores of the rows "seen by the biological variables". The significance of the co-inertia analysis was tested after randomizing the results, using a repeated random permutation of the rows of both tables, and a comparison of these results obtained with a standard PCA. The resulting distribution of 2000 replicated matches of the two arrays gave an estimated significance of $p < 0.001$ for the difference with the original value. All data processing was performed using ADE-4 software (Thioulouse et al., 1997).

3. Results

3.1. Environmental parameters

Physico-chemical conditions were similar for every site during the two surveys with no significant differences ($p > 0.05$) between the wet and dry seasons (November and May) whatever the station (Table 1). The vertical profiles of values obtained by the CTD profiler did not show significant differences ($p > 0.05$) along the profile. The surface temperature ranged from 27.0°C (GLO2 in

November) to 28.6 °C (GLO7 in May) whereas the salinity ranged from 34.95 (GLO1 in May) to 35.19 (GLO2 in May). The dissolved oxygen concentrations ranged from 5.80 mg/L (GLO5 in May) to 7.06 mg/L (GLO2 in May). The pH values varied little with the season, with values comprised between 7.52 and 8.26. Significant differences were found for phosphorus (PO_4) and dissolved organic carbon (DOC) concentrations, with the greatest values reported in November, whereas ammonia and nitrate concentrations were not significantly different between the two periods (U-test, $p < 0.05$; Table 2). Concentrations of chlorophyll-a (CHL-A) were significantly higher in November than in May (U-test, $p = 0.028$), and the difference was more marked with the values based on cell-size inferior to 3 µm (CHL<3; U-test, $p = 0.001$), with the highest concentrations for the GLO2 site. CHL<3 µm contribution to total pigment concentrations was 78% in November and 59% in May.

Accessory pigment concentrations of the total fraction were dominated by four pigments: chlorophyll-b (CHL-B), fucoxanthin (FUCO), diadinoxantin (DIAD) and zeaxanthin (ZEAX), and their concentrations were always higher in November than in May. However, no significant difference in means was observed between the two surveys (Table 2). Accessory pigments diversity was higher in May (11 pigments without CHL-A) than in November (6 pigments without CHL-A), (Figure 2). The GLO5 and GLO7 stations exhibited the lowest concentrations of pigments in May and November, associated with an absence of CHL-B in November (Table 2). Zeaxanthin concentrations were detected in all stations whatever the season, with an important contribution in May (between 30% and 60% of total pigments, except at GLO1; Figure 2). Fucoxanthin concentrations (FUCO) were also detected in all stations, especially at GLO2 and GLO5 in November. It was obvious that GLO-1 (oceanic site) showed higher pigment diversity than GLO7 (coral reef site), whatever the season. The GLO7 station was characterized by the marked contribution of 19'Hex-fucoxanthin (HEXF) in May and November (almost 30% of total accessory pigment). Peridinin (PERI) contribution was only detected at GLO2 in May.

3.2. Biological variables

The abundance of heterotrophic prokaryotes (bacteria and archaea; HPROK) was similar between the two seasons (mean of $7.28 \pm 3.14 \times 10^8$/L in November and $8.78 \pm 1.40 \times 10^8$/L in May; Table 3). The percentage of active cells with high nucleic acid content (% HNA) was not significantly different between November and May (U-test, $p > 0.05$), with a mean of $55.2 \pm 10.2\%$ and $48.9 \pm 5.44\%$, respectively.

Among phytoplankton, the dominant groups in terms of abundance were the picocyanobacteria (CYAN), represented by the *Synechococcus* (SYN) and *Prochlorococcus* (PRO) genus, with no significant difference between the two surveys. SYN was the most represented with a SYN/PRO mean ratio of 7.43 ± 1.12 in November and 6.60 ± 3.13 in May. Significant differences in

nanophytoplankton (NANO) abundance were reported between the two surveys, with the greatest values noted in November (mean of 1.86 ± 0.25 x 10^5/L *versus* 0.09 ± 0.02 x 10^5/L; U-test, p = 0.008). Inversely, picoeukaryote (PICO) abundance was significantly higher in May (mean of 7.23 ± 4.98 x 10^4/L; U-test, p = 0.016), with the highest values observed in GLO2. The last autotrophic group identified as phytoplankton was microphytoplankton (ALG), with a total of 30 different species belonging to 3 algal classes considered as dominant (with a selected occurrence above 2 %) during the two surveys (Table 4). No significant difference in the mean values of microphytoplankton abundance was observed between the surveys (Table 3). Although the number of species per station (RICH) was significantly higher in May (mean of 40.6) than in November (mean of 29.4) (U-test, p = 0.037), no significant difference was observed in terms of diversity through the Shannon-Wiever index (U-test; p = 0.690; Table 3). However, species evenness index (EVEN), referring to how close in numbers each species in an environment is, showed significant difference between the two surveys (U-test, p = 0.036; Table 3) with mean values of 0.818 in November and 0.712 in May. Bacillariophyceae were the most diverse with 66% of total species, followed by Dinophyceae (Table 4). Centric Bacillariophyceae were largely represented by *Coscinodiscus* sp., *Chaetoceros* sp., and *Cocconeis* spp. Most of the abundance was represented by two species (present at each station throughout the surveys) belonging to Bacillariophyceae: *Cylindrotheca closterium* and an unidentified species of *Navicula* sp1, representing > 20% of total microalgal abundance. The genus *Pseudo-Nitzschia* was also present with a density greater than 2%, except at GLO6 in November. Globally, Dinophyceae exhibited higher species richness (number of species) in November than in May, except for one station (GLO5).

The mean abundance of heterotrophic nanoflagellates (HNF) was significantly higher in November than in May (U-test, p = 0.008) (mean of 8.85 ± 3.95 x 10^5/L versus 1.78 ± 2.07 x 10^5/L). The ratio of heterotrophic prokaryotes and heterotrophic nanoflagellates (HPROK/HNF) was thus significantly higher in May (mean ratio of 10753 ± 8974) than in November (mean ratio of 1514 ± 1993) (U-test, p = 0.049), with the highest ratio observed in GLO2 (Table 3). The ciliate community was identified as two groups (aloricates and loricates) and their abundance were very low for all the stations for both surveys (Table 3). As for HNF, aloricate abundance (ALOR) were significantly higher in November (mean of 18.4 ± 3.6 ind/L) than in May (mean of 1.2 ± 2.7 ind/L) (U-test, p = 0.008), and were absent in 4 stations. Thus, the protozooplankton community was very poorly represented in May compared to November.

3.3. Bacterial diversity by sequence processing

A mean value of 327 (std = 49) OTU sequences per station was discriminated using sequence processing. The taxonomic composition of the bacterial assemblages within the surveys was

dominated by three phyla: cyanobacteria, alpha- and gammaproteobacteria, with means of 25%, 26% and 21%, respectively (Figure 3). Bacteroides represented 37.3% of total sequences at GLO2 in May and clearly dominated the total microbial assemblage. This class represented low values in November (mean of 9%). Inversely, GLO2 was characterized by the lowest percentage of cyanobacteria in May (16%). Other microorganisms such as eukaryotes were detected, with the presence of chloroplasts, representing 9% in November and 10% in May of the total sequences on average. The spatial variation of the OTU microbial sequences by station and by survey was determined using Non-Metric Multi-Dimensional Scaling (NMDS), with a stress value of 0.01 indicating a strong ordination (Figure 4). A clear difference was observed with a cluster including only the stations sampled in May 2016, with a similarity of 78%. Interestingly, similarity between GLO5 and GLO6 was high in November 2015 (72%), while the similarity at the survey level was only 60.15%. Dissimilarity between stations sampled in November 2015 and May 2016 was 46.43 %.

3.4. Link between environmental and biological variables: Co-inertia analysis

The two PCAs on environmental and biological variables were performed on the grouped data sets of the two seasons (5 stations; 10 environmental parameters and 12 biological variables). The first two eigenvalues of the co-inertia analysis accounted for 90.9% of the total variability (Figures 5 and 6), therefore the analysis focused on the first 2 axes. The values of the projected variables on the environmental and biological tables on the axes (F1, F2) of the co-inertia analysis (Iner E and Iner B) were close to the values of projected variables on the same axes of the standard (PCA) analysis (Var E and Var B) (Table 5). The co-inertia analysis demonstrated a co-structure between the two data sets. The correlation between the new environmental and biological ordination of the stations, reflecting the degree of association between the scores of the sampling points (stations-periods) on the first two axes of the two systems (Axis F1 Environment/ Axis F1 Biology; Axis F2 Environment/ Axis F2 Biology), demonstrated the high and significant degree of association between Environmental and Biological systems, with R-values of 0.886 and 0.955 (Table 5). Figures 5 and 6 show the plots of the variables and stations, in November 2015 and May 2016, in the first factorial plane for the Environmental and the Biological Systems, respectively.

In the Environmental System (Figure 5), the first axis (F1) clearly shows an opposition in the parameters studied between November 2015 and May 2016, with higher concentrations of all the parameters analyzed in 2015, such as dissolved organic matter and chlorophyll-a concentrations (DOC, CHL-A, CHL<3), with significant correlations between them (Table 6). The location of stations sampled in 2015 was clearly differentiated by the second axis (F2), with a marked opposition between GLO2 and GLO7. The highest concentrations of zeaxanthin (ZEAX) were significantly correlated with the majority of chlorophyll variables (CHL-A and CHL<3) (Table 6).

In the Biological System (Figure 6), the F1 axis also showed the clear opposition between the stations sampled in 2015 *versus* in 2016, with grouped sites in 2016 characterized by higher abundance of heterotrophic prokaryotes (HPROK, HNA, HPROK/HNF) and autotrophic picoeukaryotes (PICO). A significant positive correlation was observed between HPROK/HNF and PICO ($r = 0.635$, $p < 0.05$; Table 7). Inversely, picocyanobacteria variables (CYAN, SYN/PRO), nanophytoplankton (NANO), heterotrophic nanoflagellates (HNF) and ciliates (LOR, ALOR) exhibited higher abundance in 2015. The nanoplanktonic variables (HNF, ALOR, LOR, NANO) were significantly correlated to each other (Table 7). The F2 axis revealed large discrepancies between the stations sampled in 2015 (Figure 6), with a marked opposition between GLO2 and GLO7, as reported in the Environmental System (Figure 5). It was clear that stations sampled in 2016 were more strongly characterized by a dominance of picoplankton compared to the situation in 2015, with a dominance of nanoplankton. Otherwise, abundance of microphytoplankton (ALG) and Shannon index (SHAN) revealed no significant difference between the two situations with an opposite contribution of these two variables to Axis 1 (ALG: - 0.56; SHAN: + 0.70).

The relationship between the normalized coordinates of the stations on the first axis of the two systems ("Environmental" and "Biological"), reflecting the degree of association between Biology and Environment, mostly in terms of time variation (ie opposition between 2015 and 2016), was highly significant ($r^2 = 0.79$). Figure 7 shows the plots of the stations with most of them sampled in 2015 situated close to the equality line, whereas the position of some stations sampled in 2016 are far from the equality line. The same relationships between the coordinates from the second axis of the two systems, mostly reflecting the space variability, was also highly significant ($r^2=0.91$) but much more explained by the plots of 2015 ($r^2=0.94$) than by those of 2016 ($r^2=0.39$).

4. Discussion

4.1. Context

Coral reef environments are generally recognized as being among the most threatened of the fragile marine ecosystems (Mellin et al., 2008), and ecological changes take place slowly, driven by anthropogenic pressure and climate change (Costanza et al., 1997). However, it is extremely difficult to distinguish the effects of climate change from other forcing forces. As a place that is remote from human activities, the Iles Eparses are considered as "pristine" ecosystems with respect to anthropogenic pressures (Bouvy et al., 2016), allowing us to establish ecological baselines for coral reef ecosystems. Previous regional studies conducted in the Iles Eparses provided preliminary insights into the spatial distribution of the plankton community (Bouvy et al., 2016), and the trophic relationships between communities (Dupuy et al., 2016). So far as we are aware, studies of microbial communities conducted in these islands are few (Riaux-Gobin et al., 2011; Bouvy et al., 2016; Dupuy

et al., 2016; Bouvy et al., 2021), and in a context of global change, it is necessary to assess water quality and their changes through potential planktonic components linked to these fragile coral-reef environments.

Our study reveals a high stability of the water column, based on non-significant differences in parameters between surface and bottom samples confirming the data obtained in 2011 (Bouvy et al., 2016) and the qualification of these islands as a relatively stable environment by Quetel et al. (2016). Environmental conditions were characterized by very low nutrient levels, consistent with previous observations (Bouvy et al., 2016; 2021). Chlorophyll-a concentrations (CHL-A) were always under 1µg/L, revealing the classification of Glorieuses waters as being ultra-oligotrophic. This status was confirmed by very low levels of dissolved nutrients (especially orthophosphates, nitrate, dissolved organic carbon) similar to data acquired in 2011 (Bouvy et al., 2016).

4.2. Biological components of the water column

Regarding biological variables, heterotrophic prokaryotes (bacteria and archaea; HPROK) were the major component of plankton communities both in November 2015 and May 2016, with abundance values comparable to those occurring in coral-reef systems (Seymour et al., 2005; Dinsdale et al., 2008; Patten et al., 2011; Bouvy et al., 2012). Picocyanobacteria (CYAN; particularly *Prochloroccoccus* and *Synechococcus*) dominated the phytoplankton community, due to their efficiency in integrating nutrients available in their lowest concentrations (Karl et al., 2001). This dominance also confirmed the ultraoligotrophic status of Glorieuses islands, with a predominance of *Synechococcus* characterizing the coastal coral reefs in Iles Eparses (Bouvy et al., 2016; 2021). SYN/PRO ratios were always between 12.2 and 4.8, confirming that *Prochlorococcus* can be considered as being an oceanic marker in nutrient-low waters (Charpy, 1996). Indeed, *Synechococcus* was found numerically dominant in coastal waters in the Mozambique Channel, followed by *Prochlorococcus* and then picoeukaryotes in lower abundance (Zubkov and Quartly, 2003). Bidigare et al. (1992) reported that accessory pigments accounted for 60% of the light absorbed in the surface, and 90% at the base of euphotic zones (low-light depth) in the Sargasso Sea. Zeaxanthin associated with cyanobacteria (*Prochloroccoccus* and *Synechococcus;* Vidussi et al., 2001) represented the major pigments in both surveys; other specific pigments of green algae (CHL-B, Violaxanthine, Neoxanthine), also detected, confirmed the presence of Chlorophyceae. Haptophytes presence was detected by 19'Hex-Fucoxanthin and 19'But-Fucoxanthin (Paerl et al., 2003), confirmed by microscopic observation with the identification of the coccolithophoride *Emiliania huxleyi*, known as a paleoclimatic display. The life cycle of *E. huxleyi* is complex, involving several different phases such as haploid and diploid stages with many cell sizes (Laguna et al., 2001). The smallest forms of the flagellate can be detected with autotrophic picoeukaryotes in the Deep Chlorophyll Maximum

(DCM) in the Mozambique Channel, also contributing to surface populations (Barlow et al., 2014). Adaptive mechanisms were required by these communities in low light environments with low absorption efficiency by chlorophyll-a, corresponding to environmental conditions in the DCM (at 40-110 m depth) (Barlow et al., 2014). The elevated proportion of photosynthetic carotenoids (PSC) would have enabled the picoeukaryotes to adapt to a range of irradiance conditions in the euphotic zone like the Haptophytes (Bricaud et al., 2004; Bouman et al., 2011), and it is obvious that picoeukaryotes (PICO) had an advantage over picocyanobacteria (CYAN) in more turbulent environment. Thus, the large significant difference in picoeukaryote abundance between November and May ($p = 0.016$) can be explained by the drastic environmental modifications linked to a climatic event (such a cyclone, see below), with high turbulence activities inducing greater turbidity and lower light availability in May (wet season).

Microphytoplankton presented a very high diversity of taxa observed as confirmed by Shannon index value and species richness i.e. more than 150 taxa identified for both surveys at the 5 stations sampled. Their abundance exhibited no significant difference between May and November, whereas changes in the community composition were evidenced. Pielou's evenness index (EVEN) showed significant differences between the two periods also suggesting that the station grouping was due to the presence or absence of certain key factors rather differences in abundances of several species. The survey in May 2016 was mainly characterized by pennate Bacillariophyceae (diatoms) taxa, whereas the November 2015 survey revealed more Dinophyceae (dinoflagellates) taxa. Among the Bacillariophyceae, the occurrence of *Cylindrotheca closterium*, *Navicula* spp (2-5 x 8-15 µm) and *Pseudo-nitzschia* spp were dominant at all stations. It is important to note that *Cylindrotheca closterium* is considered to be responsible for harmful algal blooms (HAB) in many regions (Najdek et al., 2005); however, HAB events have not been reported in Glorieuses marine waters, certainly explained due to the absence of nutrient inputs from the islands. The genus *Pseudo-nitzschia* contains several harmful species that are well recognized as potential producers of the domoic acid toxin (Kudela et al., 2010). Pigment features confirmed this diatom dominance with mean ratios of fucoxanthin:Chl-A of 0.283 and 0.263, respectively in 2015 and 2016. Diadinoxanthin pigment (DIAD) is a protecting pigment in diatoms, minimizing photoinhibitory damage due to high-light intensity (Levaux et al., 2002), and no difference of mean concentration was noted between the two surveys. Lower fucoxanthin:Chl-A ratios (0.18) were reported for Prymnesiophyceae than for diatoms (0.31) (Descy et al., 2009). The low availability of nitrate did not favor the growth of the Prymnesiophyceae, and this smaller phytoplankton is generally well adapted to low light and dominates in turbulent waters (Sarma et al., 2020). The non-identified dinoflagellate called « Dinophyceae sp» (Table 4) was detected at a same level in all stations, with occurrence percentages always under 7%. Other dinoflagellates species characterized some stations and included

heterotrophic species such as *Protoperidinium bipes* and *Protoperidinium quiquecorne*, as well as the mixotrophic dinoflagellate *Gymnodinium* spp. However, peridinin, a pigment marker for autotrophic dinoflagellates, was only significantly detected in GLO2 in May. Zeaxanthin (ZEAX) is considered as being a protecting pigment of cyanobacteria (Brunet et al., 2011), but Chlorophytes can also contain zeaxanthin along with Chl-B (Jeffery and Vesk, 1997). Indeed, Chl-B is an indicator pigment for Chlorophytes and was observed in higher concentrations in November 2015 (0.588 µg/L at GLO6), whereas it was totally absent in GLO7 (lagoon site).

The high significant correlations observed between CHL-A, CHL<3, CHL-B and ZEAX suggested a dominance of picocyanobacteria (*Prochlorococcus* and *Synechococcus*) in Glorieuses, especially in 2015, as also demonstrated by the co-inertia analysis (Fig. 5). These results confirmed that the small cells of picocyanobacteria (CYAN) are more efficient in synthesizing high levels of zeaxanthin (ZEAX) compared to diadinoxanthin (DIAD) synthesis in eukaryotes such as Bacillariophyceae as also demonstrated by Barlow et al. (2017).

Overall, very low concentrations of total ciliates were observed in Glorieuses, with aloricate forms (ALOR) being more abundant than loricate ones (LOR), supporting the data reported by Bouvy et al. (2016). In May 2016, a very low presence of ciliates and heterotrophic nanoflagellates was detected, presumably affecting the flow of organic matter in the microbial food web. This hypothesis might explain the high susceptibility to stress and disturbance of these fragile marine ecosystems (Mellin et al., 2008). The microbial food web can be characterized by the trophic relationships between bacteria and their predators (Berglund et al., 2007), and it is now recognized that protozoa, and especially heterotrophic nanoflagellates (HNF), are the most important grazers on bacteria in most environments (Sanders et al., 2000). Experimental approaches conducted in Iles Eparses showed a wide range of bacterial growth rates and grazing rates by HNF, with the lowest values observed in Glorieuses and Tromelin islands (Bouvy et al., 2016). Nevertheless, low concentrations of HNF were recorded in Glorieuses, confirming the data reported for the Mayotte reef (Houlbréque et al., 2006), the great reef of Toliara, Madagascar (Bouvy et al., 2015), and the Iles Eparses systems (Bouvy et al., 2016). Moreover, the ratio between HPROK and HNF can provide insight into trophic food webs, with a low ratio suggesting a high grazing pressure exerted by HNF, implying that organic biomass passes through active microbial food webs (Bouvy et al., 2010). That was likely the case in November with a significantly lower HPROK/HNF ratio than in May (means of 1514 and 10753, respectively, t-test: 0.049), indicating a drastic modification of the pelagic trophic food web towards less HNF predation pressure. It is obvious that the protist community (ciliates and HNF) was very sparsely represented in May, highlighting the cascading effect generated by zooplankton predators (Bouvy et al., 2006), or linked to an exceptional climatic event such as a cyclone.

The molecular approach (16S rRNA) revealed dominant phyla, with cyanobacteria and proteobacteria (alpha and gamma), without significant differences between the stations. The unique study considering molecular studies on bacterial diversity on the scale of Iles Eparses showed a clear difference for archaea, bacteria and picoeukaryotes (from DGGE analysis) between the islands studied, with a great homogeneity between stations within each island (Bouvy et al., 2016). Large ubiquitous distribution seems uncommon among marine bacterioplankton, with a dominance of alpha-protobacteria and picocyanobacteria in the tropical open ocean (Chisholm et al., 1988; Morris et al., 2002). In the tropical lagoon of Ahe atoll (Tuamotu Archipelago), the OTUs belonged to Cyanobacteria and to heterotrophic groups with proteobacteria (alpha and gamma) and *Flavobacteria* (Michotey et al., 2012). The microbial distribution defined by the integrative approach, using co-inertia analysis, is further corroborated by the NMDS ordination based on bacterial OTU sequences (Fig. 4), where the OTU distribution was found to be significantly different in May 2016 *versus* November 2015. It is obvious that the environmental and biological context encountered in May 2016 shows a great homogeneity in the stations, compared to the context in November 2015, with greater diversity of habitats. Co-inertia analysis confirmed this spatial pattern of microbial components linked to the habitat, with a clear opposition between GLO2 (located on oceanic site) and GLO7 (located on a coral reef lagoon), especially in November 2015. The absence of CHL-B, the very low concentration of FUCO (diatoms) and lower concentrations of PICO (*Prochlorocccus* and *Synechococcus*) confirmed the location of GLO7 in an enclosed lagoon compared to GLO2 on the outer slopes of the lagoon. In term of habitat, one of the main differences between the two stations is the absence of soft coral in GLO7 (Chabanet et al., 2016). These authors reported great differences between stations in fish biomass and diversity in the Glorieuses, with the highest fish abundance in GLO2 due to the presence of planktivores, consuming the planktonic communities present in the water column.

4.3. Characterization of the Glorieuses marine waters

Overall, the microbial distribution defined by the integrative approach, using co-inertia analysis, demonstrated that the differences in environmental conditions explained the relative abundance of biological components in Glorieuses island, with two distinct situations (May and November). Figure 7 stressed the relationship between the stations and the two systems (Environmental and Biological), suggesting (i) a clear opposition between the two situations (May and November) and (ii) a distinct spatial zonation of stations in November 2015, with the biological components strongly related to those stemming from environmental variables. On the other hand, the “Biological system” seemed more independent from the “Environmental system” in May 2016, corroborating the data reported using the metabarcoding approach (Figure 4). Thus, the analysis revealed a reef system characterized

by a richer nutrient context and higher algal pigment concentrations, in November 2015, associated with a higher abundance of protozoa and nanophytoplankton; whereas in May 2016, the reef system was dominated by a high abundance of active heterotrophic prokaryotes (H-PROK) and picoeukaryotes (PICO), in a poorer nutritive context. The significant correlation between H-PROK and HNA cell abundance ($r = 0.839$; $p<0.001$; Table 7) confirmed the dominant contribution of HNA cells in the remineralization of organic matter, as already demonstrated by Servais et al. (2003) and Bouvy et al. (2010). Thus, the complexity of reef systems in Glorieuses results in a fluctuating availability of growth limiting resources and light, with a large mineralization of organic matter in May 2016 *versus* an active microbial food web with the presence of bacterial predators in November 2015. Our results illustrate the relative importance of top-down dynamics, based on the observed relationships between bacterial and HNF abundance (Gasol, 1994; Sanders et al., 2000), and demonstrate a large modification of the environmental context, with a very low active microbial food web in May 2016 compared to November 2015.

4.4 Potential influence of Fantala cyclone on plankton structure

It is important to note that reef islands are widely acknowledged to be highly vulnerable to extreme climate events, such as tropical and non-tropical cyclones (Hoeke et al., 2013), the frequency and intensity of which might be affected by climate change. These processes can displace biological communities such as phytoplankton along isopycnal gradients (Liccardo et al., 2013).

The Mozambique Channel and the Western Indian Ocean (WIO) are subjected to periodical extreme weather events such as cyclones, with serious impact on shoreline and sediment transport, as described by Duvat et al. (2017) on Farquhar Atoll (Seychelles), after the passage of a category 5 tropical cyclone (Fantala) in April 2016. To the best of our knowledge, there is no available study in literature concerning the impact of cyclones on plankton structure in an isolated coral-reef system, whereas numerous studies pertain to the influence of cyclones on coastal lagoons positioned at the interface between rivers and the sea (McKinnon et al., 2003; Tsuchiya et al., 2013; Srichandan et al., 2015). Very few studies considered the influence of mesoscale eddies on pelagic biological compartments in the WIO and Mozambique Channel, and generally concluded to a drastic effect on turbidity and nutrient regime, which play a role in phytoplankton assemblage (Barlow et al., 2014; Ternon et al., 2014).

As already mentioned above, the drastic ecological modification reported in May can probably be linked to the passage of Fantala cyclone in April 2016. However, the absence of relationships between the environmental and biological contexts encountered in May 2016 (see Figure 7) can be explained by the impact of the Fantala cyclone, linked to the high turbulence of lagoon and oceanic waters, disturbing the habitats and modifying the trophic interactions inside the trophic food web. No increase

of nutrient concentration was observed after the cyclone, as generally mentioned after cyclonic eddies in the Mozambique Channel (Tew Kai and Marsac, 2009) and in the South West Indian Ocean (Noyon et al., 2019). Due to the absence of tide gauges on Glorieuses and on nearby islands, both the wave height and the storm surge remain unknown. Nevertheless, Duvat et al. (2017) clearly described the track direction and increasing intensity of the Fantala cyclone when approaching Farquhar atoll (Seychelles islands), with the maximum wind speed reaching 352 km/h and rain falling close to 300 mm/h on the southwestern side of the cyclone eye (Météo-France Réunion, 2016). Duvat et al. (2017) concluded on a high contrast in the cyclone's impact, both spatially and in terms of ecological-morphological impacts, and reported continued sediment transfer to islands four months after the cyclone. This study corroborates previous conclusions with regard to disturbances (eddies, cyclones, seamounts), whereby the impacts of these turbulences can contribute to the changes and disequilibrium we observed in WIO microbial community (Rocke et al., 2020).

4.5. Potential variables reflecting the change of ecological status in Glorieuses islands

Plankton communities are often used to alert scientists and stakeholders about ecological changes in aquatic systems, due to their rapid response to multiple environmental stressors (Lemley et al., 2016). Microbial communities provide sensitive, meaningful and quantifiable indications of ecological changes (Hayes et al., 2015). With the weakness of our database, it is not reasonable to apply standard indexes and bioindicators developed by many authors (e.g. Teixeira et al., 2016; Cozzoli and Basset, 2017; Lampert and Hernandez-Farinas, 2018; Varkitzi et al. 2018). Moreover, the Glorieuses marine waters are not submitted to anthropic pressures, eutrophication, chemical pollution (Quetel et al., 2016); thus, water quality assessment using regional reference values of indicator variables is not appropriate. Index values are generally likely to be associated with a relative contribution of local and regional forcing functions (Herrera-Silvera and Morales-Ojeda, 2009; Varkitzi et al., 2018). Finally, in agreement with Goffart (2020), the relationships between phytoplanktonic variables and proxies (diversity indices) are difficult to establish mainly due to (i) the ultra-oligotrophic nature of marine waters in the region and (ii) the low amplitude of seasonal variations in nutrient concentrations.

In this context, we propose the relevance of three potential variables to assess the impact of environmental changes on ecosystem functioning in the Glorieuses marine waters :

- Small flagellates such as picoeukaryotes (PICO) exhibit a large variability between the two surveys, and dominate the microbial community with heterotrophic prokaryotes in May, knowing that PICO have moderate efficiency in light absorption, and have an advantage over picocyanobacteria (CYAN) in more turbulent environments (Bouman et al., 2011). Although picoplankton studies are sparse in WIO, picoeukaryote communities

can dominate the carbon biomass, and characterize the microbial structure, as demonstrated in the anticyclonic region of the Madagascar Ridge (Rocke et al., 2020).

- Pigment analyses could be good candidates as descriptors of community composition (Soane et al., 2011) and be incorporated into environmental monitoring for the purposes of ecological status (Lampert and Hernandez-Farinas, 2018; Goffart, 2020). Cyanobacterial pigment such as zeaxanthin (ZEAX), considered as the biomarker for *Synechococcus* and *Prochlorococcus* which are the dominant primary producers, seems to be a good candidate to assess environmental change, with a high abundance in May compared to November. The presence of this pigment may be attributed to high levels of light and stable conditions (Barlow et al., 2017; Rii et al., 2018).
- The ratio of heterotrophic prokaryotes to heterotrophic nanoflagellates (HPROK/HNF) can also be applied to detect changes in microbial structure of the food web. This ratio is frequently used to characterize environmental conditions and provides considerable insight into the dominant trophic webs (Gasol, 1994; Bouvy et al., 2010). The drastic modification of the microbial food web observed in May can be explained by the very low ratio, implying a low active microbial food web compared to November.

Of course, these biological variables, based upon floristic composition (species or pigment diversity), can be influenced by spatial variability among sites (rainfall, currents, eddies…), and may not be due to different ecological states, but rather to geographic and natural factors, according to Degerlund and Eilertsen (2010). Nevertheless, these three variables can potentially be considered to characterize the ecological status in the Glorieuses islands, knowing that an exceptional event (a category 5 tropical cyclone) occurred between May and November.

5. Conclusions

This study is the first report to describe the physico-chemical and microbial components of the Glorieuses islands, considered as pristine ecosystems similarly to the other Iles Eparses in WIO. The study will provide useful insights into microbial structure (diversity, distribution, and abundance), as well as their relationships with the environmental conditions. The implementation of microbial variables for monitoring the ecological status of Glorieuses islands can be highly beneficial for studying the effects of severe climatic perturbations such as cyclones, as well as helping face eventual anthropogenic events in these pristine systems. However, given the limited information from two surveys, with an exceptional event between them, long-term studies are required to better evaluate

the usefulness of the proposed biological variables for assessing climatic perturbations, without forgetting the eventual anthropogenic impacts on marine environments.

Conflicts of interest

All authors submit that they have no actual or potential conflict of interest that could inappropriately influence, or be perceived to influence, this study.

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Figure 1 : Location of the Glorieuses Islands (Grande Glorieuse and Île du Lys) in the Mozambique Channel, Western Indian Ocean. The five stations are indicated by a blue star.

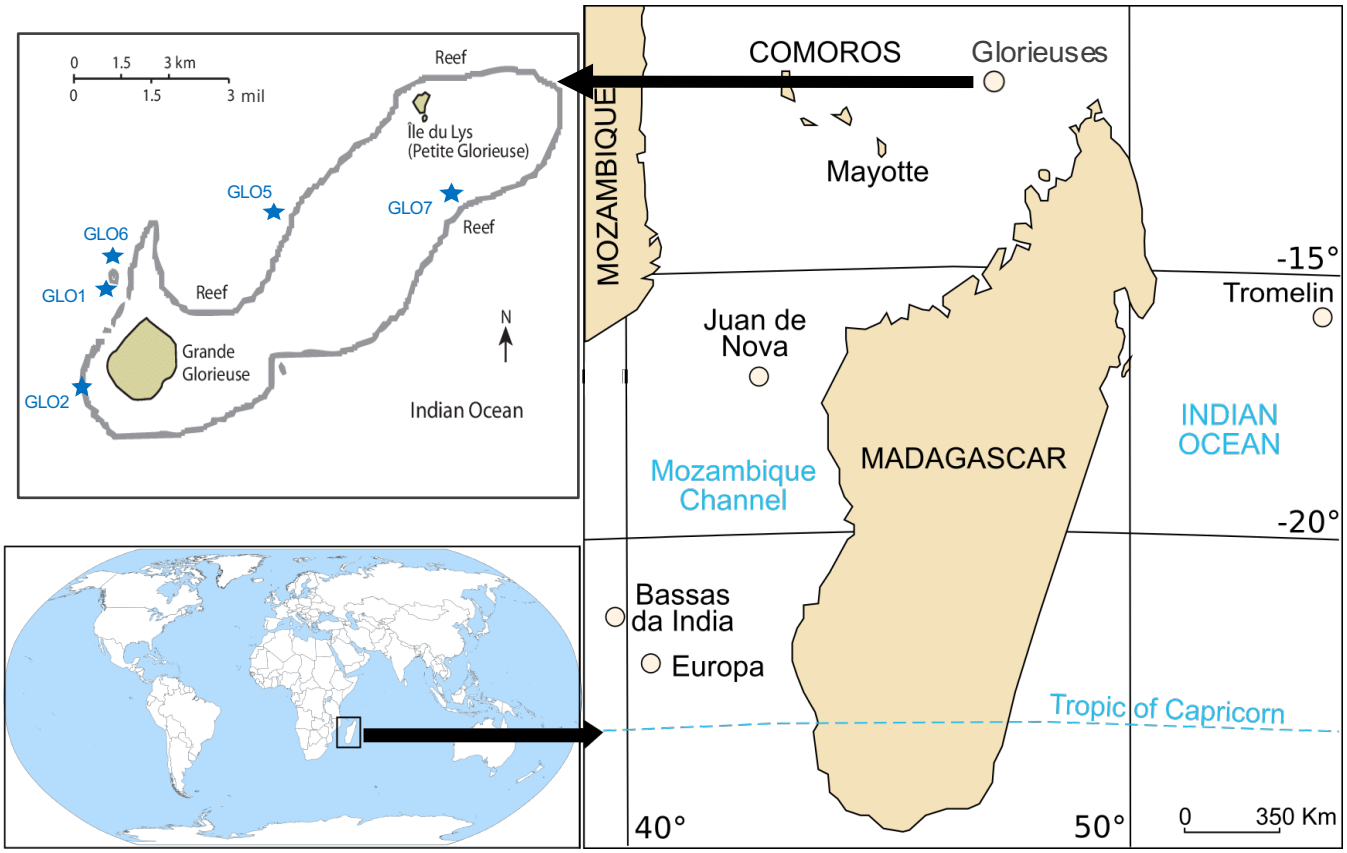

Figure 2 : Proportions of chlorophyll a and accessory pigments in the five stations in November 2015 (A and B) and in May 2016 (C and D). Corresponding relative contributions (%) of different accessory pigments without chlorophyll a date in November 2015 (B) and May 2016 (D).

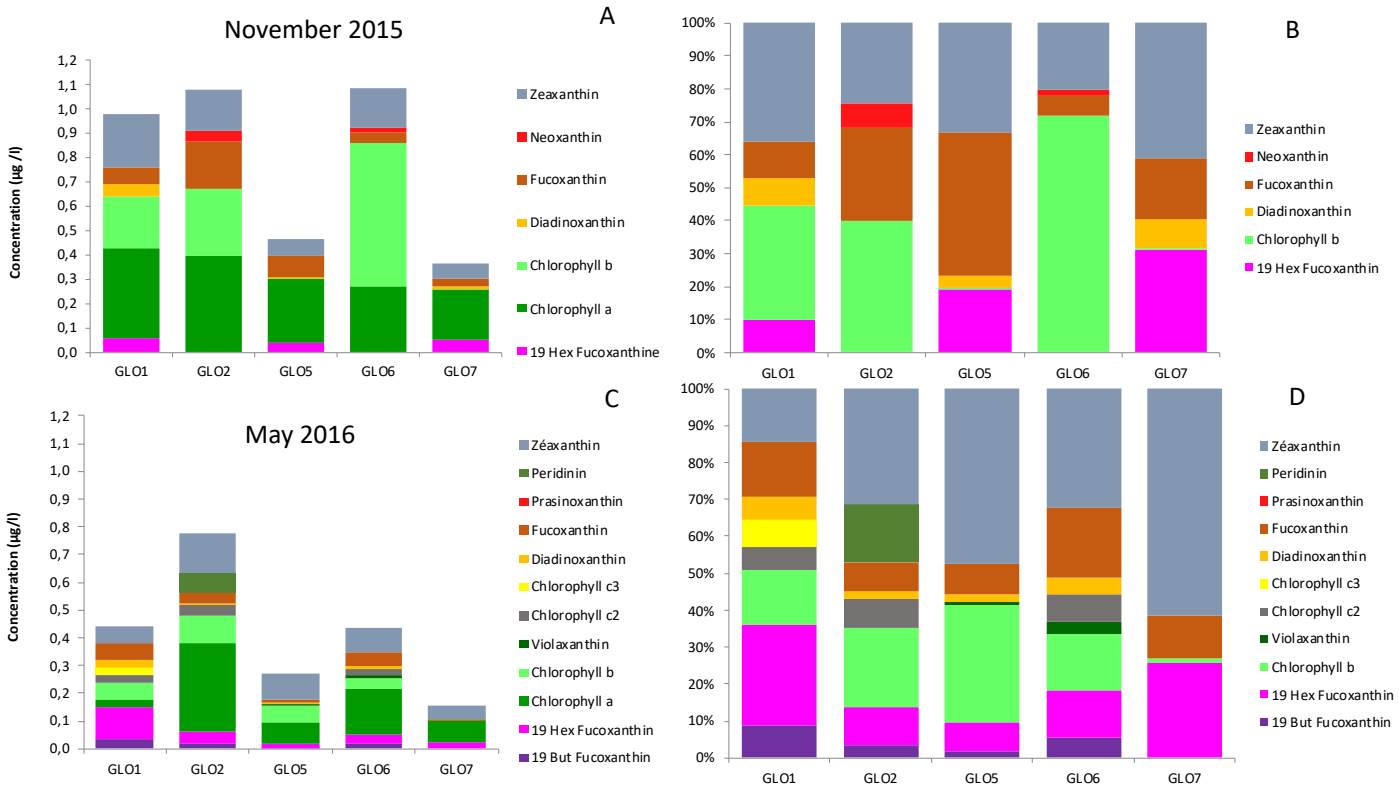

Figure 3 : Relative abundance (% of sequences) of microbial phyla in the five stations during the two periods (November 2015 and May 2016). No data for GLO1 in May 2016.

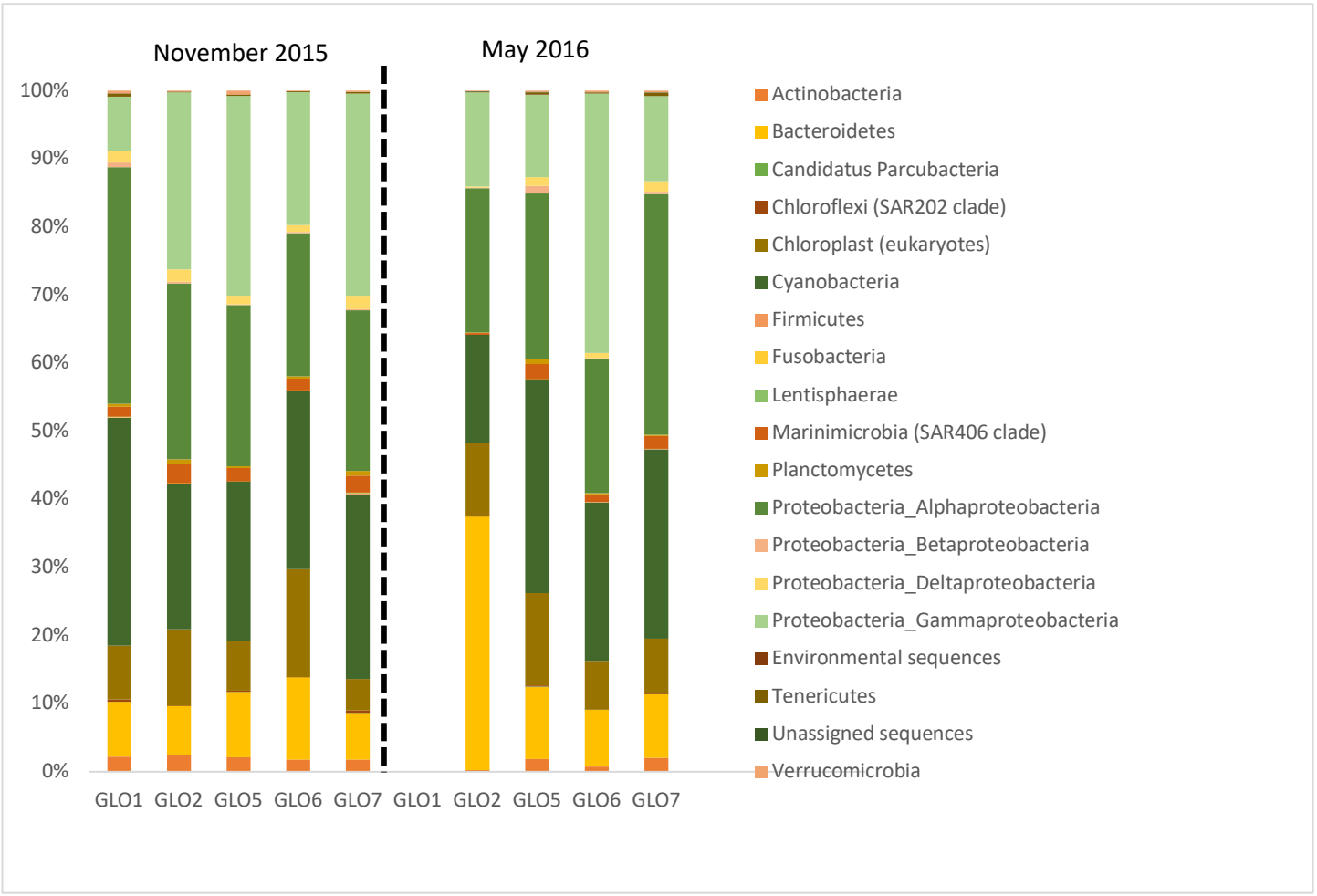

Figure 4 : NMDS ordination performed on Bray Curtis dissimilarity matrices based on the OTU sequences (Square root transformed data) (five stations; November 2015 and May 2016). The number refers to the label of the station and the year (November 2015 and May 2016).

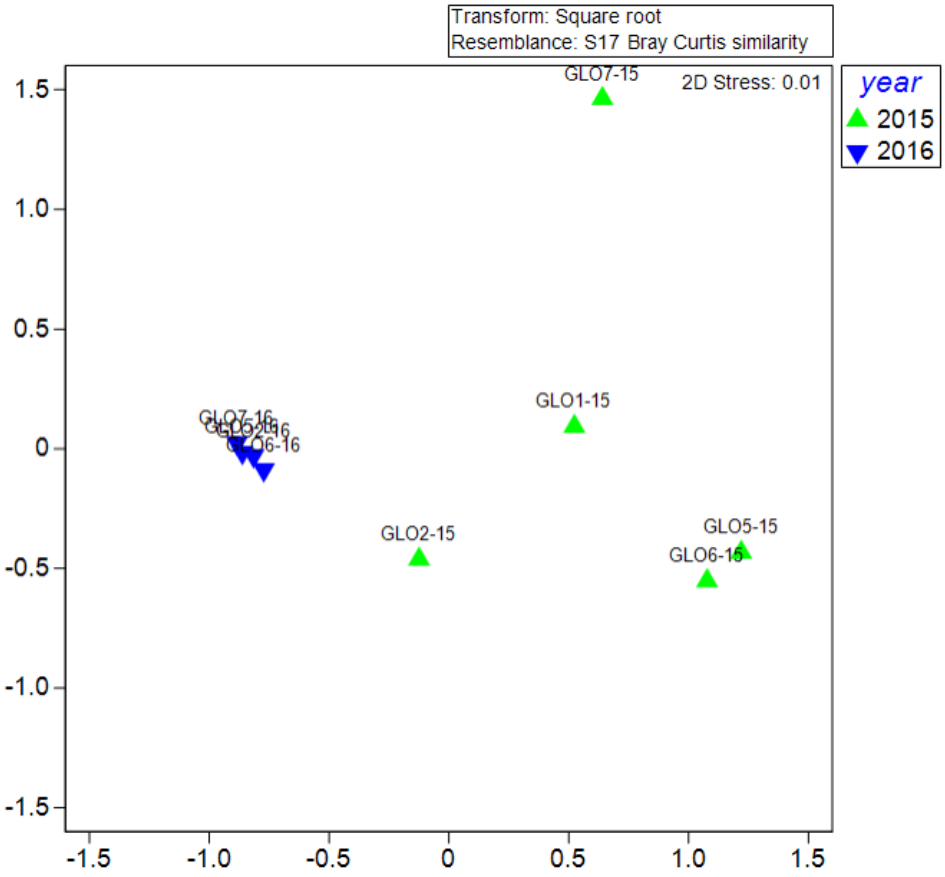

Figure 5 : Co-inertia analysis with the position of the environmental variables on the F1 X F2 plane. Position of the sites (red colour) was linked to biological variables co-inertia weights. The number refers to the label of the station and the year (November 2015 and May 2016). See abbreviations in Table 1 for the sites. Abbreviations: PO_4 : dissolved phosphorus ; NO_3 : nitrate ; NH_4 : ammonia ; DOC : dissolved organic carbon; CHL-A: chlorophyll-a; CHL<3: chlorophyll-a of cell size < 3µm; CHL-B: chlorophyll-b; FUCO: fucoxanthin; DIAD: diadinoxanthin; ZEAX: zeaxanthin.

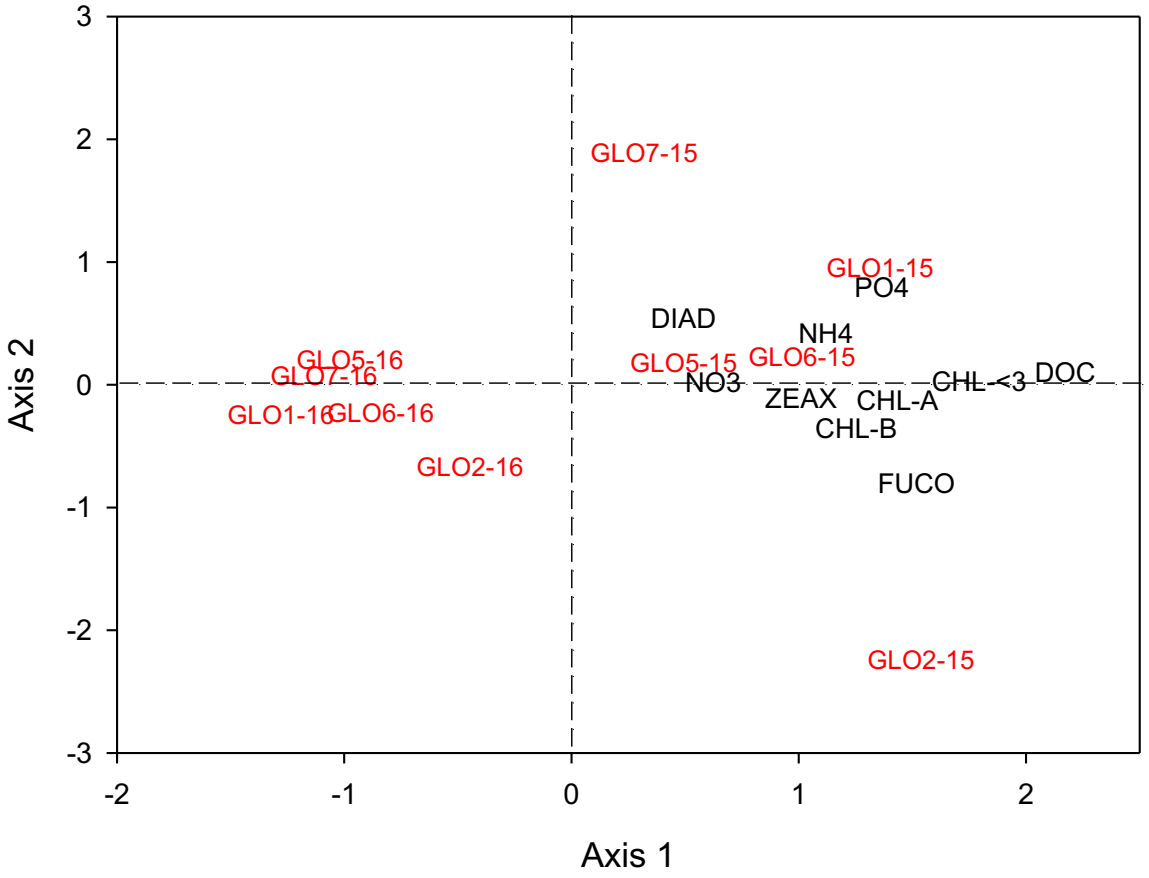

Figure 6 : Co-inertia analysis with the position of the biological variables on the F1 x F2 plane. Position of the sites (red colour) was linked to biological variables co-inertia weights. The number refers to the label of the station and the year (November 2015 and May 2016). See abbreviations in Table 1 for the sites.
Abbreviations: HPROK : heterotrophic prokaryotes; PICO: picoeukaryotes; NANO : nanophytoplankton; CYAN: picocyanobacteria ; SYN/PRO: ratio *Synechococcus* / *Prochlorococcus*; HNF: heterotrophic nanoflagellates; HNA: heterotrophic prokaryotes with high nucleic acid level. ALOR: aloricate ciliates; LOR: loricate ciliates; SHAN: Shannon index; ALG: microphytoplankton.

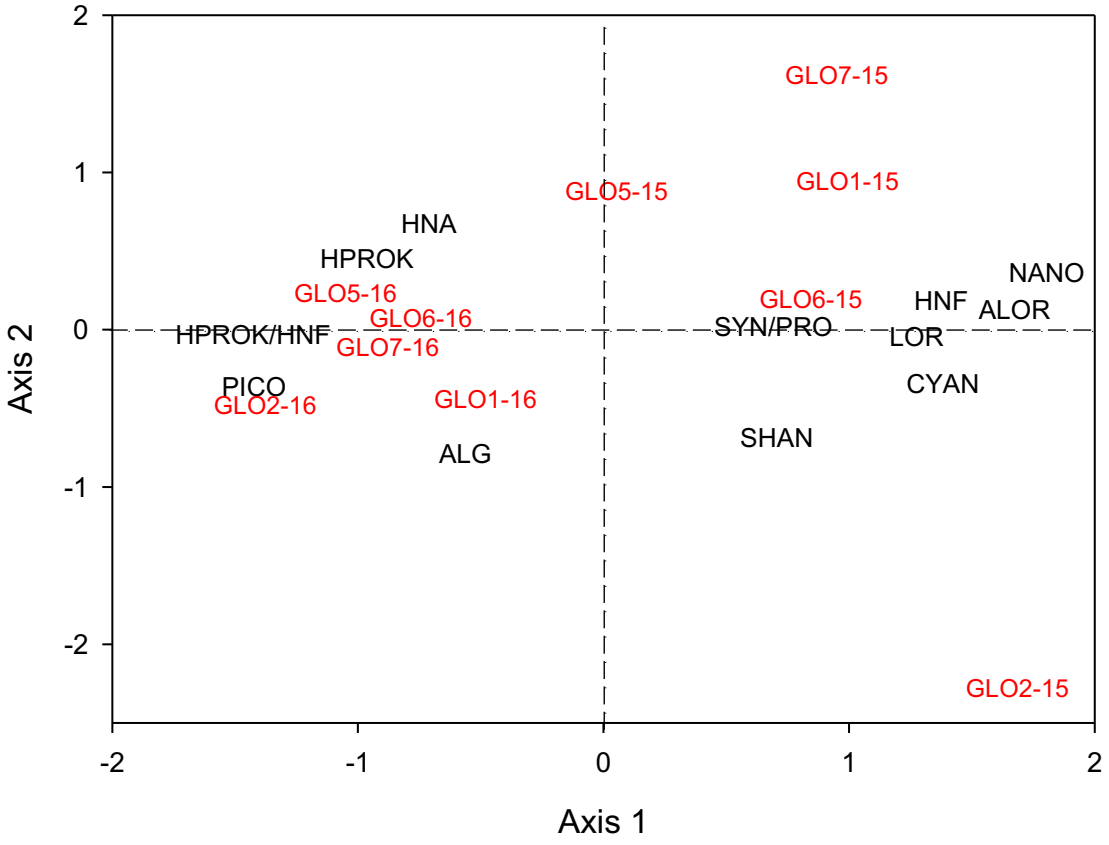

Figure 7: Co-inertia analysis: relationship between the normalized coordinates of the stations on the first axis of the two systems (“Environment” and “Biology”; The line represents the equality between the coordinates on the two systems. The number refers to the label of the station and the year (November 2015 and May 2016).

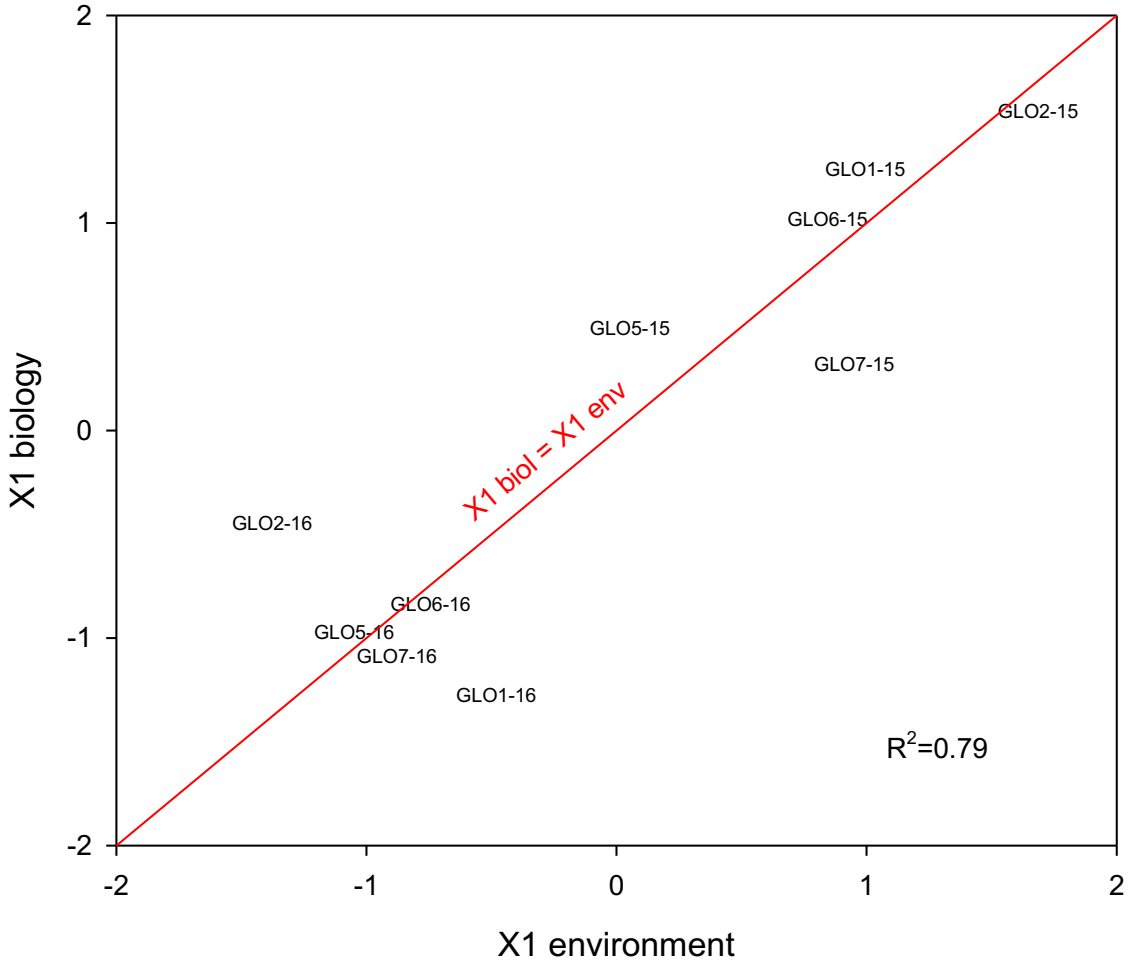

Table 1: List of stations studied in Glorieuses islands in West Indian Ocean in November 2015 and May 2016.
Sampling data, station code, geographical coordinates, station maximal depth and physico-chemical parameters (temperature, salinity, dissolved oxygen concentration and pH) are reported as averaged over the water column (undet : not determined).
All water samples were taken at 2 m depth.

Code	Latitude	Longitude	Max depth	Sampling	Temp	Salinity	Oxygen	pH
	South	East	m	Date	°C		mg/L	
GLO 1	11° 33.673'	47° 17.474'	3	26/11/2015	27.4	35.02	undet	7.67
				19/05/2016	27.6	34.95	6.15	7.98
GLO 2	11° 34.880'	47° 16.862'	8	20/11/2015	27.0	35.06	7.33	8.26
				20/05/2016	27.7	35.19	7.06	undet
GLO 5	11° 32.388'	47° 20.458'	6	23/11/2015	27.8	35.07	undet	8.11
				18/05/2016	28.4	34.97	5.80	7.99
GLO 6	11° 32.973'	47° 17.755'	14	22/11/2015	27.8	34.99	6.88	8.07
				19/05/2016	27.9	34.97	6.05	7.97
GLO 7	11° 32.227'	47° 22.402'	4.5	25/11/2015	27.3	35.02	6.56	7.52
				17/05/2016	28.6	35.48	6.74	8.11

Table 2: Values, mean and standard deviation (Std) of chemical parameters from the 5 studied sites during the two surveys (November 2015 and May 2016). Differences between surveys were tested using the non-parametric Mann-Whitney U-test (*: $p<0.05$). Abbreviations: PO_4 : dissolved phosphorus ; NO_3 : nitrate ; NH_4 : ammonium ; DOC : dissolved organic carbon; CHL-A: chlorophyll-a; CHL<3: chlorophyll-a of cell size < 3µm; CHL-B: chlorophyll-b; FUCO: fucoxanthin; DIAD: diadinoxanthin; ZEAX: zeaxanthin;

Parameters	**Code**	**PO_4**	**NO_3**	**NH_4**	**DOC**	**CHL-A**	**CHL<3**	**CHL-B**	**FUCO**	**DIAD**	**ZEAX**
Units		µM	µM	µM	µM	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
November 2015											
	GLO1	0.130	0.133	0.409	126	0.368	0.281	0.211	0.066	0.052	0.219
	GLO2	0.114	0.307	0.261	149	0.396	0.278	0.272	0.195	0.001	0.138
	GLO5	0.142	0.444	0.303	117	0.262	0.242	0.001	0.087	0.007	0.069
	GLO6	0.199	0.117	0.365	117	0.269	0.221	0.588	0.048	0.001	0.164
	GLO7	0.216	0.268	0.223	140	0.206	0.144	0.001	0.029	0.014	0.066
Mean 2015		*0.160*	*0.253*	*0.312*	*130*	*0.300*	*0.233*	*0.215*	*0.085*	*0.015*	*0.131*
Std 2015		*0.044*	*0.134*	*0.075*	*14*	*0.079*	*0.056*	*0.241*	*0.065*	*0.021*	*0.065*
May 2016											
	GLO1	0.100	0.160	0.123	87	0.028	0.030	0.061	0.061	0.025	0.060
	GLO2	0.105	0.130	0.165	93	0.319	0.143	0.097	0.037	0.008	0.142
	GLO5	0.109	0.196	0.332	95	0.079	0.034	0.061	0.015	0.004	0.091
	GLO6	0.130	0.182	0.089	95	0.163	0.076	0.041	0.053	0.012	0.088
	GLO7	0.099	0.213	0.251	98	0.077	0.084	0	0.009	0	0.049
Mean 2016		*0.104*	*0.174*	*0.188*	*94*	*0.133*	*0.074*	*0.052*	*0.035*	*0.010*	*0.086*
Std 2016		*0.015*	*0.030*	*0.101*	*4*	*0.114*	*0.045*	*0.035*	*0.022*	*0.009*	*0.036*
Test (2015-2016)		**0.027***	**0.256**	**0.059**	**0.008***	**0.028***	**0.001***	**0.421**	**0.145**	**0.498**	**0.215**

Table 3: Values, mean and standard deviation (Std) of biological parameters from the 5 studied sites during the two surveys (November 2015 and May 2016). Differences between surveys were tested using the non-parametric Mann-Whitney U-test (*: $p<0.05$). Abbreviations: HPROK : heterotrophic prokaryotes; HNA: percentage of heterotrophic prokaryotes with high nucleic acid content . PICO: picoeukaryotes; NANO : nanophytoplankton; CYAN: picocyanobacteria ; SYN/PRO: ratio *Synechococcus* / *Prochlorococcus*; HNF: heterotrophic nanoflagellates; HNA: heterotrophic prokaryotes with high nucleic acid level. ALOR: aloricate ciliates; LOR: loricate ciliates; ALG: microphytoplankton and 3 diversity indices : RICH : Species richness; SHAN: Shannon diversity index; EVEN : Pielou's evenness index;

Parameters	**Code**	**PICO**	**NANO**	**CYAN**	**SYN/**	**RICH**	**SHAN**	**EVEN**	**ALG**	**HPROK**	**HNF**	**HNA**	**HPROK**	**ALOR**	**LOR**
Units		10^4/L	10^5/L	10^7/L	**PRO**				10^4/L	10^8/L	10^5/L	%	**/HNF**	/L	/L
November 2015															
	GLO1	1.22	2.17	5.71	7.95	21.0	2.532	0.832	1.70	8.88	8.62	41.3	1030	16	8
	GLO2	2.14	1.87	3.99	8.25	35.0	3.231	0.902	3.12	2.75	10.8	59.4	255	20	18
	GLO5	2.66	2.04	2.92	5.59	35.0	2.652	0.746	2.29	10.9	2.15	67.4	5041	16	4
	GLO6	1.76	1.62	5.30	8.36	37.0	2.601	0.720	3.09	8.25	10.6	48.9	779	24	4
	GLO7	1.53	1.61	0.93	6.93	19.0	2.616	0.889	0.78	5.64	12.1	58.8	465	16	16
Mean 2015		*1.84*	*1.86*	*3.77*	*7.43*	*29.4*	*2.732*	*0.818*	*2.20*	*7.28*	*8.85*	*55.2*	*1514*	*18.4*	*10.0*
Std 2015		*0.59*	*0.25*	*1.93*	*1.12*	*8.6*	*0.126*	*0.082*	*0.99*	*3.14*	*3.95*	*10.2*	*1993*	*3.6*	*6.6*
May 2016															
	GLO1	10.3	0.12	2.32	4.80	42.0	2.653	0.709	3.98	10.0	5.40	58.1	1860	6	4
	GLO2	14.1	0.09	2.21	5.19	49.0	2.514	0.646	3.12	9.31	0.38	45.2	24582	0	2
	GLO5	2.78	0.68	0.82	5.16	38.0	2.824	0.776	2.29	7.22	0.84	50.1	8615	0	0
	GLO6	2.64	0.10	2.54	5.69	37.0	2.594	0.718	3.09	9.99	0.71	46.1	14009	0	10
	GLO7	6.22	0.76	1.10	12.17	37.0	2.567	0.711	2.64	7.33	1.56	45.6	4701	0	0
Mean 2016		*7.23*	*0.09*	*1.80*	*6.60*	*40.6*	*2.631*	*0.712*	*3.02*	*8.78*	*1.78*	*48.9*	*10753*	*1.2*	*3.2*
Std 2016		*4.98*	*0.02*	*0.78*	*3.13*	*5.1*	*0.119*	*0.046*	*0.65*	*1.40*	*2.07*	*5.44*	*8974*	*2.7*	*4.1*
Test (2015-2016)		**0.016***	**0.008***	**0.067**	**0.067**	**0.037***	**0.690**	**0,036***	**0.154**	**0.357**	**0.008***	**0.985**	**0.049***	**0.008***	**0.088**

Table 4 : Heatmap of the contribution of the dominant microalgal species (> 2%) at each station in November (Nov) 2015 and May 2016. Shading in the boxes indicates the percentage of total microalgal density represented by each dominant taxon. Identification was made at the highest level possible, numbers after genus indicate different but unidentified taxa. Shading legend is indicated on bottom.

Period	***Nov***	***May***	***Nov***	***May***	***Nov***	***May***	***Nov***	***May***	***Nov***	***May***
Station	GLO1	GLO1	GLO2	GLO2	GLO5	GLO5	GLO6	GLO6	GLO7	GLO7
Bacillariophyceae										
Amphora sp.										
Bacteriastrum sp.										
Cerataulina pelagica										
Cylindrotheca closterium										
Chaetoceros sp1										
Chaetoceros sp2										
Cocconeis spp.										
Coscinodiscus spp.										
Dactyliosolen sp.										
Leptocylindrus danicus										
Leptocylindrus minimus										
Licmophora sp.										
Navicula sp1 *(5 x 15 µm)*										
Navicula sp2										
Navicula sp3										
Nitzschia sp1										
Nitzschia sp2										
Pennate spp. *(5 x 40 µm)*										
Pseudonitzschia spp.										
Skeletonema sp1										
Dinophyceae										
Prorocentrum cordatum										
Scrippsiella spp.										
Protoperidinium spp.										
Dinophyceae sp.										
Oxytoxum laticeps										
Heterocapsa niei										
Gymnodinium sp1										
Gymnodinium sp2										
Gymnodinium sp3										
Cyanophyceae										
Oscillatoriales spp.										
Colour scale		> 20 %		15-20 %		10-15 %		5-10 %		2-5 %

Table 5: Comparison of inertia from the 2 data sets based on Environmental (E) and Biological (B) variables resulting from the co-inertia analysis.
Two co-inertia axes (F1 and F2) are selected. Var E and Var B: inertia of each table projected on the co-inertia axes. Iner E and Iner B: maximal projected inertia of each table.
Covar: covariance of the 2 sets of coordinates projected on the co-inertia axes.
R-value represents the correlation between the 2 new sets of coordinates resulting from the co-inertia analysis.

Axis	Var E	Var B	Iner E	Iner B	Covar	R-value
F1	4.268	5.56	4.471	5.742	4.318	0.8863
F2	1.089	1.863	2.057	2.311	1.361	0.9551

Table 6: Mann-Whitney rank correlations between the 10 chemical descriptors studied at the 5 stations. Significant values are given in bold (*p<0.05; **p<0.01; ***p<0.001).
Abbreviations: PO_4 : dissolved phosphorus ; NO_3 : nitrate ; NH_4 : ammonium ; DOC : dissolved organic carbon; CHL-A: chlorophyll-a; CHL<3: chlorophyll-a with a cell size < 3µm; CHL-B: chlorophyll-b; FUCO: fucoxanthin; DIAD: diadinoxanthin; ZEAX: xeaxanthin;

	PO_4	NO_3	NH_4	DOC	CHL-A	CHL<3	CHL-B	FUCO	DIAD	ZEAX
PO_4	1.000	0.107	0.250	0.545	0.256	0.334	0.379	-0.063	0.001	0.121
NO_3		1.000	0.061	0.433	0.140	0.316	-0.375	0.438	-0.305	-0.437
NH_4			1.000	0.448	0.391	0.592	0.483	0.056	0.180	0.543
DOC				1.000	**0.682***	**0.764****	0.314	**0.614***	0.063	0.332
CHL-A					1.000	**0.895*****	0.469	0.606	0.176	**0.756***
CHL<3						1.000	0.503	**0.622***	0.196	**0.646***
CHL-B							1.000	0.295	-0.063	**0.681***
FUCO								1.000	-0.037	0.277
DIAD									1.000	0.470
ZEAX										1.000

Table 7: Mann-Whitney rank correlations between the 12 biological descriptors studied at the 5 stations. Significant values are given in bold (*p<0.05; **p<0.01; ***p<0.001).
Abbreviations: HNF: heterotrophic nanoflagellates; ALOR: aloricate ciliates; LOR: loricate ciliates; NANO : nanophytoplankton; PICO: picoeukaryotes; ; SYN/PRO: ratio *Synechococcus*/*Prochlorococcus*; HPROK : heterotrophic prokaryotes; HNA: heterotrophic prokaryotes with high nucleic acid content; HPROK/HNF : ratio heterotrophic prokaryotes / heterotrophic nanoflagellates; CYAN: picocyanobacteria; SHAN: Shannon index; ALG: microphytoplankton

	HNF	ALOR	LOR	NANO	PICO	SYN/ PRO	HPROK	HNA	HPROK/ HNF	CYAN	SHAN	ALG
HNF	1.000	**0.849****	**0.687***	**0.712***	-0.479	0.251	-0.567	-0.446	**-0.732***	0.476	0.302	-0.324
ALOR		1.000	0.517	**0.914*****	-0.563	0.184	-0.324	-0.006	**-0.687***	**0.678***	0.289	-0.247
LOR			1.000	0.531	-0.454	0.018	-0.607	-0.428	-0.369	0.209	0.503	-0.303
NANO				1.000	**-0.632***	0.173	-0.255	-0.048	-0.601	**0.647***	0.226	-0.471
PICO					1.000	-0.259	0.329	0.068	**0.635***	-0.317	-0.284	0.543
SYN/PRO						1.000	-0.413	-0.417	-0.402	0.141	0.024	-0.155
HPROK							1.000	**0.839****	0.383	0.056	**-0.744***	0.240
HNA								1.000	0.176	-0.023	-0.493	0.037
HPROK/HNF									1.000	-0.312	-0.319	0.258
CYAN										1.000	0.055	0.145
SHAN											1.000	0.170
ALG												1.000