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1 Do microbial planktonic communities reflect the ecological changes of
2 Glorieuses coral reefs (Iles Eparses, Western Indian Ocean)?
3

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19 *Keywords:* Plankton, Microorganisms, Ecological changes, Cyclone, Pristine ecosystem, Indian
20 Ocean
21

22 *Highlights:*

- 23 - Glorieuses islands can be considered as pristine ecosystems, not subject to human pressure
- 24 - Planktonic microbial communities were studied in terms of ecological descriptors for tracking
25 changes in the way coral-reef systems function
- 26 - A category 5 tropical cyclone could explain the post-event environmental situation
- 27 - A low active microbial food web was observed after the cyclone.
- 28 - Three variables among the autotrophic and heterotrophic compartments can be considered for
29 assessing environmental changes in Glorieuses marine waters.
30

31 *Author Contributions:*

32 Conceptualization: MB, CD, PC, LB

33 Data curation: MB, AB, CC, MP, CD,

34 Formal analysis: MB, AB, CC, PG, MP, HA, BB, CR, CD,

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37 Methodology: MB, CC, PG, HA, CD, MP,

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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)

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

5 Ecological indicators are seen as a way to reduce the complexity of ecosystems to a small number of
6 key criteria that could retain the essential information needed for tracking changes in the state of the
7 environment, as pointed out by Hayes et al. (2015). These authors cited several international studies
8 on a global scale, including those mandated in 2008 by the European Marine Strategy Framework
9 Directive (EC, 2008). Microbial planktonic communities encompass a great diversity of organisms
10 such as bacteria, phytoplankton and protozoa (ciliates and nanoflagellates). The composition and
11 structure of microbial communities are basic indicators of the state of the ecosystem, including the
12 type of factors regulating the dynamics of these communities (Suttle, 2005; Estrada and Vaque,
13 2014). Phytoplankton, roughly defined as the autotrophic component of the planktonic food web, are
14 considered as having an important ecological function as primary producers that directly and
15 indirectly fuels the food webs (Domingues et al., 2008). Recently, Gittings et al. (2019) evaluated
16 tropical phytoplankton phenology using satellite data. They proposed to categorize phytoplankton as
17 an “ecosystem indicator” to assess “ecosystem health” in response to environmental and climatic
18 disturbances.

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

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

3 Interactions between anthropogenic and climatic stressors represent a serious challenge for managing
4 and predicting the water quality and ecological status of interface ecosystems (estuaries, coastal
5 lagoons) (Hemraj et al., 2017). However, the effects of climate change can lead to constantly changing
6 ocean dynamics, and so an understanding of biological indices would be appreciable to follow the
7 ecological status of fragile ecosystems such as coral-reef systems (Burke et al., 2001). Generally, it
8 is extremely difficult to identify the effects of climate change among other forcing stressors, mainly
9 because ecosystems that are isolated from human pressure are rare. Glorieuses islands (Grande
10 Glorieuse, Île du Lys), belonging to the Iles Eparses and part of the TAAF (Terres Australes et
11 Antartiques Françaises), located in the Western Indian Ocean (WIO), display an extraordinary and
12 rich biodiversity in terms of marine species (Durville et al., 2003; Poupin et al., 2014; Conand et al.,
13 2014; Chabanet et al., 2015; Bouvy et al., 2016; Dupuy et al., 2016; Quetel et al., 2016). These islands
14 are associated with a tropical wet climate (Quod et al., 2007; Quetel et al., 2016) and are recognized
15 as being uninhabited (other than a limited military presence) as well as geographically isolated
16 (located 222 km north of Madagascar and 253 km north of Mayotte). As a result, Glorieuses islands
17 can be considered as equivalent tropical pristine ecosystems, only being touched by natural
18 disturbances such as climatic actions. Geographically, the Mozambique Channel and the Western
19 Indian Ocean (WIO) are dominated by a series of southward drifting anti-cyclonic and cyclonic
20 eddies, which cause the upwelling of cooler, nutrient-rich waters, resulting in an elevated
21 phytoplankton biomass (Barlow et al., 2014; Lamont et al., 2014). In addition, the WIO is also
22 subjected to periodical extreme weather events such as cyclones, with serious impact on shorelines
23 and sediment transport, as described by Duvat et al. (2017) on Farquhar Atoll (Seychelles), after the
24 passage of a category 5 tropical cyclone (Fantala) in April 2016. There is a limited understanding of
25 the influence of mesoscale eddies and cyclones on pelagic biological compartments in the WIO and
26 Mozambique Channel, essentially focusing on phytoplankton (Barlow et al., 2014; Ternon et al.,
27 2014). Nevertheless, cyclones can cause a dramatic change in the ecological functioning of tropical
28 coastal lagoons in Australia (McKinnon et al., 2003) and in Bengal Bay (Srichandan et al., 2015),
29 with drastic effects on phytoplankton assemblages, caused by modifications in turbidity and nutrient
30 regime.

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

1 2015; Quétel et al., 2016). Therefore, the TAAF decided to set up monitoring and management tools
2 to efficiently protect the biodiversity of the Glorieuses islands.

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

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

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

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

24

25 **2. Material and Methods**

26 *2.1. Study site and samplings*

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

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

7

8 *2.2. Physical-chemical variables*

9 At each sampling site, a CTD profiler (YSI 600 XLM) was deployed to record temperature, depth,
10 pH, and dissolved oxygen concentrations. Dissolved organic carbon (DOC) analyses were performed
11 on 30 ml sub-samples collected in pre-combusted (450 °C overnight) glass vials, preserved with 35
12 µl of 85% phosphoric acid. Samples were stored in the dark until analysis, using a Shimadzu TOC
13 VCPH analyzer (Rochelle Newall et al., 2008). Samples for measuring dissolved nutrients (NH₄-N,
14 NO₃-N, NO₂-N, PO₄-P) were filtered onto Whatman GF/F fiberglass filters, stored at - 20°C and
15 analyzed as described by Strickland and Parsons (1968).

16

17 *2.3. Biological variables*

18 For archaeal and bacterial abundance (heterotrophic prokaryotes: HPROK), samples were fixed with
19 prefiltered (0.2 µm) buffered formaldehyde (2% final concentration), stored in liquid nitrogen (-
20 196°C) and analyzed upon return to Montpellier University. HPROK cells were enumerated by flow
21 cytometry, according to the protocol described by Marie et al. (1997), slightly modified with the use
22 of a higher fluochrome concentration (Bouvy et al., 2016). One milliliter of fixed sub-samples was
23 incubated with SYBR Green I (Molecular Probes, Eugene, OR, USA) at a final concentration of
24 1/375, for 15 min at 4°C in the dark. For each subsample, three replicate counts were performed using
25 a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an air-cooled
26 argon laser (488 nm, 15 mW). Stained bacterial cells, excited at 488 nm, were enumerated according
27 to their right angle light scatter (RALS), and the green fluorescence (FL1) was measured using a
28 530/30 nm filter. These cell parameters were recorded on a four-decade logarithmic scale mapped
29 onto 1024 channels. Fluorescent beads (1-2 µm, Polysciences, Warrington, PA, USA) were
30 systematically added to each sample. True count beads (Becton Dickinson, San Jose, CA, USA) were
31 added to determine the volume analyzed. Standardized RALS and FL1 values (cell RALS and FL1
32 divided by 1 µm beads, RALS and FL1 respectively) were used to estimate the relative size and the
33 nucleic acid content of bacterial cells respectively (Troussellier et al., 1999). List-mode files were
34 analyzed using BD Cellquest Pro software (ver.5.2.1). HPROK cells with High Nucleic Acid (HNA)
35 content were identified and considered as active cells in the HPROK community (Gasol et al., 1999).

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

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

28

29 *2.4. Photosynthetic pigments*

30 The identification and quantification of photosynthetic pigments included in phytoplankton (total and
31 below 3 µm fraction) was performed through HPLC (High Performance Liquid Chromatography),
32 using Waters D600 equipment and an ODS C-18 column only with UV-detection (Wright et al.,
33 1991). Pigments were detected by a Waters 2996 photo-diode detector (optic resolution 1.2 nm) from
34 400 to 700 nm for chlorophylls and carotenoids, and by a 2475 Multi λ fluorescence detector (Leruste
35 et al., 2016). Photosynthetic pigments composition in samples was determined by using Empower

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

12 Photosynthetic pigments that are dominant in phytoplanktonic cells can be seen as fingerprint markers
13 of algal groups: chlorophyll-a (CHL-A) is a biomarker of the total phytoplankton biomass, peridin
14 (PERI) for Dinophytes, fucoxanthin (FUCO) for fucoxanthin-rich diatoms. Diadinoxanthin (DIAD)
15 is typical to marine planktonic diatoms (Lavaud et al., 2002). Alloxanthin (ALLO) is then used as a
16 biomarker for Cryptophytes, whereas prasinoxanthin (PRAS) is typical to Prasinophytes (Bustillos-
17 Guzman et al., 2004). Violaxanthin (VIOL), neoxanthin (NEO), and chlorophyll b (CHL-B) are found
18 in Chlorophytes, also known as green algae (Roy et al., 2011). Zeaxanthin (ZEAX) is mainly found
19 in cyanobacteria especially in coastal tropical water ecosystems, but can also be found in
20 Chlorophytes and Prasinophytes (Vidussi et al., 2001). Finally, 19'But-fucoxanthin (BUTF) and
21 19'Hex-fucoxanthin (HEXF) were used to identify Haptophytes (Roy et al., 2011).

22

23 *2.5. Sequence processing – Bacterial diversity*

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

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

12

13 *2.6. Data processing - co-inertia analysis*

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

29

30 **3. Results**

31 *3.1. Environmental parameters*

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

1 November) to 28.6 °C (GLO7 in May) whereas the salinity ranged from 34.95 (GLO1 in May) to
2 35.19 (GLO2 in May). The dissolved oxygen concentrations ranged from 5.80 mg/L (GLO5 in May)
3 to 7.06 mg/L (GLO2 in May). The pH values varied little with the season, with values comprised
4 between 7.52 and 8.26. Significant differences were found for phosphorus (PO₄) and dissolved
5 organic carbon (DOC) concentrations, with the greatest values reported in November, whereas
6 ammonia and nitrate concentrations were not significantly different between the two periods (U-test,
7 $p < 0.05$; Table 2). Concentrations of chlorophyll-a (CHL-A) were significantly higher in November
8 than in May (U-test, $p = 0.028$), and the difference was more marked with the values based on cell-
9 size inferior to 3 µm (CHL<3; U-test, $p = 0.001$), with the highest concentrations for the GLO2 site.
10 CHL<3 µm contribution to total pigment concentrations was 78% in November and 59% in May.
11 Accessory pigment concentrations of the total fraction were dominated by four pigments:
12 chlorophyll-b (CHL-B), fucoxanthin (FUCO), diadinoxantin (DIAD) and zeaxanthin (ZEAX), and
13 their concentrations were always higher in November than in May. However, no significant
14 difference in means was observed between the two surveys (Table 2). Accessory pigments diversity
15 was higher in May (11 pigments without CHL-A) than in November (6 pigments without CHL-A),
16 (Figure 2). The GLO5 and GLO7 stations exhibited the lowest concentrations of pigments in May
17 and November, associated with an absence of CHL-B in November (Table 2). Zeaxanthin
18 concentrations were detected in all stations whatever the season, with an important contribution in
19 May (between 30% and 60% of total pigments, except at GLO1; Figure 2). Fucoxanthin
20 concentrations (FUCO) were also detected in all stations, especially at GLO2 and GLO5 in
21 November. It was obvious that GLO-1 (oceanic site) showed higher pigment diversity than GLO7
22 (coral reef site), whatever the season. The GLO7 station was characterized by the marked contribution
23 of 19'Hex-fucoxanthin (HEXF) in May and November (almost 30% of total accessory pigment).
24 Peridinin (PERI) contribution was only detected at GLO2 in May.

25

26 3.2. *Biological variables*

27 The abundance of heterotrophic prokaryotes (bacteria and archaea; HPROK) was similar between the
28 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).
29 The percentage of active cells with high nucleic acid content (% HNA) was not significantly different
30 between November and May (U-test, $p > 0.05$), with a mean of $55.2 \pm 10.2\%$ and $48.9 \pm 5.44\%$,
31 respectively.

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

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

22 The mean abundance of heterotrophic nanoflagellates (HNF) was significantly higher in November
23 than in May (U-test, $p = 0.008$) (mean of $8.85 \pm 3.95 \times 10^5/L$ versus $1.78 \pm 2.07 \times 10^5/L$). The ratio
24 of heterotrophic prokaryotes and heterotrophic nanoflagellates (HPROK/HNF) was thus significantly
25 higher in May (mean ratio of 10753 ± 8974) than in November (mean ratio of 1514 ± 1993) (U-test,
26 $p = 0.049$), with the highest ratio observed in GLO2 (Table 3). The ciliate community was identified
27 as two groups (aloricates and loricates) and their abundance were very low for all the stations for both
28 surveys (Table 3). As for HNF, aloricate abundance (ALOR) were significantly higher in November
29 (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
30 in 4 stations. Thus, the protozooplankton community was very poorly represented in May compared
31 to November.

32

33 3.3. Bacterial diversity by sequence processing

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

1 dominated by three phyla: cyanobacteria, alpha- and gammaproteobacteria, with means of 25%, 26%
2 and 21%, respectively (Figure 3). Bacteroides represented 37.3% of total sequences at GLO2 in May
3 and clearly dominated the total microbial assemblage. This class represented low values in November
4 (mean of 9%). Inversely, GLO2 was characterized by the lowest percentage of cyanobacteria in May
5 (16%). Other microorganisms such as eukaryotes were detected, with the presence of chloroplasts,
6 representing 9% in November and 10% in May of the total sequences on average. The spatial variation
7 of the OTU microbial sequences by station and by survey was determined using Non-Metric Multi-
8 Dimensional Scaling (NMDS), with a stress value of 0.01 indicating a strong ordination (Figure 4).
9 A clear difference was observed with a cluster including only the stations sampled in May 2016, with
10 a similarity of 78%. Interestingly, similarity between GLO5 and GLO6 was high in November 2015
11 (72%), while the similarity at the survey level was only 60.15%. Dissimilarity between stations
12 sampled in November 2015 and May 2016 was 46.43 %.

13

14 *3.4. Link between environmental and biological variables: Co-inertia analysis*

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

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

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

15 The relationship between the normalized coordinates of the stations on the first axis of the two
16 systems (“Environmental” and “Biological”), reflecting the degree of association between Biology
17 and Environment, mostly in terms of time variation (ie opposition between 2015 and 2016), was
18 highly significant ($r^2 = 0.79$). Figure 7 shows the plots of the stations with most of them sampled in
19 2015 situated close to the equality line, whereas the position of some stations sampled in 2016 are far
20 from the equality line. The same relationships between the coordinates from the second axis of the
21 two systems, mostly reflecting the space variability, was also highly significant ($r^2=0.91$) but much
22 more explained by the plots of 2015 ($r^2=0.94$) than by those of 2016 ($r^2=0.39$).

23

24 **4. Discussion**

25 *4.1. Context*

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

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

4 Our study reveals a high stability of the water column, based on non-significant differences
5 in parameters between surface and bottom samples confirming the data obtained in 2011 (Bouvy et
6 al., 2016) and the qualification of these islands as a relatively stable environment by Quetel et al.
7 (2016). Environmental conditions were characterized by very low nutrient levels, consistent with
8 previous observations (Bouvy et al., 2016; 2021). Chlorophyll-a concentrations (CHL-A) were
9 always under 1µg/L, revealing the classification of Glorieuses waters as being ultra-oligotrophic. This
10 status was confirmed by very low levels of dissolved nutrients (especially orthophosphates, nitrate,
11 dissolved organic carbon) similar to data acquired in 2011 (Bouvy et al., 2016).

12

13 4.2. Biological components of the water column

14 Regarding biological variables, heterotrophic prokaryotes (bacteria and archaea; HPROK) were the
15 major component of plankton communities both in November 2015 and May 2016, with abundance
16 values comparable to those occurring in coral-reef systems (Seymour et al., 2005; Dinsdale et al.,
17 2008; Patten et al., 2011; Bouvy et al., 2012). Picocyanobacteria (CYAN; particularly
18 *Prochlorococcus* and *Synechococcus*) dominated the phytoplankton community, due to their
19 efficiency in integrating nutrients available in their lowest concentrations (Karl et al., 2001). This
20 dominance also confirmed the ultraoligotrophic status of Glorieuses islands, with a predominance of
21 *Synechococcus* characterizing the coastal coral reefs in Iles Eparses (Bouvy et al., 2016; 2021).
22 SYN/PRO ratios were always between 12.2 and 4.8, confirming that *Prochlorococcus* can be
23 considered as being an oceanic marker in nutrient-low waters (Charpy, 1996). Indeed, *Synechococcus*
24 was found numerically dominant in coastal waters in the Mozambique Channel, followed by
25 *Prochlorococcus* and then picoeukaryotes in lower abundance (Zubkov and Quartly, 2003). Bidigare
26 et al. (1992) reported that accessory pigments accounted for 60% of the light absorbed in the surface,
27 and 90% at the base of euphotic zones (low-light depth) in the Sargasso Sea. Zeaxanthin associated
28 with cyanobacteria (*Prochlorococcus* and *Synechococcus*; Vidussi et al., 2001) represented the
29 major pigments in both surveys; other specific pigments of green algae (CHL-B, Violaxanthine,
30 Neoxanthine), also detected, confirmed the presence of Chlorophyceae. Haptophytes presence was
31 detected by 19'Hex-Fucoxanthin and 19'But-Fucoxanthin (Paerl et al., 2003), confirmed by
32 microscopic observation with the identification of the coccolithophoride *Emiliana huxleyi*, known
33 as a paleoclimatic display. The life cycle of *E. huxleyi* is complex, involving several different phases
34 such as haploid and diploid stages with many cell sizes (Laguna et al., 2001). The smallest forms of
35 the flagellate can be detected with autotrophic picoeukaryotes in the Deep Chlorophyll Maximum

1 (DCM) in the Mozambique Channel, also contributing to surface populations (Barlow et al., 2014).
2 Adaptive mechanisms were required by these communities in low light environments with low
3 absorption efficiency by chlorophyll-a, corresponding to environmental conditions in the DCM (at
4 40-110 m depth) (Barlow et al., 2014). The elevated proportion of photosynthetic carotenoids (PSC)
5 would have enabled the picoeukaryotes to adapt to a range of irradiance conditions in the euphotic
6 zone like the Haptophytes (Bricaud et al., 2004; Bouman et al., 2011), and it is obvious that
7 picoeukaryotes (PICO) had an advantage over picocyanobacteria (CYAN) in more turbulent
8 environment. Thus, the large significant difference in picoeukaryote abundance between November
9 and May ($p = 0.016$) can be explained by the drastic environmental modifications linked to a climatic
10 event (such a cyclone, see below), with high turbulence activities inducing greater turbidity and lower
11 light availability in May (wet season).

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

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

8 The high significant correlations observed between CHL-A, CHL<3, CHL-B and ZEAX suggested a
9 dominance of picocyanobacteria (*Prochlorococcus* and *Synechococcus*) in Glorieuses, especially in
10 2015, as also demonstrated by the co-inertia analysis (Fig. 5). These results confirmed that the small
11 cells of picocyanobacteria (CYAN) are more efficient in synthesizing high levels of zeaxanthin
12 (ZEAX) compared to diadinoxanthin (DIAD) synthesis in eukaryotes such as Bacillariophyceae as
13 also demonstrated by Barlow et al. (2017).

14 Overall, very low concentrations of total ciliates were observed in Glorieuses, with aloricate forms
15 (ALOR) being more abundant than loricate ones (LOR), supporting the data reported by Bouvy et al.
16 (2016). In May 2016, a very low presence of ciliates and heterotrophic nanoflagellates was detected,
17 presumably affecting the flow of organic matter in the microbial food web. This hypothesis might
18 explain the high susceptibility to stress and disturbance of these fragile marine ecosystems (Mellin et
19 al., 2008). The microbial food web can be characterized by the trophic relationships between bacteria
20 and their predators (Berglund et al., 2007), and it is now recognized that protozoa, and especially
21 heterotrophic nanoflagellates (HNF), are the most important grazers on bacteria in most environments
22 (Sanders et al., 2000). Experimental approaches conducted in Iles Eparses showed a wide range of
23 bacterial growth rates and grazing rates by HNF, with the lowest values observed in Glorieuses and
24 Tromelin islands (Bouvy et al., 2016). Nevertheless, low concentrations of HNF were recorded in
25 Glorieuses, confirming the data reported for the Mayotte reef (Houlbréque et al., 2006), the great reef
26 of Toliara, Madagascar (Bouvy et al., 2015), and the Iles Eparses systems (Bouvy et al., 2016).
27 Moreover, the ratio between HPROK and HNF can provide insight into trophic food webs, with a
28 low ratio suggesting a high grazing pressure exerted by HNF, implying that organic biomass passes
29 through active microbial food webs (Bouvy et al., 2010). That was likely the case in November with
30 a significantly lower HPROK/HNF ratio than in May (means of 1514 and 10753, respectively, t-test:
31 0.049), indicating a drastic modification of the pelagic trophic food web towards less HNF predation
32 pressure. It is obvious that the protist community (ciliates and HNF) was very sparsely represented
33 in May, highlighting the cascading effect generated by zooplankton predators (Bouvy et al., 2006),
34 or linked to an exceptional climatic event such as a cyclone.

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

25

26 4.3. Characterization of the Glorieuses marine waters

27 Overall, the microbial distribution defined by the integrative approach, using co-inertia analysis,
28 demonstrated that the differences in environmental conditions explained the relative abundance of
29 biological components in Glorieuses island, with two distinct situations (May and November). Figure
30 7 stressed the relationship between the stations and the two systems (Environmental and Biological),
31 suggesting (i) a clear opposition between the two situations (May and November) and (ii) a distinct
32 spatial zonation of stations in November 2015, with the biological components strongly related to
33 those stemming from environmental variables. On the other hand, the “Biological system” seemed
34 more independent from the “Environmental system” in May 2016, corroborating the data reported
35 using the metabarcoding approach (Figure 4). Thus, the analysis revealed a reef system characterized

1 by a richer nutrient context and higher algal pigment concentrations, in November 2015, associated
2 with a higher abundance of protozoa and nanophytoplankton; whereas in May 2016, the reef system
3 was dominated by a high abundance of active heterotrophic prokaryotes (H-PROK) and
4 picoeukaryotes (PICO), in a poorer nutritive context. The significant correlation between H-PROK
5 and HNA cell abundance ($r = 0.839$; $p < 0.001$; Table 7) confirmed the dominant contribution of HNA
6 cells in the remineralization of organic matter, as already demonstrated by Servais et al. (2003) and
7 Bouvy et al. (2010). Thus, the complexity of reef systems in Glorieuses results in a fluctuating
8 availability of growth limiting resources and light, with a large mineralization of organic matter in
9 May 2016 *versus* an active microbial food web with the presence of bacterial predators in November
10 2015. Our results illustrate the relative importance of top-down dynamics, based on the observed
11 relationships between bacterial and HNF abundance (Gasol, 1994; Sanders et al., 2000), and
12 demonstrate a large modification of the environmental context, with a very low active microbial food
13 web in May 2016 compared to November 2015.

14

15 *4.4 Potential influence of Fantala cyclone on plankton structure*

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

20 The Mozambique Channel and the Western Indian Ocean (WIO) are subjected to periodical extreme
21 weather events such as cyclones, with serious impact on shoreline and sediment transport, as
22 described by Duvat et al. (2017) on Farquhar Atoll (Seychelles), after the passage of a category 5
23 tropical cyclone (Fantala) in April 2016. To the best of our knowledge, there is no available study in
24 literature concerning the impact of cyclones on plankton structure in an isolated coral-reef system,
25 whereas numerous studies pertain to the influence of cyclones on coastal lagoons positioned at the
26 interface between rivers and the sea (McKinnon et al., 2003; Tsuchiya et al., 2013; Srichandan et al.,
27 2015). Very few studies considered the influence of mesoscale eddies on pelagic biological
28 compartments in the WIO and Mozambique Channel, and generally concluded to a drastic effect on
29 turbidity and nutrient regime, which play a role in phytoplankton assemblage (Barlow et al., 2014;
30 Ternon et al., 2014).

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

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

13

14 *4.5. Potential variables reflecting the change of ecological status in Glorieuses islands*

15 Plankton communities are often used to alert scientists and stakeholders about ecological changes in
16 aquatic systems, due to their rapid response to multiple environmental stressors (Lemley et al., 2016).
17 Microbial communities provide sensitive, meaningful and quantifiable indications of ecological
18 changes (Hayes et al., 2015). With the weakness of our database, it is not reasonable to apply standard
19 indexes and bioindicators developed by many authors (e.g. Teixeira et al., 2016; Cozzoli and Basset,
20 2017; Lampert and Hernandez-Farinas, 2018; Varkitzi et al. 2018). Moreover, the Glorieuses marine
21 waters are not submitted to anthropic pressures, eutrophication, chemical pollution (Quetel et al.,
22 2016); thus, water quality assessment using regional reference values of indicator variables is not
23 appropriate. Index values are generally likely to be associated with a relative contribution of local
24 and regional forcing functions (Herrera-Silvera and Morales-Ojeda, 2009; Varkitzi et al., 2018).
25 Finally, in agreement with Goffart (2020), the relationships between phytoplanktonic variables and
26 proxies (diversity indices) are difficult to establish mainly due to (i) the ultra-oligotrophic nature of
27 marine waters in the region and (ii) the low amplitude of seasonal variations in nutrient
28 concentrations.

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

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

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

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

17
18 Of course, these biological variables, based upon floristic composition (species or pigment
19 diversity), can be influenced by spatial variability among sites (rainfall, currents, eddies...),
20 and may not be due to different ecological states, but rather to geographic and natural factors,
21 according to Degerlund and Eilertsen (2010). Nevertheless, these three variables can
22 potentially be considered to characterize the ecological status in the Glorieuses islands,
23 knowing that an exceptional event (a category 5 tropical cyclone) occurred between May and
24 November.

25 26 **5. Conclusions**

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

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

3
4

5 **Conflicts of interest**

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

8

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22

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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.

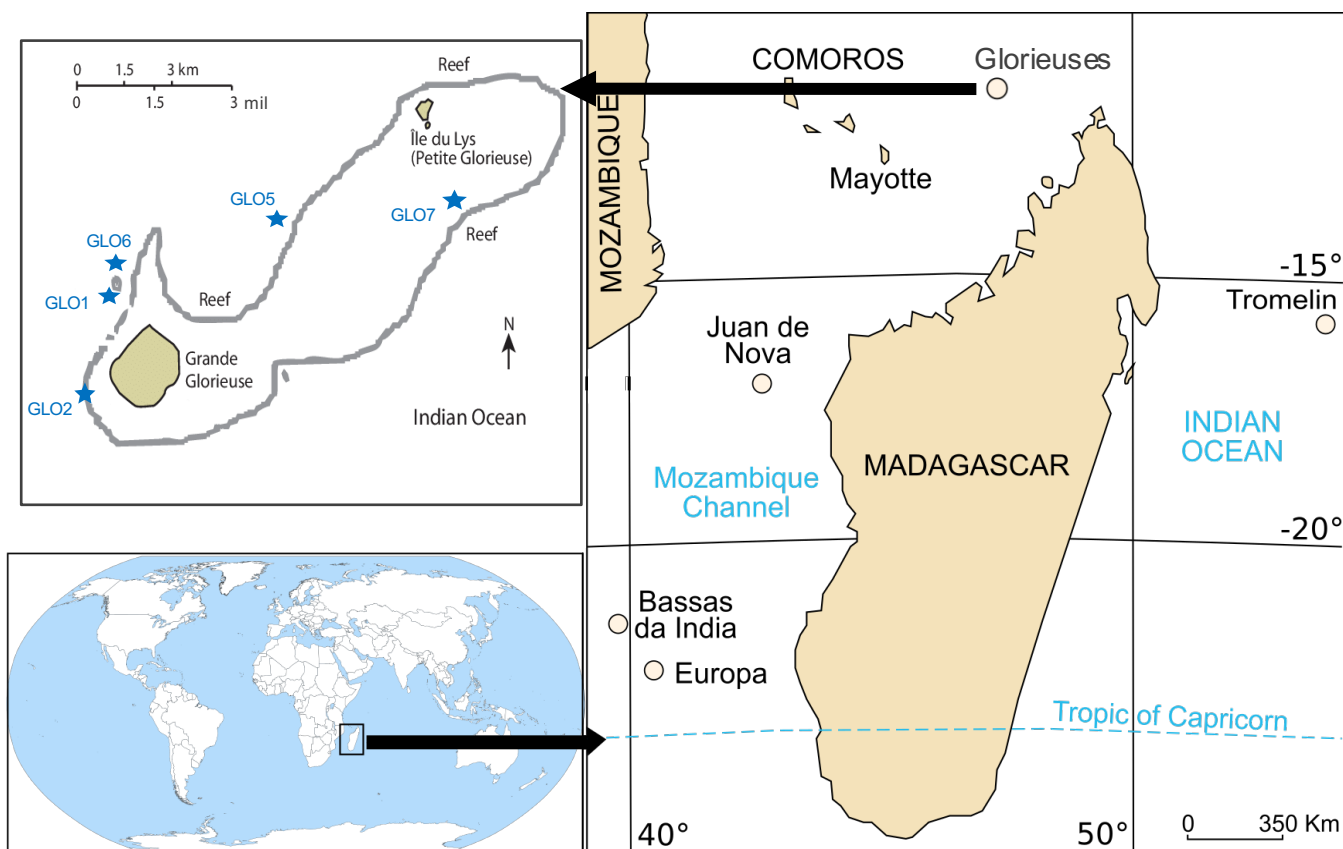


Figure 2 : Proportions of chlorophyll a and accessory pigments in the five stations in November 2015 (A and B) and in May 2016 (C and D). Corresponding relative contributions (%) of different accessory pigments without chlorophyll a date in November 2015 (B) and May 2016 (D).

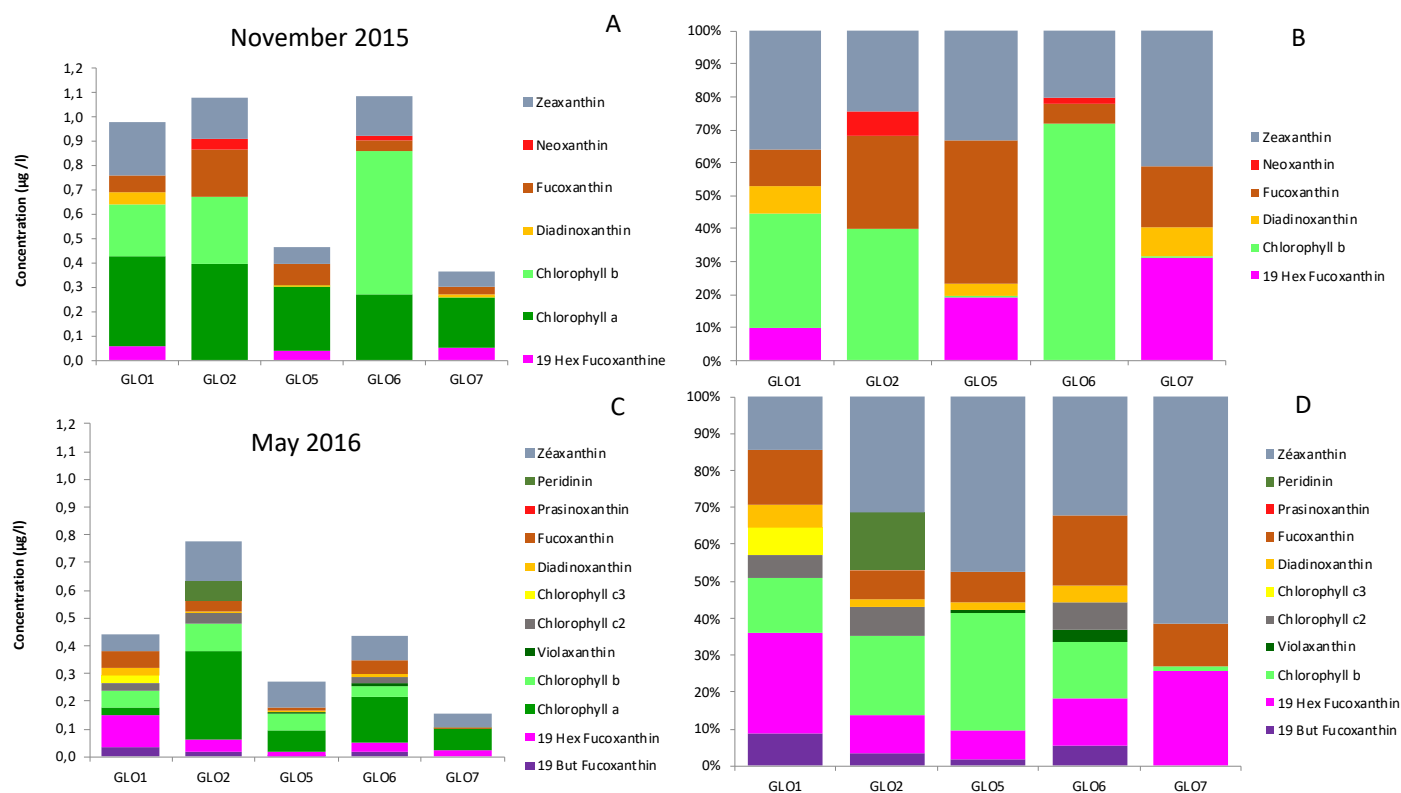


Figure 4 : NMDS ordination performed on Bray Curtis dissimilarity matrices based on the OTU sequences (Square root transformed data) (five stations; November 2015 and May 2016). The number refers to the label of the station and the year (November 2015 and May 2016).

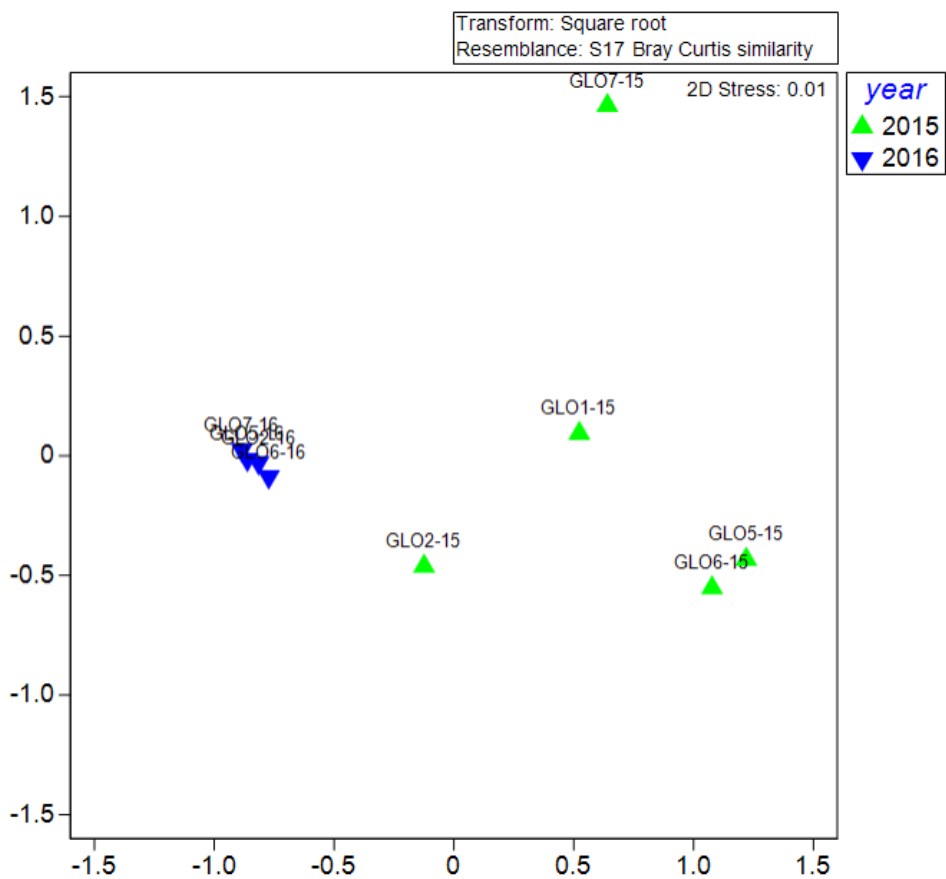


Figure 5 : Co-inertia analysis with the position of the environmental variables on the F1 X F2 plane. Position of the sites (red colour) was linked to biological variables co-inertia weights. The number refers to the label of the station and the year (November 2015 and May 2016). See abbreviations in Table 1 for the sites.

Abbreviations: PO₄ : dissolved phosphorus ; NO₃ : nitrate ; NH₄ : 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.

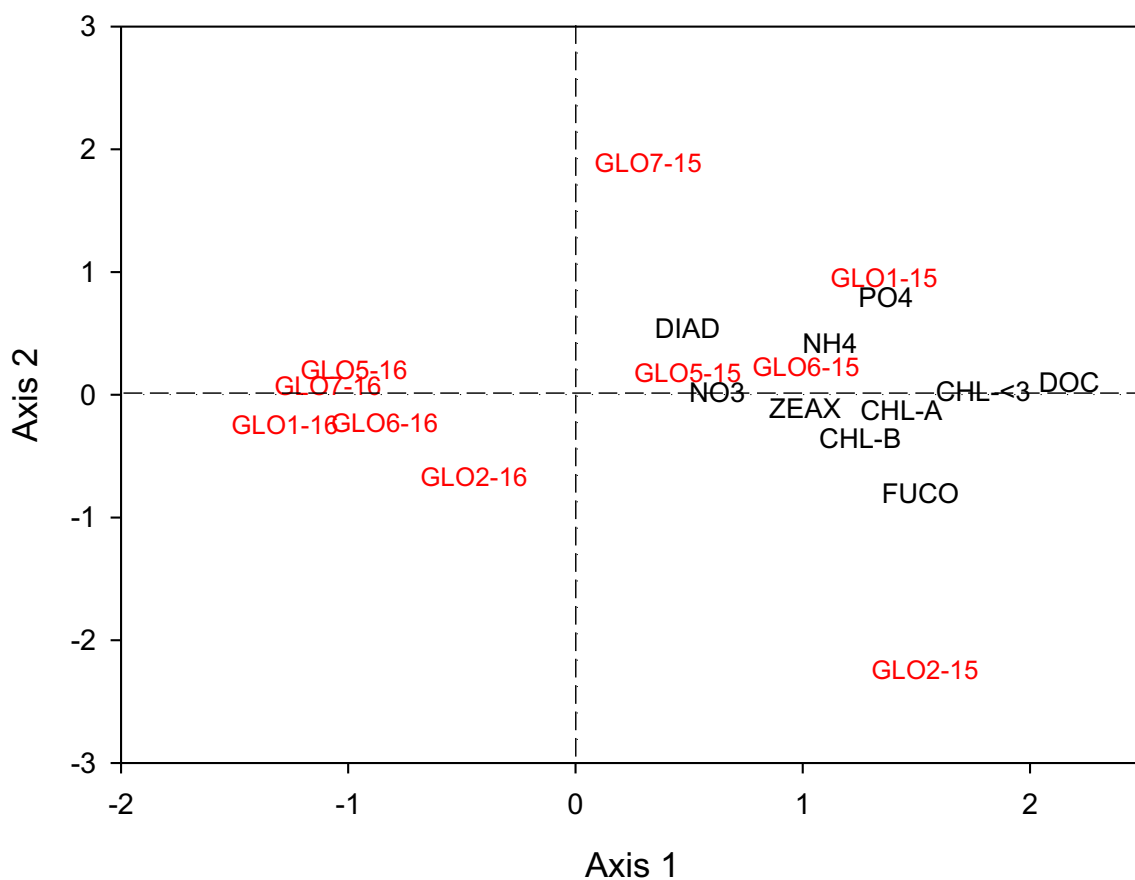


Figure 6 : Co-inertia analysis with the position of the biological variables on the F1 x F2 plane. Position of the sites (red colour) was linked to biological variables co-inertia weights. The number refers to the label of the station and the year (November 2015 and May 2016). See abbreviations in Table 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.

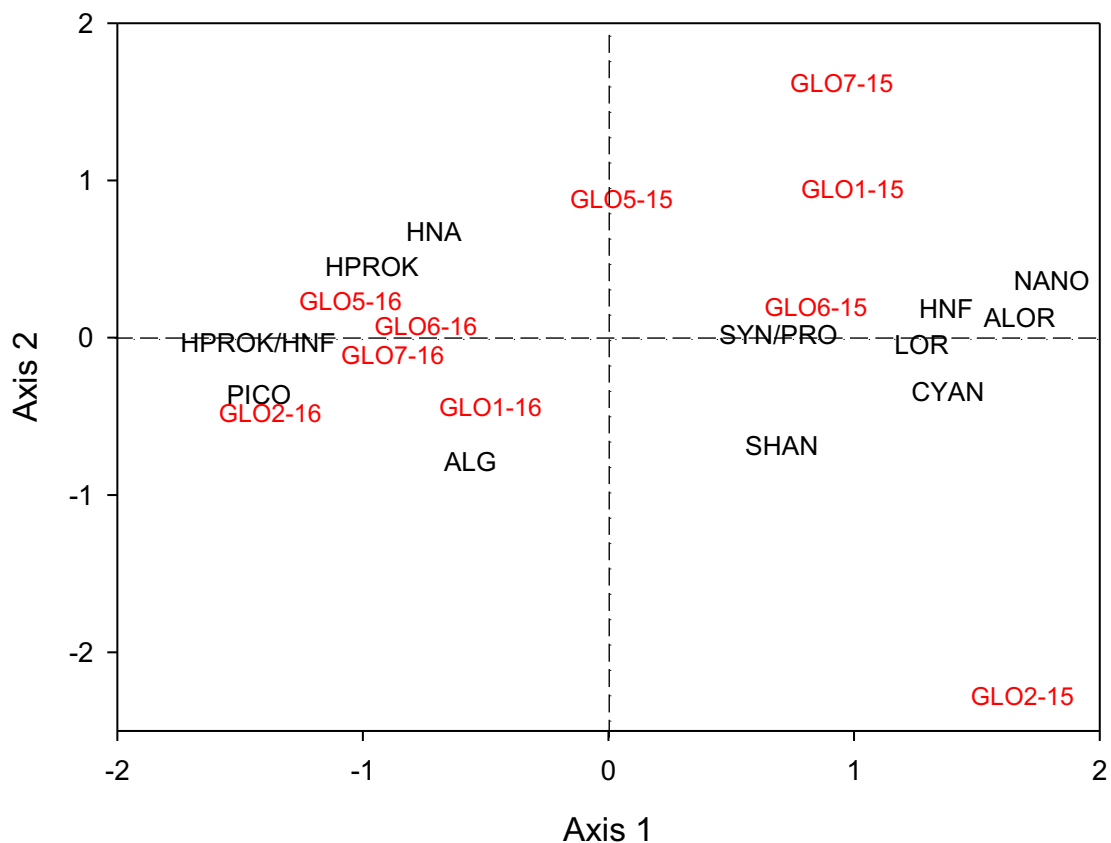


Figure 7: Co-inertia analysis: relationship between the normalized coordinates of the stations on the first axis of the two systems ("Environment" and "Biology"); The line represents the equality between the coordinates on the two systems. The number refers to the label of the station and the year (November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

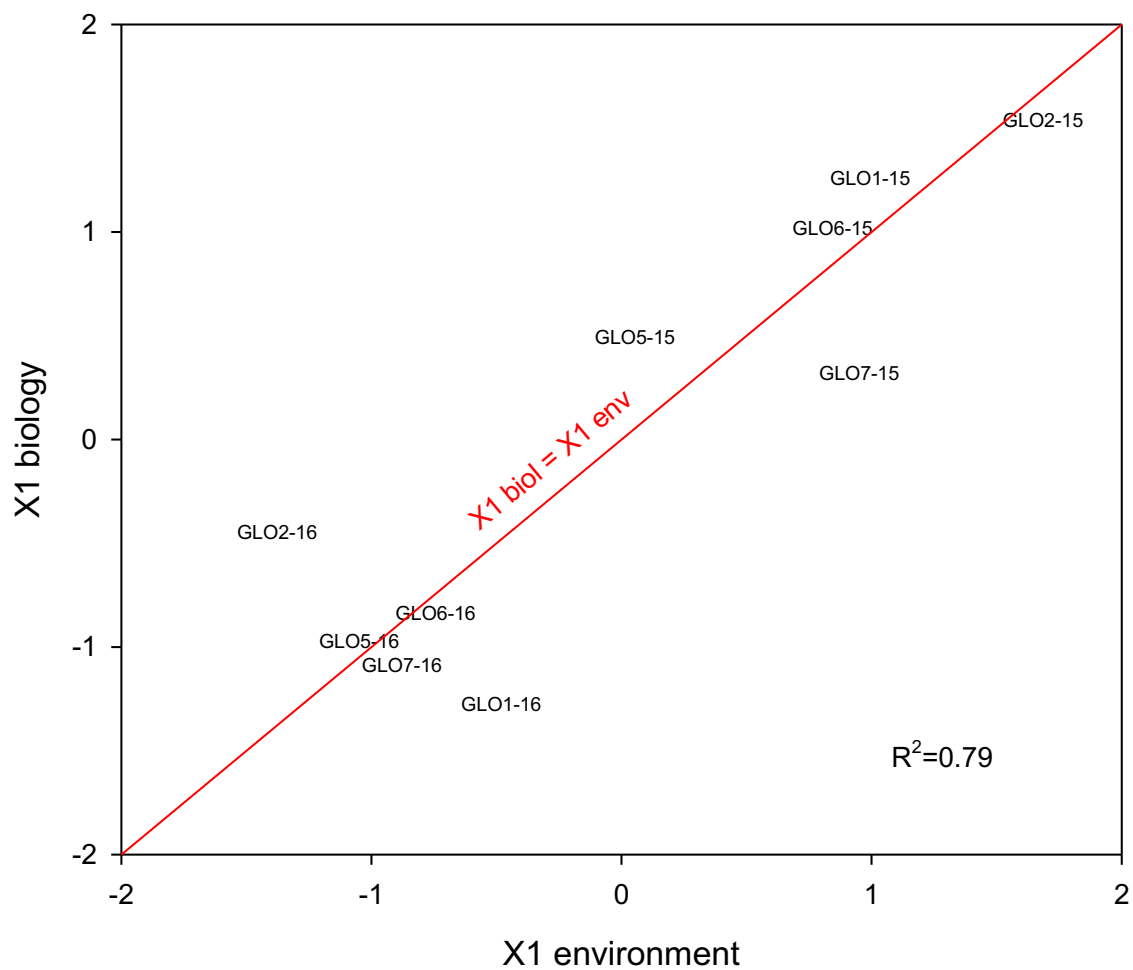


Table 1: List of stations studied in Glorieuses islands in West Indian Ocean in November 2015 and May 2016. Sampling data, station code, geographical coordinates, station maximal depth and physico-chemical parameters (temperature, salinity, dissolved oxygen concentration and pH) are reported as averaged 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: PO₄ : dissolved phosphorus ; NO₃ : nitrate ; NH₄ : 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
<i>Mean 2015</i>		<i>0.160</i>	<i>0.253</i>	<i>0.312</i>	<i>130</i>	<i>0.300</i>	<i>0.233</i>	<i>0.215</i>	<i>0.085</i>	<i>0.015</i>	<i>0.131</i>
<i>Std 2015</i>		<i>0.044</i>	<i>0.134</i>	<i>0.075</i>	<i>14</i>	<i>0.079</i>	<i>0.056</i>	<i>0.241</i>	<i>0.065</i>	<i>0.021</i>	<i>0.065</i>
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
<i>Mean 2016</i>		<i>0.104</i>	<i>0.174</i>	<i>0.188</i>	<i>94</i>	<i>0.133</i>	<i>0.074</i>	<i>0.052</i>	<i>0.035</i>	<i>0.010</i>	<i>0.086</i>
<i>Std 2016</i>		<i>0.015</i>	<i>0.030</i>	<i>0.101</i>	<i>4</i>	<i>0.114</i>	<i>0.045</i>	<i>0.035</i>	<i>0.022</i>	<i>0.009</i>	<i>0.036</i>
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/ PRO	RICH	SHAN	EVEN	ALG	HPROK	HNF	HNA	HPROK	ALOR	LOR
Units		10 ⁴ /L	10 ⁵ /L	10 ⁷ /L					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
	<i>Mean 2015</i>	<i>1.84</i>	<i>1.86</i>	<i>3.77</i>	<i>7.43</i>	<i>29.4</i>	<i>2.732</i>	<i>0.818</i>	<i>2.20</i>	<i>7.28</i>	<i>8.85</i>	<i>55.2</i>	<i>1514</i>	<i>18.4</i>	<i>10.0</i>
	<i>Std 2015</i>	<i>0.59</i>	<i>0.25</i>	<i>1.93</i>	<i>1.12</i>	<i>8.6</i>	<i>0.126</i>	<i>0.082</i>	<i>0.99</i>	<i>3.14</i>	<i>3.95</i>	<i>10.2</i>	<i>1993</i>	<i>3.6</i>	<i>6.6</i>
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
	<i>Mean 2016</i>	<i>7.23</i>	<i>0.09</i>	<i>1.80</i>	<i>6.60</i>	<i>40.6</i>	<i>2.631</i>	<i>0.712</i>	<i>3.02</i>	<i>8.78</i>	<i>1.78</i>	<i>48.9</i>	<i>10753</i>	<i>1.2</i>	<i>3.2</i>
	<i>Std 2016</i>	<i>4.98</i>	<i>0.02</i>	<i>0.78</i>	<i>3.13</i>	<i>5.1</i>	<i>0.119</i>	<i>0.046</i>	<i>0.65</i>	<i>1.40</i>	<i>2.07</i>	<i>5.44</i>	<i>8974</i>	<i>2.7</i>	<i>4.1</i>
	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.

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

