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Diversity, density and photo-physiology of micro-phytoplankton from degraded and non-degraded reefs around Rodrigues Island, Western Indian Ocean

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Abstract. *Soondur M, Kaullysing K, Ramah S, Bhagooli R. 2023. Diversity, density and photo-physiology of micro-phytoplankton from degraded and non-degraded reefs around Rodrigues Island, Western Indian Ocean. Indo Pac J Ocean Life 7: 108-121.* This study aimed at investigating the variations in micro-phytoplankton diversity, density and photo-physiology between a degraded, Anse Aux Anglais (AAA) in the north, and a non-degraded, Port Sud-Est (PSE) in the southeast, reefs around Rodrigues Island, Republic of Mauritius. Sampling was carried out in summer and winter seasons from 2016 to 2019 at the two sites, PSE and AAA. Micro-phytoplankton samples were collected using a plankton net of 5µm and conserved with Lugol's solution. Physico-chemical parameters namely temperature, dissolved oxygen, salinity, and ph were recorded in situ. Seawater samples were collected for nutrient and chlorophyll-*a* concentration analyses. The Total Micro-Phytoplankton Densities (TMPD) did not differ seasonally for the whole period from 2016 until 2019 but there was a significant difference station-wise ($P < 0.01$), with higher TMPD recorded at the non-degraded reef of PSE. PSE exhibited the higher species diversity. Diatoms and dinoflagellates were more abundant at PSE while the cyanobacteria showed higher densities at AAA. Genera like *Licmorphora*, *Gonyaulax*, *Polykrikos*, *Trichodesmium*, and *Lygnbya*, reported worldwide to possibly cause mortality and/or changing community structure of corals, were recorded at both PSE and AAA though not at blooming densities. Photo-physiological assessment of micro-phytoplankton, using a Diving Pulse-Amplitude-Modulated (D-PAM) fluorometer, varied significantly both seasonally and spatially. These findings indicated that the non-degraded reef site had higher micro-phytoplankton density, diversity and photo-physiological performance than the degraded one, implying that degraded reefs may not only be characterized by the benthic cover and/or their health status but also the biological parameters from the water column.

Keywords: Chlorophyll *a*, coral reef, micro-phytoplankton, non-degraded reef, PAM

INTRODUCTION

The coral reef is an ecosystem that can be recognized as a sanctuary of marine biodiversity. Unfortunately, worldwide, it is being recognized that the coral reefs have suffered an astonishing decline (Jackson et al. 2014). Among the major threats to coral reefs are ocean acidification, global warming, induced anthropogenic eutrophication, and physically damaged in response to fishing activities by a human. The hard coral covered at the Great Barrier Reef decreased by 70% (Bell et al. 2013). Over 80% of the coral reef in Southeast Asia (Burke et al. 2002) is under severe threat, and the Caribbean has had an estimated 80% loss in coral cover during the last three decades (Gardner et al. 2003). Notably, the coral reef provides over 400 million people with their required protein, and the amount of world's reef fish has already declined by one third (Edgar et al. 2014). These findings indicate the urgency to apply protective measures and come up with studies for better management and restoration of the unique coral reef ecosystems worldwide. However, many studies have been focusing more on the physical aspect, like pressure induced by a human rather than reef-fishing that damages reefs compared to changes in the surrounding biological environment that influences the reef ecosystem.

Nutrient enrichment related to anthropogenic activities in the coastal regions is one of the major threats to the coral reefs. This event is related to the abundance of algal formation via eutrophication (D'Angelo and Wiedenmann 2014). The nutrient increase will promote algae growth, namely the phytoplankton, which eventually changes the coral-reef community structure. Owing to the rise in temperature globally, the whole coral community is suffering. One of the oldest phytoplankton groups, cyanobacteria, has a high-temperature tolerance and thus takes over the dead reefs by forming huge mats (Hallock 2005). One study has found that cyanobacteria use different methods like space occupation and dominance to hinder the recruitment of new juvenile corals during phase shifts, whereby promoting a decrease in coral recovery leading to a decrease in coral coverage (Charpy et al. 2010). Among the most studied filamentous cyanobacteria, the *Trichodesmium* is responsible for about 0.03-20% of Carbon dioxide fixation in the Tanzanian waters. Moreover, the study indicates that this particular genus of cyanobacteria has a nitrogen-fixing rate of about 2 pmol N trichome^{-1} h^{-1} (Karl et al. 2002). Therefore, it has been

found that there is usually a high density of cyanobacteria in degrading reef regions.

Furthermore, many studies have used the phytoplankton community structure as an index to determine the health status of the different marine ecosystems. The healthier an ecosystem, the more diverse it is. Several studies demonstrated that the abundance of phytoplankton in the ocean positively correlates with fish abundance (Longhurst 1976; Kraus and Supić 2011; Stock et al. 2017), which is considered to be high in healthy regions. Ultimately, phytoplankton can be used to assess the ecosystem status of coral reefs due to their sensitivity to fluctuations in water quality parameters like dissolved oxygen, ph, salinity, and temperature. Phytoplankton acts as a good indicator of ecological change. These microorganisms are not too difficult to detect, identify, and quantify. They cater to a large share of primary production while being very sensitive to diverse environmental stressors. Changes in the diversity and density of the phytoplankton indicate some alteration in the environmental parameters (Paerl et al. 2007; Wang et al. 2021).

Despite the numerous and important ecosystem services that the corals are providing and the frightening rate of their decline on the Great Barrier Reef and worldwide (Steffen et al. 2009; Hughes et al. 2017, 2018), and in the seas of Mauritius (Bhagooli and Kaullysing 2019; Bhagooli et al. 2021a), very limited data has been documented on the phytoplankton community in association with the coral reef. The productivity of phytoplankton governs the health status of the coral reef (Saravanakumar et al. 2008). We have some studies on the phytoplankton in the Republic of Mauritius (Sadally et al. 2014a,b, 2012, 2015, 2016; Armance et al. 2019; Sandooyea et al. 2020; Soondur et al. 2021a,b, 2022) but in the waters around Rodrigues Island micro-phytoplankton studies appear to be unchartered.

Therefore, this study aimed to determine a baseline for the micro-phytoplankton community structure and its photo-physiology around Rodrigues Island, including comparing non-degraded and degraded reef sites. The diversity and density of the micro-phytoplankton were also assessed. Furthermore, the chlorophyll-*a* concentration was determined and used to calculate the estimated primary productivity. The photo-physiology of the micro-phytoplankton was investigated using chlorophyll fluorescence technique. Moreover, the relationships of nutrients (nitrate, phosphate, and silicate) and physico-chemical parameters like dissolved oxygen, salinity, ph, and temperature with the biological parameters like the different groups of micro-phytoplankton namely, diatom, dinoflagellates, and cyanobacteria were determined.

MATERIALS AND METHODS

Study site, stations, and sampling strategy

The Southern Indian Ocean is a region that is still under study (Figure 1A). Rodrigues Island, located at latitude 19.72 45° S and longitude 63.42 72° E, which belongs to the Republic of Mauritius, is among those islands with limited published research on the marine environment. The Island is surrounded by a fringing reef of around 90km (Ahamada et al. 2008). Hardman et al. (2004) reported severe and mass mortality of corals around the island but more consequently in the Northern region. Therefore, this particular study was based at two stations namely Anse Aux Anglais (AAA) in the North and Port Sud-Est (PSE) in the South (Figure 1B). Based on the healthy coral cover, AAA was classified as a degraded zone (Figure 1C) and on the other hand, PSE was a non-degraded zone (Figure 1D). These two sites were chosen due to different percentages of live coral cover based on quadrat estimation method (Jokiel et al. 2015). The coral cover was estimated using a 1 m^2 quadrat frame, which was moved along three 25 m transect lines without overlapping, summing to a total area of 25 m^2 per transect line. The coral cover was estimated and recorded in situ on an underwater writing slate and eventually the quadrat.

This study investigated the changes in the micro-phytoplankton community structure at the degraded and non-degraded stations. Samples were collected for the chlorophyll *a* and nutrient concentration analysis and also physico-chemical parameter was recorded in situ. Sampling was done during the day on a seasonal basis, twice a year, whereby winter samples were collected in June and summer samples in December and it began in summer month of December 2016 and ended in summer of December 2019, whereby only sampling for winter 2019 was not carried out. The sampling was completed in summer of 2019, whereby a survey of both sites was conducted to investigate any potential sign of an outbreak of epizoic micro-phytoplankton attached to corals. The sampling at the two sites was done on two different subsequent days between 9:00 to 12:00 a.m. At three stations along the reef. All samples were collected in triplicates.

Micro-phytoplankton sampling, preservation and analysis

Micro-phytoplankton samples were collected based on 10L of surface seawater filtration through a plankton net of 5 µm. The concentrated sample was preserved using 1% Lugols' solution in situ. The sample was centrifuged at 3500rpm for 10min, further concentrated into pellets of around 1 mL ex-situ back in laboratory conditions, and stored at 4°C (Zarauz and Irigoien 2008; Mukherjee et al. 2014; Sadally et al. 2014a,b). To analyze the micro-phytoplankton, the sample was loaded on a Sedgwick Rafter counting chamber (Woelkerling et al. 1976), and analysis was done under a light microscope (Devassy and Goes 1991; Sadally et al. 2014a,b). Furthermore, the identification of micro-phytoplankton was done according to Tomas (1996). Micro-phytoplankton was counted at magnifications x200 and x400 and classified into three groups of diatoms, dinoflagellates and cyanobacteria. The groups were further classified into different genera. The density of micro-phytoplankton was calculated as cells L-1 whereby the total micro-phytoplankton density was taken as the sum of the different groups of micro-phytoplankton.

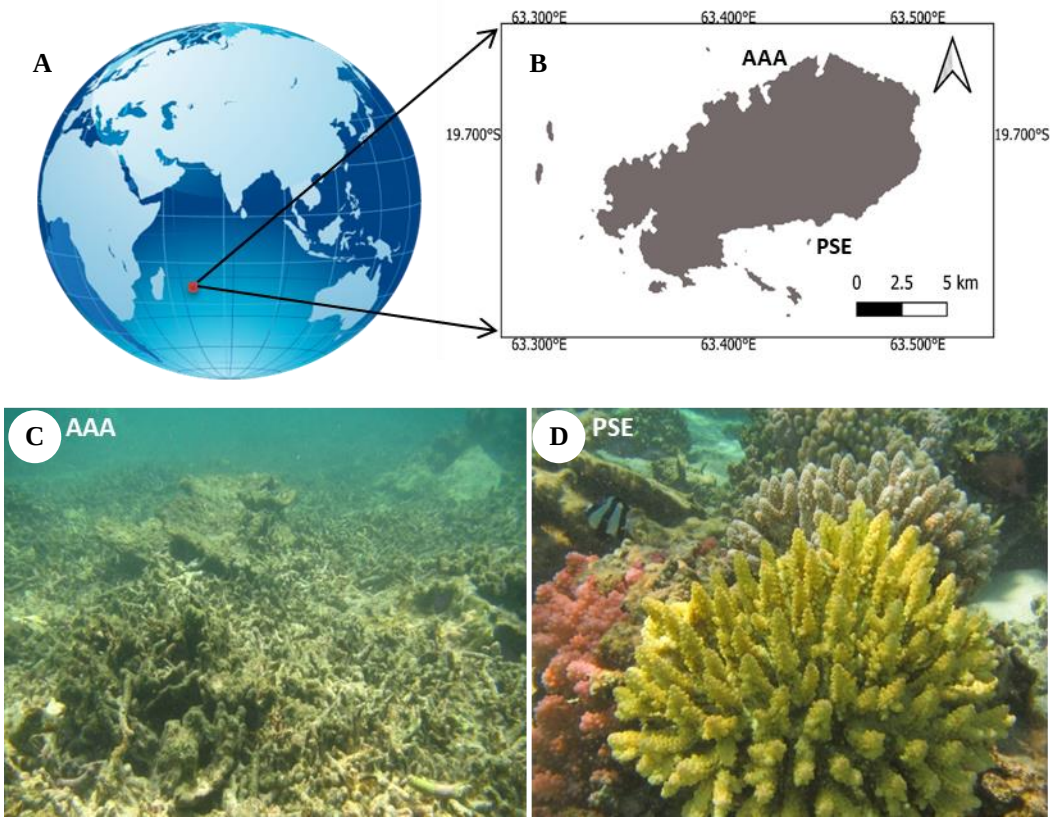

Figure 1. A. The World's globe with the red dot representing the study site in the Southern Indian Ocean, B. is the map of Rodrigues Island, Republic of Mauritius with the studied stations' AAA (Anse Aux Anglais) -Degraded reef and PSE (Port Sud-Est) - Non-degraded reef, C. Degraded reef at AAA and D. Non-degraded reef at PSE

Sea surface chlorophyll-*a* and photophysiological analysis

The in situ Chlorophyll-a (Chla) concentration was determined based on the acetone extraction. First, 500 mL of sea surface water was collected and filtered through Whatman glass fiber filters of pore size 0.45 µm using an electrical pump. The filter paper was then placed in 10 mL of 90% acetone to perform the extraction of chlorophyll-*a* pigment. The extraction process was left overnight for 24 hours before the Chla concentration was determined using a spectrophotometer (Spectronic® genesystm 8) at 4 different wavelengths (630, 647, 664 and 750 nm) based on the formula:

$$\text{Chlorophyll } a = (11.85^* (E_{664} - E_{750}) - 1.54^* (E_{647} - E_{750}) - 0.08 (E_{630} - E_{750})) ^*V_e/L^*V_f$$

Where, L: Cuvette light-path in centimeter; V_e: Extraction volume in milliliter; V_f: Filtered volume in liter and concentrations were in unit mgm^{-3} (Jeffrey and Humphrey 1975) .

Moreover, the satellite sea surface Chlorophyll *a* was extract from the level 3 aquamodis NASA database and data extraction and processing was performed using the seadass software (Acker and Leptoukh 2007) .

The photo-physiology and estimated productivity were assessed for samples collected. The Diving Pulse Amplitude Modulator (D-PAM) fluorometer (Submersible Photosynthesis Yield Analyzer, Walz, Germany) was used to assess the photo-physiology of micro-phytoplankton by measuring the fluorescence of Chla. This technique allowed us to determine the relative electron transport rate (rETR) and Non-Photochemical Quenching (NPQ) when exposed to a series of Rapidly (10 s) changing Light Climates (RLC) (McMinn et al. 2005, 2012). Through the preparation phase, phytoplankton at the 2 sites were adsorbed on filter papers by filtering seawater samples collected, through the Whatman® glass fiber filters of pore size 0.45 µm, and immediately analyzed using the D-PAM (Bhagooli and Hidaka 2003; Bhagooli et al. 2021a,b). Using the rlcs, the rETR and NPQ were estimated at each irradiance. At each irradiance, the respective relative electron transport rate (rETR) was calculated using the formula rETR= 0.5 x ΦPSII x PAR, where PAR is the photosynthetically active radiance. The different PAR used were 110, 150, 300, 400, 500, 800, 1000 and 1325. The NPQ was calculated based on the formula NPQ = (Fm-Fm') /Fm'. The maximum rETR and NPQ were calculated using sigma plots (Platt and Jassby 1976). The estimated relative productivity for each sample at respective sites was calculated using the formula for Estimated productivity, P, defi ned as P = (rETRmax× Chla) (McMinn and Hegseth 2004; McMinn et al. 2005, 2010, 2012).

Nutrient and physico-chemical analysis

Nutrient analysis was performed based on the absorbance technique using the spectrophotometer for the years 2016 and 2017 during summer and winter. Sea surface water samples were collected in triplicates at each station and analyzed for nitrate and phosphate using the cadmium reduction and the ascorbic acid methods (Greenberg et al. 1992) whereby the wavelength for absorption was measured at 493nm and 840nm, respectively. For the determination of the silicate concentration, the seawater sample was left to stabilize and absorbance was read at 810 nm (Caspers 1970) . Moreover, the physical parameter sea surface temperature was measured in situ using a hand-held thermometer (Cormac 314), the chemical parameter salinity was measured using a refractometer (ERMA), dissolve oxygen measured using DO-meter (Hanna HI 9142) and ph via a portable ph-meter (Sadally et al. 2014a,b; Soondur et al. 2020)

Statistical analysis

The software PASW Statistics 18 was used to analyze the data. The data was first to check for normality before any other processing or evaluation. Non-normally distributed data were transformed via $\log_{10}$ or Arcsine. The statistical analysis two-way ANOVA was used to determine the significant difference among the different stations and seasons for the biological parameter total micro-phytoplankton, diatom, dinoflagellates, cyanobacteria, and sea surface Chla. Moreover, the Principle Correspondence Analysis (PCA) was performed for all the biological and physico-chemical parameters to determine the overall correlation strength among the parameters and it was coupled by Pearson's Correlation coefficient to have individual R-values. Shannon-Wiener, Equitability, and Evenness diversity indices were used to determine the variability of the different micro-phytoplankton genera season-wise and station-wise.

RESULTS AND DISCUSSION

Coral cover at degraded and non-degraded sites

The rapid survey conducted only in 2016 revealed that the reef ecosystem at AAA was degraded compared to PSE based on the live and dead coral coverage. The survey revealed that among the most common coral genera present at both sites were: *Pocillopora*, *Acropora*, *Montipora*, *Porites*, *Pavona*, *Fungia* and *Helipora*. These common species align with the survey conducted by Fenner et al. (2004) . As estimated from the quadrat method, it was found that the live coral cover at AAA ranged between 20 to 25% and that in PSE was above 50%.

In situ chlorophyll-a concentration and photophysiology of micro-phytoplankton

The in situ sea surface Chla concentration revealed higher values during summers of 2016 (1.63 ± 0.41 mgm^{-3}), 2017 (1.64 ± 0.70 mgm^{-3}), 2018 (1.68 ± 0.55 mgm^{-3}) and 2019 (1.74 ± 0.24 mgm^{-3}). A gradual increase in Chla concentration was noted from the summer season of 2016 to 2019. The lowest recorded Chla concentration was in winter 2018 at AAA (1.24 ± 0.37 mgm^{-3}). Station-wise, the Chla concentration at AAA ranged between 1.24 ± 0.33 mgm^{-3} and 1.54 ± 0.40 mgm^{-3} and at PSE it was between 1.54 ± 0.27 mgm^{-3} and 1.74 ± 0.24 mgm^{-3} (Figure 2). The two-way ANOVA revealed strong significant difference site-wise ($P < 0.01$) and non-significant difference season-wise and also when both the effect of site and season were combined ($P > 0.05$).

In 2018, the photo-physiological assessment of the functioning of micro-phytoplankton did reveal significant differences seasonally and spatially ($P < 0.05$) (Figure 3). The effective quantum yield (ΦPSII) was mostly below 0.5 with the highest at PSE during summer (0.51 ± 0.23) which may be indicative of good photoinhibition and photoprotection efficiency. As for rETRmax, slightly higher values were recorded at PSE (11.21 ± 3.44) resulting in higher acclimatization potential and thus higher light saturation point. The higher rETRmax aligned with the higher estimated productivity at PSE (15.84 ± 3.17 mgm^{-2}). Moreover, on average, npqmax at both sites revealed higher values during summer, indicating a seasonal adjustment to higher light levels.

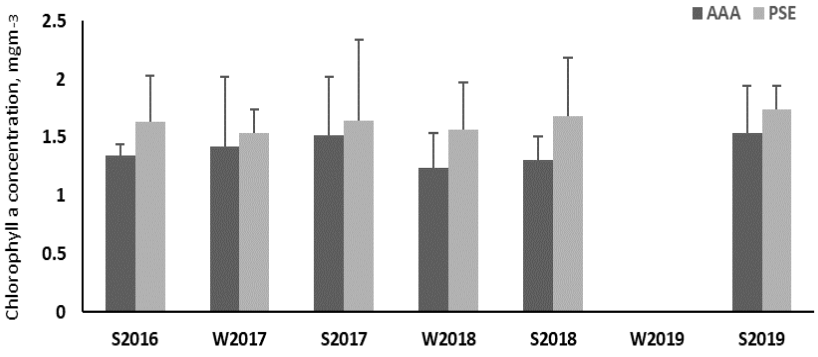

Figure 2. In situ chlorophyll-*a* concentration at Anse Aux Anglais (AAA) and Port Sud-Est (PSE) from summer 2016 (S2016) up to summer 2019 (S2019) in Summer (S) and winter (W) samplings

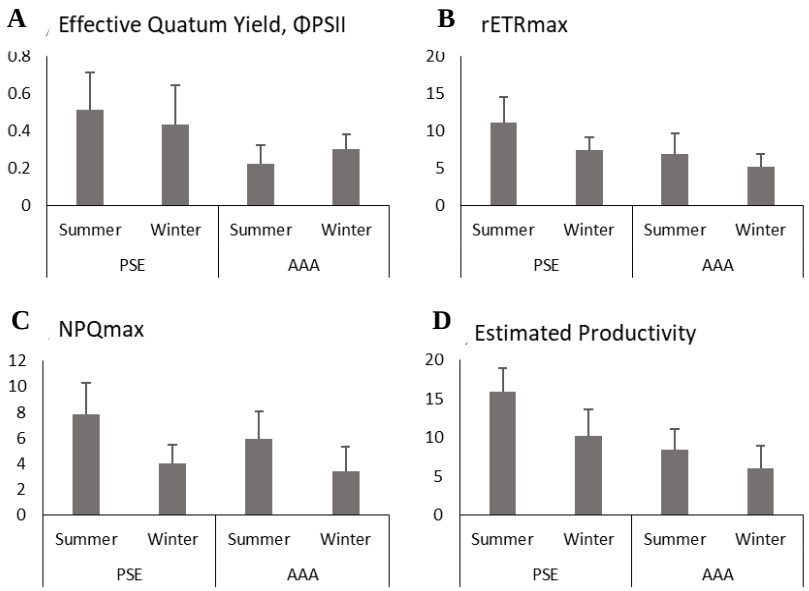

Figure 3. Photo-physiological parameters (y-axis): (A) Effective quantum yield (ΦPSII), (B) rETRmax, (C) npqmax and (D) Estimated Productivity, where the x-axis represent the sites, PSE and AAA, during summer and winter 2018

Spatial and Seasonal variation of micro-phytoplankton densities

The total micro-phytoplankton densities ranged between 12.1 ± 1.6 $\times 10^4$cells.L^{-1} and 19.7± 1.0 $\times 10^4$cells.L^{-1} and these highest and lowest values were recorded at AAA during the summer 2017 and AAA during winter 2018 respectively. As for PSE, the highest density of TMPD that was recorded was 17.7 ± 1.5 $\times 10^4$cells.L^{-1} in summer 2018 and lowest in winter 2017 of 14.6 ± 1.7 $\times 10^4$cells.L^{-1} (Figure 2). Out of all the number of samplings, on average, PSE recorded the highest densities of TMPD. The two-way ANOVA revealed that there was significant difference at (P <0.01) among the two different sites for the TMPD and this difference was not significant season-wise (Table 1).

Throughout the study period, irrespective of sites, the densities of diatom were the highest followed by the dinoflagellates and cyanobacteria. The highest density of diatom at AAA was during summer 2017 of 9.8 ± 0.5 $\times 10^4$cells.L^{-1} and the lowest was during winter 2018 of 6.1 ± 0.8 $\times 10^4$cells.L^{-1} (Figure 4). Site-wise, the difference among the diatom densities was not significant but season-wise, there was a significant difference at (P <0.05) (Table 1). Moreover, for the dinoflagellates, at AAA the highest (summer 2017: 6.9 ± 0.4 $\times 10^4$cells.L^{-1}) and lowest (winter 2018: 3.2 ± 0.4 $\times 10^4$cells.L^{-1}) densities were recorded at the same period as to where diatom densities were highest and lowest (Figure 4). The two-way ANOVA did not reveal any significant differences for the dinoflagellates; neither between sites nor between seasons and this followed the same trend for the cyanobacteria densities (Table 1). At AAA the highest density of cyanobacteria was 4.0 ± 0.4 $\times 10^4$cells.L^{-1} during the first sampling of summer 2016 whereas the lowest densities were recorded in winter 2017 of 2.5 ± 0.3 $\times 10^4$cells.L^{-1}. As for PSE, the highest density was in summer 2017 2.7 ± 0.5 $\times 10^4$cells.L^{-1} and lowest in winter 2018 1.4 ± 0.3 $\times 10^4$cells.L^{-1} (Figure 4).

Throughout this study, for the diatom and dinoflagellates, out of the six samplings, four revealed higher diatom and dinoflagellates densities at PSE compared to AAA whereas for the cyanobacteria, for the whole sampling period, higher densities were recorded at AAA compared to PSE (Table 1). Moreover, the highest densities TMPD, diatom, dinoflagellates, and cyanobacteria at both AAA and PSE were recorded in the summer season compared to winter (Figure 4).

It was found through the PCA biplot analysis that there were four different clusters formation of the sampling periods in response to the biological parameters, namely winter 2017 and 2018 at PSE, summer 2016, 2017, 2018 and 2019 at PSE, winter and summer 2018 at AAA, summer 2016 and 2017 at AAA and summer 2017 and 2019 at AAA (Figure 4).

Diversity of micro-phytoplankton

During this study, a baseline was set for the identification of the micro-phytoplankton whereby, in total 47 genera of micro-phytoplankton was identified of which 28 were diatom, 12 were dinoflagellates and 7 were cyanobacteria. The diatom group dominated not only in terms of the number of genera and on the density distribution. Out of the 28 genera of diatom, only 8 genera showed higher densities at AAA compared to PSE and the rest 20 genera had higher densities at PSE. For the dinoflagellates, out of the 12 genera, 4 genera had higher densities at AAA compared to PSE, and for cyanobacteria; all the 7 genera had higher densities at AAA itself.

The genera of dominant diatom at both PSE and AAA based on the densities were *Coscinodiscus* (2.3 $\times 10^3$cells.L^{-1}), *Navicula* (9.7 ± 2.6 $\times 10^3$cells.L^{-1}), *Nitzschia* (7.7 ± 2.3

$\times 10^3$cells.L^{-1}), *Chaetoceros* (7.1 ± 2.5 $\times 10^3$cells.L^{-1}), *Licmorphora* (6.5 ± 1.9 $\times 10^3$cells.L^{-1}) and *Fragillaria* (5.9 ± 1.8 $\times 10^3$cells.L^{-1}) (Figure 4A). For the dinoflagellates, it was the genera *Ceratium* (10.9 ± 2.6 $\times 10^3$cells.L^{-1}) and *Peridinium* (9.7 ± 2.5 $\times 10^3$cells.L^{-1}) (Figure 4B). Eventually, for the cyanobacteria, the four major genera were *Anabaena* (5.9 ± 1.6 $\times 10^3$cells.L^{-1}), *Trichodesmium* (5.3 ± 2.0 $\times 10^3$cells.L^{-1}), *Nodularia* (5.3 ± 1.5 $\times 10^3$cells.L^{-1}) and *Lyngbya* (4.3 ± 1.2 $\times 10^3$cells.L^{-1}). Lesser prominent cyanobacteria genera were the *Phormidium*, *Snowella* and *Spirulina* (Figure 5C).

The Shannon-wiener (H') showed higher values at PSE compared to AAA for all the different groups of micro-phytoplankton analyzed. This implies that the diversity at PSE is more than at AAA. Moreover, the diversity of the diatom group was higher than dinoflagellates and cyanobacteria (Figure 6A). The Equitability indices (E_H) revealed that the community of diatom, dinoflagellates, and cyanobacteria at PSE was highly diverse compared to AAA (Figure 6B).

The highest H' and E_H were during summer for all the groups of micro-phytoplankton. For the diatom it was during summer 2016 (H' =3.02; E_H =0.91) and 2019 (H'= 3.02; E_H =0.91), for the dinoflagellates it was summer 2017 (H'= 2.41; E_H =0.97) and 2019 (H'= 2.39; E_H =0.91; E_H =0.96) and for cyanobacteria it was during summer 2016 (H'= 1.88; E_H =0.97) and 2017 (H'= 1.92; E_H =0.99). The lowest H' and E_H values were recorded in the winter signifying that summer promoted a higher diversity compared to winter (Figure 6).

Nutrient, physico-chemical parameters and their correlations with biological parameters

The nutrient analysis was performed to determine the concentration of nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate (Si (OH) $_4$) in the sea surface water at AAA and PSE only during summer 2016 and winter 2017. The highest recorded concentration for all the three nutrients were during the winter 2017 whereby NO_3^- = 14.7 ± 1.8 µmol.L^{-1}, PO_4^{3-} = 5.0 ± 1.0 µmol.L^{-1} and Si (OH) $_4$= 6.4 ± 0.3 µmol.L^{-1} and it was at PSE (Figure 7A).

The ph varied between 7.45 ± 0.12 and 8.37 ± 0.10, with the lowest and highest values recorded at PSE. Out of the six samplings conducted between summer 2016 up to summer 2019, it was found that 5 samplings revealed higher ph at PSE compared to AAA excluding winter 2017 (Figure 7B). As for salinity, the highest values were recorded at PSE compared to AAA throughout all the samplings but the salinity variation range was minimal between 33 and 35 ppt (Figure 7C). Dissolved oxygen followed the same trend as salinity, whereby the highest concentration was always recorded at PSE. The DO ranged from the lowest of 7.25 ± 0.5 ppm and the highest of 8.25 ± 0.3 ppm (Figure 7D). PSE recorded the highest sea surface temperature compared to AAA except during summer 2019. On average the winter SST was 26.4 ± 0.3°C and in summer it was 29.9 ± 0.2°C (Figure 7E).

All the nutrient concentrations had a negative correlation with the biological parameter TMPD, diatom, dinoflagellates, and cyanobacteria excluding the chlorophyll *a* (Table 2). Pearson's correlation confirmed these relationships but they were not statistically significant (Figure 8). The ph had a negative non-significant correlation with all the biological parameters expect Chla concentration. Salinity showed a mostly negative correlation with the biological parameters expect with Chla concentration where the correlation was positive, r = 0.699 (P<0.05). DO had significant negative correlation only with TMPD at r = -0.627 (P<0.05). Except with the TMPD, SST showed a significant positive correlation with all the biological parameters (P<0.05). It was revealed from the PCA that all the biological parameters TMPD, diatom, dinoflagellates, cyanobacteria, and Chla had a negative correlation with the chemical parameters nitrate, phosphate, silicate, salinity, DO and ph but positive correlation with the physical parameter SST (Figure 8).

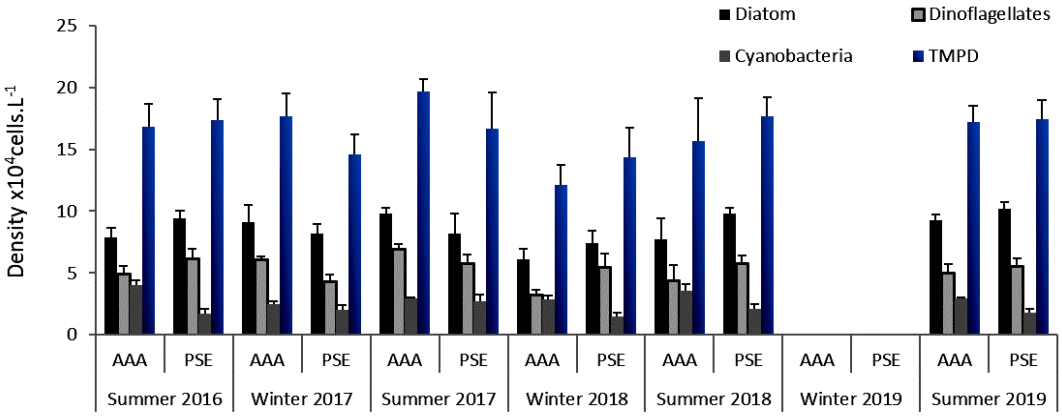

Figure 4. The densities of the TMPD (Total Micro-Phytoplankton Density), diatom, dinoflagellates, and cyanobacteria at the sites AAA (Anse Aux Anglais) and PSE (Port Sud-Est) from summer 2016 up to summer 2019 in the absence of the winter 2019 sampling

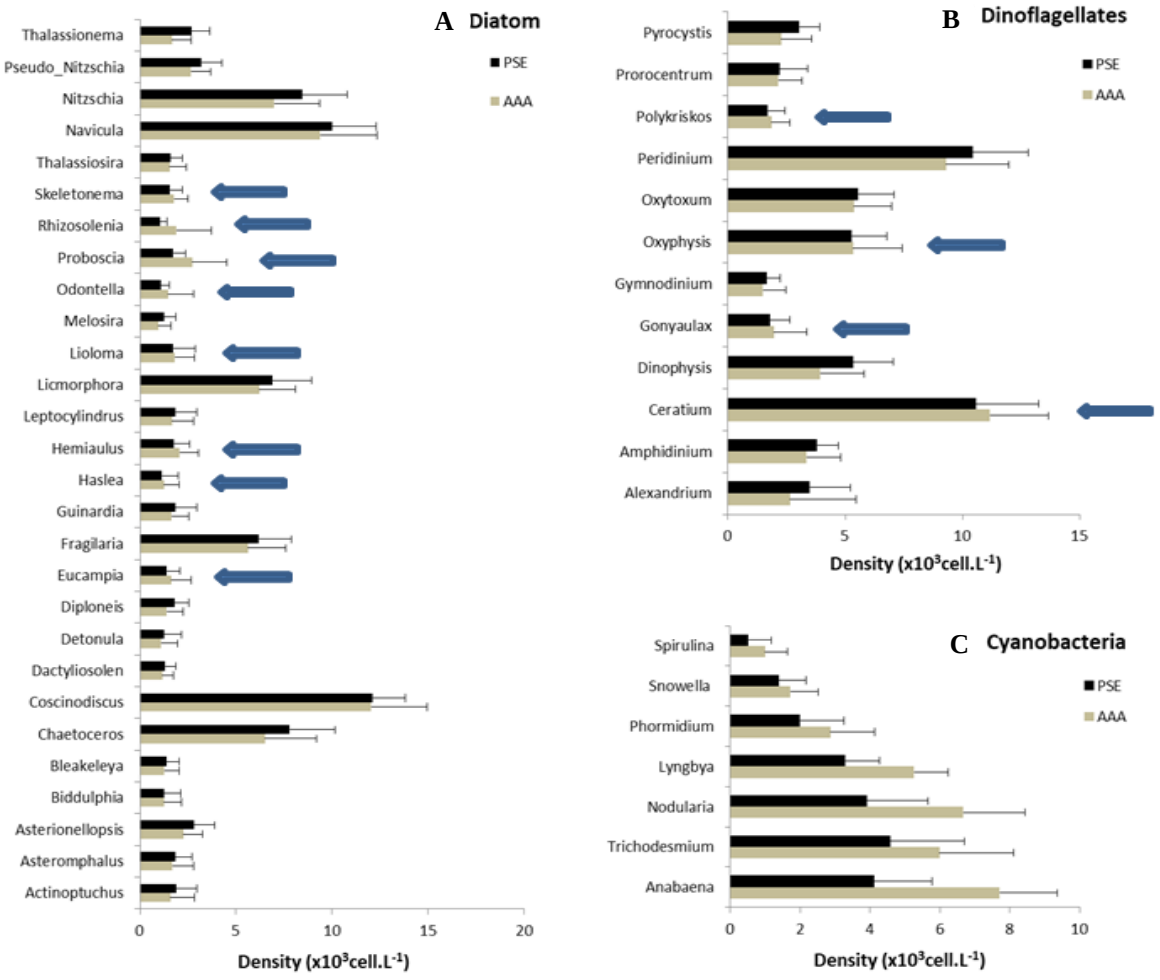

Figure 5. Densities of the 47 genera of micro-phytoplankton at PSE and AAA: (A) 28 genera of diatoms, (B) 12 genera of dinoflagellates, and (C) 7 genera of cyanobacteria. The blue arrows indicate the genera where the densities were higher at AAA compared to PSE

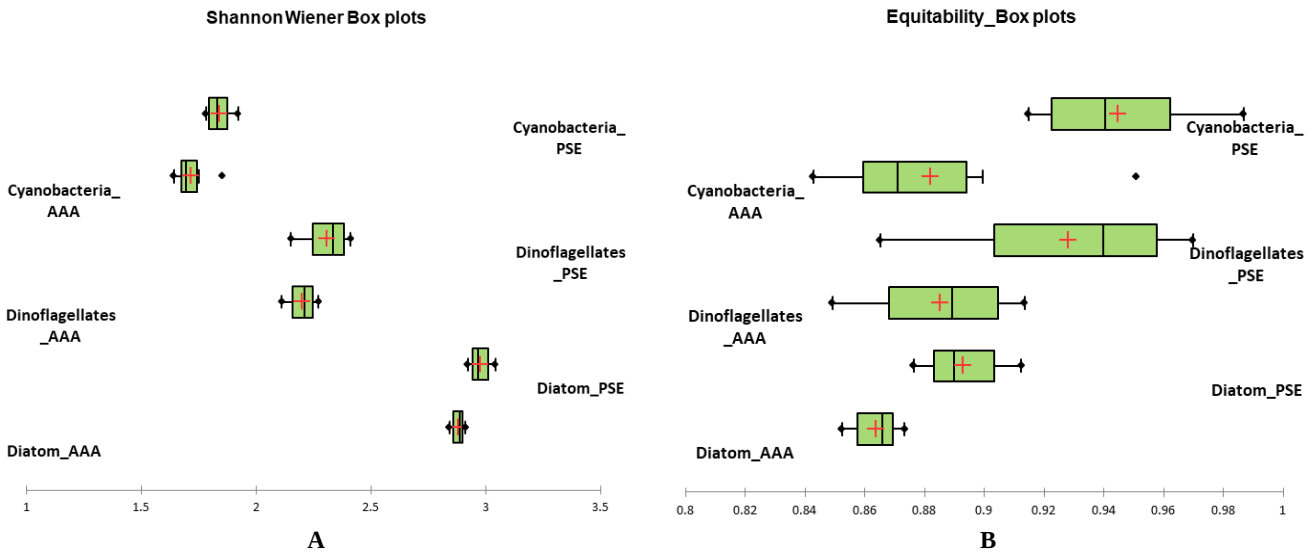

Figure 6. Representation of box plots for the diversity indices of the biological parameters diatom, dinoflagellates, and cyanobacteria at Anse Aux Anglais (AAA) and Port Sud-Est (PSE) where (A) is the Shannon Wiener (H') and (B) Equitability (EH)

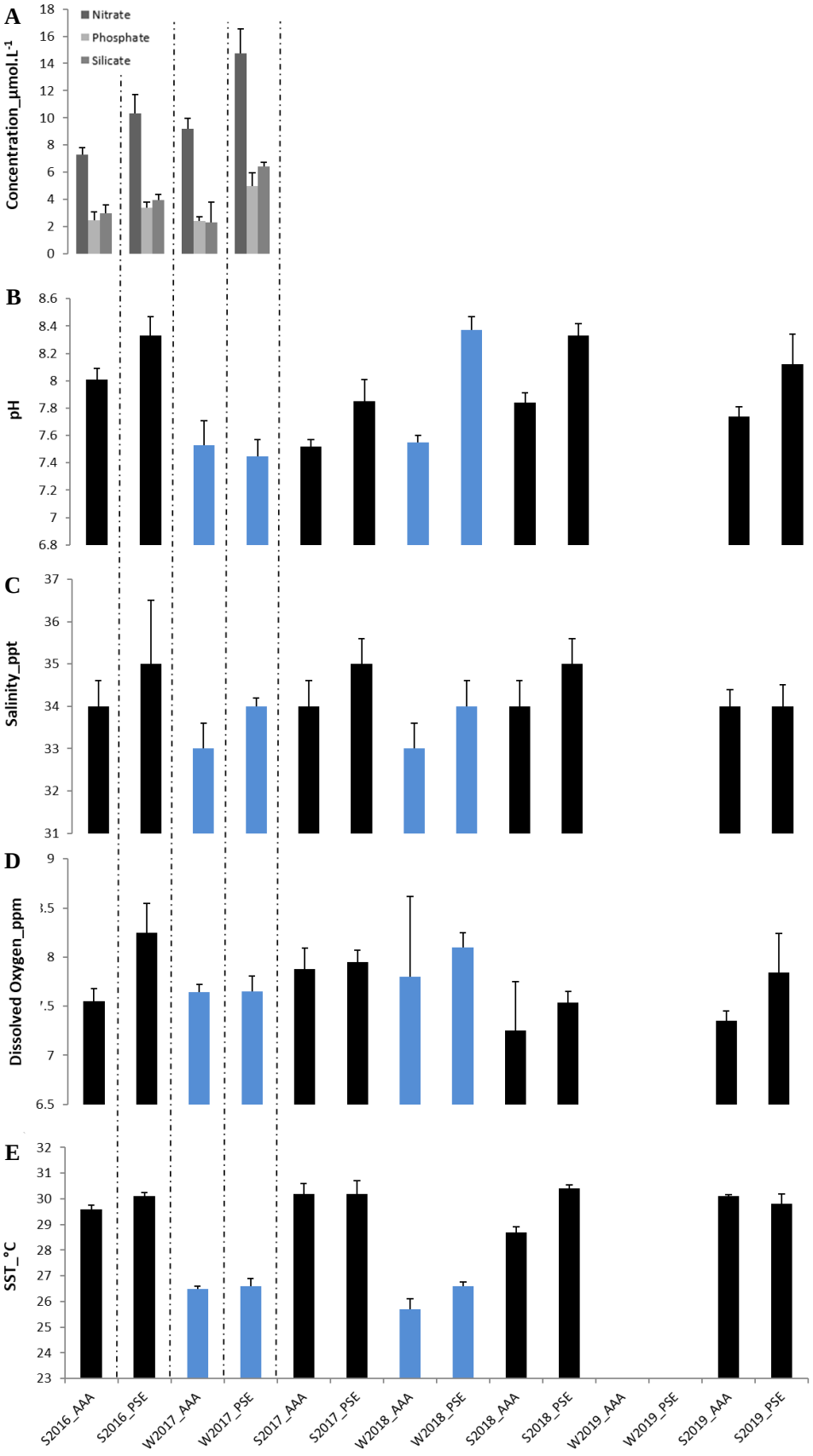

Figure 7. (A) Nutrient and (B-E) physico-chemical parameters variation during the alternate summer and winter season from 2016 up to 2019 at Anse Aux Anglais (AAA) and Port Sud-Est (PSE). The blue bars represent the winter and the black bars, summer for (B) ph, (C) Salinity, (D) Dissolved Oxygen, and (E) SST (Sea Surface Temperature)

Table 1. Represent the two-way ANOVA for the different biological parameters TMPD, diatom, dinoflagellates, cyanobacteria and chlorophyll-*a* in response to site and seasons. P<0.001= ***, P<0.01= **, P<0.05= *, NS= Not Significant

Biological parameter	Source of variation	SS	Df	MS	F	Sig.
Diatom	Site	0.155	1	0.155	0.051	NS
	Season	18.744	1	18.744	6.142	*
	Site * Season	0.100	1	0.100	0.033	NS
Dinoflagellates	Site	0.583	1	0.583	0.448	NS
	Season	4.664	1	4.664	3.585	NS
	Site * Season	0.198	1	0.198	0.152	NS
Cyanobacteria	Site	0.355	1	0.355	0.332	NS
	Season	1.707	1	1.707	1.596	NS
	Site * Season	0.029	1	0.029	0.027	NS
TMPD	Site	3.110	1	3.110	14.420	**
	Season	0.742	1	0.742	3.440	NS
	Site * Season	0.091	1	0.091	0.423	NS
Chlorophyll a	Site	0.147	1	0.147	17.637	**
	Season	0.031	1	0.031	3.691	NS
	Site * Season	0.000	1	0.000	0.032	NS

Table 2. Pearson's correlation coefficient between the different biological and physico-chemical parameters for the study period 2016 up to 2019

Pearson's correlation coefficient, r		Biological and physico-chemical parameters				
		Chla	SST	DO	Salinity	Ph
Biological parameters	TMPD	0.655*	0.067	-0.627*	-0.253	-0.442
	Diatom	0.364	0.622*	-0.063	-0.248	-0.004
	Dinoflagellates	0.645	0.675*	0.070	-0.396	-0.069
	Cyanobacteria	0.470	0.586*	0.445	-0.407	-0.191
	Chla		0.606*	0.392	0.699*	0.547

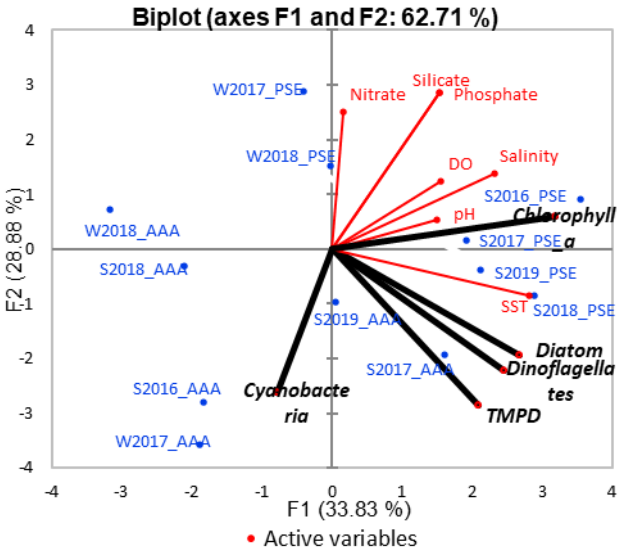

Figure 8. Principal Component Analysis (PCA) biplot analysis of the different physico-chemical parameters (nitrate, phosphate, silicate, chlorophyll-*a*, and SST-Sea Surface Temperature) and the biological parameters TMPD, diatom, dinoflagellates, cyanobacteria, and chlorophyll-*a* during the study period Summer 2016 (S2016) up to Summer 2019 (S2019), on alternate summer and winter season at Port Sud-Est (PSE) and Anse Aux Anglais (AAA)

Discussion

Over the last several decades, the coral ecosystems have been intensely threatened by complex combinations of naturogenic and anthropogenic stressors. The overall wellbeing of the coral reef depends on several factors and among these is the biological parameters phytoplankton distribution and chlorophyll-*a* concentration and other physico-chemical parameters like nutrient availability and variation in ph, salinity, dissolved oxygen and temperature of the surrounding waters.

At both sites, there were no significant differences in the photophysiological parameters. Even if the water was nutrient rich, lower ΦPSII could indicate photo-inhibition and photo-protection (Barlow et al. 2017; Oliver et al. 2003). In addition, Silsbe et al. (2015) pointed out that light had a greater influence on the ΦPSII compared to different nutrient regimes and during those days of sampling, it was always sunny and hence we can assume that the phytoplankton had adequate light. Samples were collected in the near-surface layer of 0-4 m where the micro-phytoplankton faced high light intensity and responded by making optimal use of this light and eventually dissipating the excess. Higher rETRmax values during summer period indicated that the micro-phytoplankton adapted to a higher acclimatization potential and thus the higher light saturation point (Ralph and Gademann 2005; Wagner et al. 2006). Aligning with the rETRmax, the high npqmax during the summer period tally with the findings of Kashino et al. (2002) who reported an increase in npqmax

of phytoplankton when exposed to high light regimes. Such responses in dissipating excess light is natural in micro-phytoplankton exposed to high light as a high level of NPQ may act as a safety valve to photo- protect its PSII, which is indicated by lower ΦPSII.

During this study conducted at PSE and AAA from 2016 up to 2019, it was revealed that there was no significant difference in the total micro-phytoplankton density seasonally whereby the average sea surface temperature in winter was 26.4 ± 0.3°C and in summer it was 29.9 ± 0.2°C, as expected for waters around tropical islands. The study conducted by Liu (2008) had reported similar results whereby phytoplankton did not show significant difference season-wise in the tropical waters whereby the seasonal peak was absent but it was also stated that the highest density of micro-phytoplankton was recorded in summers which corroborate with the results around Rodrigues Island. These results were also confirmed by the research conducted by Sadally et al. (2014a,b) around Mauritius Island. Studies have shown that SST rise can increase the rate of nutrient assumption by phytoplankton which can be beneficial in promoting the growth (Striebel et al. 2016; Coello-Camba and Agustí 2017; Trombetta et al. 2019) and this can explain the slightly higher density of micro-phytoplankton at PSE compared to AAA. However, rise in seawater temperatures has led to mass coral bleaching and mortality yielding in coral reefs degradation worldwide (McClanahan et al. 2007; Abdo et al. 2012; Munday et al. 2012) .

The investigation of the micro-phytoplankton variation at the non-degraded reef PSE and degraded reef AAA revealed significant spatial variation. Higher densities of micro-phytoplankton were recorded at PSE compared to AAA. A degraded reef gives the sign that the surrounding environment is not in good condition. Changes in the water quality entangle alteration of several aspects like the nutrients concentrations and physico-chemical parameters which in turn influence the micro-phytoplankton community.

Nutrient concentration and salinity can both be altered by the influx of freshwater from land via runoff due to rainfall or underground seepage and are influenced by the anthropogenic activities in the coastal region. In the seawater, some groups of phytoplankton produce nutrients via nitrogen fixation and they are namely cyanobacteria. However, the phosphate and silicate from fertilizers end up in the water via runoff. The high concentration of nutrients at the reef can be attributed to the high tidal change at Rodrigues Island (Lowry et al. 2009) . During low tide, the water recedes a lot and this mechanism can be responsible for bringing the nutrients to the reef. Nutrients such as nitrate, phosphate, and silicate are quickly used up by phytoplankton for growth (Geider and La Roche 2002; Marra et al. 1990) and this is why a negative correlation between micro-phytoplankton was obtained in response to the nutrient concentration. Excess of nutrients in the water may cause phytoplankton to bloom and eventually disturbing the planktonic community and compete against corals recruitments. Around Rodrigues Island no blooming densities of phytoplankton has been reported while studies elsewhere have been reporting cases of algae/micro-algae taking over the vast coral community (Kuffner et al. 2006; Schils et al. 2008; Foster et al. 2011) .

As for salinity, it has been categorized as a strong factor governing the phytoplankton community structure (Larson and Belovsky 2013) . The comparison of salinity between both sites did not reveal any significant differences whereby the salinity ranged between 33-35ppt which did not indicate any significant influx on freshwater runoff during the sampling periods as it was outside the peak heavy rain period of February to March, apart from the lower salinity recorded in winter which can indicate some sign of runoff waters. Moreover, for the coral community, very low salinity has been determined as having detrimental effects on the coral recruits and healthy growth. Additionally, Ferrier-Pagès et al. (1999) , showed that corals eventually died when exposed to salinity above 40ppt.

The dissolved oxygen level was always higher than > 7ppm at both sites but higher at PSE compared to AAA. This may indicate the more proper functioning of the ecosystem at PSE whereby the oxygen being consumed by the surrounding is being well replaced. Moreover, the small range of DO variation is indicative of a system in equilibrium. During this study, the DO had a negative significant correlation with the total phytoplankton density which alines with the study of El Gammal et al. (2017) in the Arabian Gulf. DO saturation in the seawater is dependent on many factors like the photosynthesis rate of phytoplankton whereby O_2 molecules are produced and the strong wind blowing on the seawater surface. If we based ourselves on the photosynthesis rate by the phytoplankton in producing oxygen molecules, the higher DO was recorded at the healthy reef, PSE, then this can be indicative of a higher density of phytoplankton. Ridder and England (2014) and Yamamoto et al. (2015) reported that during high wind activities, the interacted between the wind and water surface caused a drastic increase in the DO level of the water. Moreover, at Rodrigues Island, there is a high and fast change in tides (Lowry et al. 2009) at both sites and this can most probably promote the oxygenation of the water due to more mixing. Moreover, it was found that the ph varied above 7 at both sites which indicated the well-oxygenated ecosystems whereby there is no accumulation of the acidic molecules CO_2. As far as the corals are concerned, many studies have been reporting that the decreases in ph are causing degradation and death of corals (Marubini and Atkinson 1999; Mcculloch et al. 2012; Prada et al. 2017)

Throughout this study, the group of diatom had the highest densities followed by dinoflagellates and cyanobacteria. This alines with the studies conducted by Sadally et al. (2014a,b) in the Mascarene region at Mauritius Island located near Rodrigues Island. Moreover, these finding corroborates with those of Bazin et al. (2014) and Aubry et al. (2006) . The dominance prevailed by diatoms can be attributed to their special ability to survive and thrive through different changes in environmental parameters variability like temperature fluctuation and light availability which is crucial for photosynthesis (Kokfelt et

al. 2009) . Furthermore, Levine et al. (1999) demonstrated that grazing accounts to 0-6% of diatom removal compared to dinoflagellates which are around 6-26% per day. Some on the dominant micro-phytoplankton genera (*Coscinodiscus, Chaetoceros, Navicula, Fragillaria, Nitzschia, Licmorphora, Ceratium, Peridinium* and other like *Trichodesmium*) observed at Rodrigues Island matches with those genera observed by Sadally et al. (2014a,b) around Mauritius Island. The study conducted by Karthikeyan et al. (2013) revealed that the genera *Chaetoceros* has been located in regions where there was a high influx of nutrient and this can be attributed to the high growth rate of this specific genus which is around 1.16 generations per day. Among others, the high density of the genera *Fragillaria*, considered as freshwater micro-phytoplankton can be a sign of river runoff water at both the degraded reef AAA and the non-degraded reef PSE (Dauta et al. 1990) . Moreover, it should be noted that some toxic dinoflagellates like *Dinophysis* (Tomas 1996) , *Alexandrium* (Cembella et al. 2000) , and *Ceratium* (Orellana-Cepeda et al. 2004) was found at Rodrigues Island but they were not in blooming densities.

Rasconi et al. (2017) reported a loss of phytoplankton diversity in response to the increase in temperature which can alter the whole phytoplankton community structure. Eventually, it was found that the slightly higher sea surface temperature was recorded at PSE compared to AAA but the non-degraded reef at PSE revealed higher species diversity compared to the degraded reef at AAA. Among the difference, the highest was spotted for the density of cyanobacteria. Unlike to diatom and dinoflagellates, the cyanobacteria recorded the highest densities throughout the whole study period at the degraded reef AAA compared to non-degraded reef PSE. Mccormick et al. (2017) reported evidence where cyanobacteria were taking over the dead corals. Moreover, cyanobacteria like *Trichodesmium* genera have been reported as very resistant to an increase in temperature and to have a very high nitrogen fixation rate which can eventually promote their high growth (Karl et al. 2002) . Furthermore, cyanobacteria can use different methods like space occupation and dominance which eventually obstruct the new coral recruitments on dead reefs which lead to a decrease in coral coverage (Charpy et al. 2010) .

Apart from the cyanobacteria, Schils et al. (2008) reported the mortality of gorgonian soft coral-associated to diatom bloom. Diatom bloom causing the death of coral was pointed out in Mccormick et al. (2017) . Foster et al. (2011) reported that persistent dinoflagellates bloom of the genera *Polykrikos* led to coral community shift. Guzmán et al. (1990) reported the high coral mortality associated to dinoflagellates blooms of the genera *Gonyaulax* and among other, Kuffner et al. (2006) showed evidence of the inhibition of coral recruitment by the cyanobacteria *Lyngbya*. The study conducted by Yamashiro et al. (2012) showed that epizoic diatom *Licmorphora* was taking over the *Montipora* corals whereby the diatom kills the coral through suffocation. This process can probably die to those regions having high input of nutrients thus causing bloom of phytoplankton.

In conclusion, this study revealed spatial but not seasonal variation in the micro-phytoplankton density in the waters around Rodrigues. The micro-phytoplankton at the non-degraded reef of PSE differed from that of the degraded reef of AAA. Higher species diversity at PSE compared to AAA may show signs of diversity decrease at AAA whereby a higher diverse ecosystem is considered to be more healthy and in equilibrium. Moreover, among the physico-chemical parameters, the DO level was highest at PSE which indicated higher photosynthetic rate of the micro-phytoplankton while keeping the alkalinity level above optimum 7 which shows no accumulation of the acidic gas molecule CO_2. Cyanobacteria showing the highest densities at AAA can be considered as evidence of a decrease in the biological health of the ecosystem over there. The nutrient level at both sites was high which shows signs of runoff from the mainland and the effect of anthropogenic activities interact with the coastal waters. There exist enough evidence worldwide about the diatom, dinoflagellates, and cyanobacteria taking over the coral communities leading to mortality or changing in the community structure. Some of the species reported like *Licmorphora, Gonyaulax, Polykrikos, Trichodesmium,* and *Lygnbya* were found during the study around Rodrigues Island. It is very essential to determine the distribution of micro-phytoplankton and to determine any early signs of an outbreak of certain key species. The micro-phytoplankton will serve as an indicator for the health status of the coral ecosystem from a biological perspective and thus one will be able to design proper management strategies to overcome to loss of coral reefs and all the resultant associated ecosystem services.

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